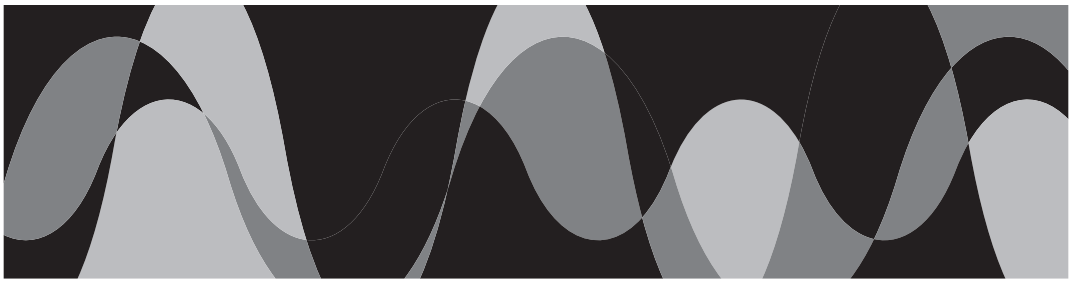

20th Anniversary Meeting
Society for Research on Biological Rhythms
Program and Abstracts

SRBR

May 17–21, 2008

Sandestin Golf and Beach Resort

Destin, Florida

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Table of Contents

President's Welcome

The Society for Research on Biological Rhythms first convened in May 1986 at Wild Dunes, South Carolina. After 20 years, we continue to fulfill founding President Fred Turek's vision of a vibrant society and a long series of successful meetings. Our goal remains to bring together researchers and their research on all aspects of biological rhythms. We encourage the exchange of information and ideas during the scheduled scientific sessions, as well as the enlightenment that comes from discussions exposed to the sun, sand, and sea breeze.

Welcome to the 11th Biennial Meeting! We are the beneficiaries of months of planning and work by many people. Program Chair Ravi Allada and his committee have put together an outstanding set of scientific sessions, diverse in form and content. Facilities Chair Shelley Tischkau choreographed the complex shifts of space through time. Ken Wright, Jr. and his committee have organized and established the first full SRBR Trainee Professional Development Day and overseen trainee awards for research accomplishment.

Various sub-groups will meet while we are here. We have two satellite symposia, one honoring Aaron Lerner's discovery of melatonin, organized by Jo Arendt and Al Lewy, and the other focused on the Systems Biology of Clocks, organized by John Hogenesch. The SRBR Biennial Meeting is an opportunity for convening the Board of Directors, our standing committees on Animal Issues, chaired by Larry Morin; on ChronoHistory, chaired by Anna Wirz-Justice; and on Communications, chaired by Frank Scheer; as well as the Editorial Board of the Journal of Biological Rhythms, chaired by Marty Zatz. Results of the election of the new SRBR board, coordinated by Rae Silver, chair of the Nominations Committee, will be announced at the Business Meeting on Wednesday. If you have ideas on ways to enhance the activities of these arms of the society, take the opportunity during the meeting to talk to any member of these groups, which are listed in the Program Book.

Special thanks are due Michelle Chappell, Karen Nichols and Amy Hubbard at Conference & Institutes of the University of Illinois, for their skill in organizing this event, coordinating with Sandestin and anticipating our needs, and ensuring that it all holds together while it runs. We are deeply grateful to our corporate and government sponsors for their generous support. Their names are listed at the front of this book, and posted throughout Sandestin Conference Center. Please take time to personally thank their representatives for their investment in the SRBR.

Last, but by no means least, I extend my thanks to each of you, the speakers, presenters, and participants. Your passion, originality, and engagement will ensure success. Best wishes for a fulfilling meeting!

Martha U. Gillette
President, SRBR, 2006–2008

General Information

Headquarters is at the Baytowne Conference Center, where all scientific activities will be held. The Conference Center is conveniently located within walking distance of all hotel rooms.

SRBR Information Desk and Message Center is in the Foyer of the Baytowne Conference Center main level.

The desk hours are as follows:

	Morning	*Afternoon*
Friday, May 16		2:00–6:00 PM
Saturday, May 17	7:30–11:00 AM	1:00–7:00 PM
Sunday, May 18	7:00–11:00 AM	3:00–7:00 PM
Monday, May 19	7:30–11:00 AM	3:00–6:00 PM
Tuesday, May 20	7:30–11:00 AM	3:00–6:00 PM
Wednesday, May 21	7:30–10:00 AM	

Messages can be left on the SRBR message board next to the registration desk. Meeting participants are asked to check the message board routinely for mail, notes, and telephone messages. The telephone number for onsite information is 217-714-9479.

Hotel check-in will be at the individual properties.

Posters will be available for viewing in the Magnolia ABC rooms.

Poster numbers 1–100	Sunday, May 18, 10:00 AM–10:30 PM
Poster numbers 101–200	Monday, May 19, 10:00 AM–10:30 PM
Poster number 201–301	Tuesday, May 20, 10:00 AM–10:30 PM

All posters must be removed by 10:00 AM on Wednesday, May 21.

The Village of Baytowne Wharf—Indulge your senses at Sandestin's charming Village of Baytowne Wharf, a picturesque pedestrian village overlooking the Choctawatchee Bay. Discover a unique collection of more than 40 specialty merchants ranging from quaint boutiques and intimate eateries to lively nightclubs—all set up against a backdrop of vibrant special events.

Special Events

Saturday, May 17

Trainee Professional Development Symposium • 9:00 AM–4:00 PM

Aaron Lerner Memorial Symposium • 2:00–5:00 PM

Welcome Reception • 7:00–9:00 PM • Grand Lawn
In case of inclement weather, the reception will be held in the Azalea Room.

Monday, May 19

Executive Committee Meeting • 12:30–2:30 PM • *Magnolia E*

ChronoHistory Committee Meeting • 2:30–3:30 PM • *Magnolia E*

Presidential Symposium • 4:30–6:00 PM • *Azalea Ballroom*

Tuesday, May 20

JBR Editors' Meeting • 2:00–4:00 PM • *Magnolia D*

Wednesday, May 21

Business Meeting • 4:00–5:00 PM • *Azalea Ballroom*
All attendees are invited to attend.

Pittendrigh/Aschoff Lecture: Ueli Schibler, University of Geneva • 5:30–6:30 PM • *Azalea Ballroom*

Closing Banquet • 8:00 PM • *Magnolia ABC*
All guests need to purchase a banquet ticket in advance at the registration desk.

Meeting at a Glance

All events will take place in the Baytowne Conference Center.

Saturday, May 17

9:00 AM–4:00 PM	**Trainee Professional Development Symposium**
2:00–5:00 PM	**Aaron Lerner Memorial Symposium**
7:00–9:00 PM	**Welcome Reception** • *Grand Lawn*

Sunday, May 18

8:00–10:00 AM	**Poster Setup** • *Magnolia ABC*
8:30–10:30 AM	**Symposium 1: Molecular Clocks 1**• *Azalea Ballroom*
	Symposium 2: Circadian Neural Networks • *Camellia I & II*
10:30–11:00 AM	**Refreshment Break**
11:00 AM–12:30 PM	**Slide Sessions A–C**
	A) Molecular Clocks I • *Azalea I & II*
	B) Feeding and Metabolism • *Camellia I & II*
	C) Sleep • *Azalea III*
12:30–4:30 PM	**Free Time**
4:30 PM–6:30 PM	**Symposium 3: Technological Advances in Circadian Biology** • *Azalea III*
	Symposium 4: Comparative Clocks • *Azalea I & II*
	Symposium 5: Circadian and Homeostatic Regulation of Human Sleep • *Camellia I & II*
8:00–10:30 PM	**Poster Session I** • *Magnolia ABC*

Monday, May 19

8:30–10:30 AM	**Symposium 6: Interplay between Circadian Clocks and Metabolism** • *Azalea Ballroom*
	Symposium 7: Disordered Human Circadian Clocks • *Camellia I & II*
10:30–11:00 AM	**Refreshment Break**
11:00 AM–12:30 PM	**Slide Session D–F**
	D) Light Signaling • *Azalea III*
	E) Neuronal Networks • *Azalea I & II*
	F) Peripheral Clocks I • *Camellia I & II*
12:30–2:30 PM	**Executive Committee Meeting** • *Magnolia E*
2:30–3:30 PM	**ChronoHistory Committee Meeting** • *Magnolia E*

12:30–4:30 PM	**Free Time**
4:30–6:00 PM	**Presidential Symposium: Is It Time for a New View of Circadian Clocks?** • *Azalea Ballroom*
8:00 PM–10:30 PM	**Poster Session II** • *Magnolia ABC*

Tuesday, May 20

8:30–10:30 AM	**Symposium 8: Post-translational Regulation of Circadian Clocks** • *Azalea Ballroom*
	Symposium 9: Circadian Clocks and Sleep • *Camellia I & II*
10:30–11:00 AM	**Refreshment Break**
11:00 AM–12:30 PM	**Slide Session G-I**
	G) Molecular Clocks II • *Azalea I & II*
	H) Peripheral Clocks II • *Camellia I & II*
	I) Human Clocks • *Azalea III*
12:30–4:30 PM	**Free Time**
2:00–4:00 PM	**JBR Editors' Meeting** • *Magnolia D*
4:30–6:30 PM	**Symposium 10: Non–image Forming Photoreception** • *Camellia I & II*
	Symposium 11: Systems Biology and Modeling of Circadian Rhythms • *Azalea I & II*
	Symposium 12: Seasonal and Circannual Rhythms • *Azalea III*
8:00–10:30 PM	**Poster Session III** • *Magnolia ABC*

Wednesday, May 21

8:30–10:30 AM	**Symposium 13: Interplay between Circadian and Social Behavior** • *Azalea I & II*
	Symposium 14: Clocks, Cell Cycle and Cancer • *Azalea III*
	Symposium 15: Clocks, Brain Function, and Dysfunction • *Camellia I & II*
10:30–11:00 AM	**Refreshment Break**
11:00 AM–12:30 PM	**Slide Session J–L**
	J) Systems Biology and Modeling • *Azalea I & II*
	K) Entrainment and Photoreception • *Azalea III*
	L) Neural Clocks and Output • *Camellia I & II*
12:30–4:00 PM	**Free Time**
4:00–5:00 PM	**Business Meeting** • *Azalea Ballroom*
5:30–6:30 PM	**Pittendrigh/Aschoff Lecture: Ueli Schibler, University of Geneva, The Mammalian Circadian Timing System: From Gene Expression to Metabolism** • *Azalea Ballroom*
8:00–11:00 PM	**Closing Banquet** • *Magnolia ABC*

Trainee Professional Development Day

Saturday, May 17

9:00–9:15 AM **Welcome and Orientation** • *Magnolia A*

9:30–10:30 AM **Keynote Address** • Martha Merrow, University of Groningen

10:40–11:30 AM **Session 1**

The Peer Review Process and How To Get Published • *Magnolia B*
Marty Zatz, Editor, *Journal of Biological Rhythms*

Grantsmanship USA—NIH • *Magnolia C*
Gary Pickard, Colorado State University, and Michael Selmanoff, National Institutes of Health

Choosing Where To Post Doc(S): How This Affects Your Search for a Faculty Position • *Jasmine*
Martha Gillette, University of Illinois at Urbana-Champaign, and Erik Herzog, Washington University, St. Louis

Circadian Techniques—Hormone Assays • *Camellia I*
Josephine Arendt, University of Surrey

Circadian Techniques—Basics of Mathematical Modeling • *Camellia II*
Elizabeth Klerman, Harvard Medical School

11:40 AM–12:30 PM **Session 2**

Grantsmanship USA—NSF • *Magnolia B*
Erik Herzog, Washington University

Grantsmanship—Europe • *Magnolia C*
Marie Currie Grant, Welcome Trust and Howard Cooper, University of Lyon

Developing and Maintaining Records of Research/Academic Performance and Interview Skills and Giving a Job Talk • *Jasmine*
David Weaver, University of Massachusetts Medical Center, and Ken Wright, University of Colorado at Boulder

Working Internationally Non-USA to USA • *Camellia I*
Horacio de la Iglesia, University of Washington and Naomi Rogers, University of Sydney

Career Paths—Industry • *Camellia II*
Lauren Shearman, Merck Research Laboratories

12:30–1:45 PM **Lunch** • *Magnolia A*

1:45–2:35 PM **Session 3**

Circadian Techniques—Electrophysiology • *Magnolia B*
Stephan Michel, Leiden University Medical School

Circadian Techniques—Genetic and Molecular Approaches for Rhythms—Flies/Zebra Fish/Neurospora • *Magnolia C*
Jay Dunlap, Dartmouth Medical School

Working Internationally USA to Non-USA • *Jasmine*
Steve Brown, University of Zurich and Derk-Jan Dijk, University of Surrey

Grantsmanship—Canadian • *Camellia I*
Ralph Mistlberger, Simon Fraser University

How To Set Up a Research Laboratory• *Camellia II*
Diane Boivin and Nico Cermakian, McGill University

2:45–3:35 PM **Session 4**

Working Internationally Non-USA to Non-USA• *Magnolia B*
Shantha Rajaratnam, Monash University, and Georg Bjarnason, University of Toronto

Circadian Techniques—Imaging • *Magnolia C*
David Welsh, University of California–San Diego

Making the Most Out of Attending Scientific Meetings • *Jasmine*
Mary Carskadon, Brown University, and Fred Turek, Northwestern University

Circadian Techniques—Genetic and Molecular Approaches for Rhythms—Mice/Humans • *Camellia I*
Till Roenneberg, Ludwig Maximilians University

Short- and Long-Term Research Program Planning • *Camellia II*
Joseph Takahashi, HHMI/Northwestern University, and Steve Lockley, Harvard Medical School

3:45–4:00 PM **Conclusion** • *Magnolia A*

Aaron Lerner Memorial Symposium

Saturday, May 17

2:00-5:00 PM

The Importance of Melatonin to Research in Biological Rhythms: A Symposium in Remembrance of Aaron Lerner, Discoverer of Melatonin • *Azalea I & II*

Organizers: Josephine Arendt, University of Surrey and Alfred Lewy, Oregon Health and Science University

Chair: Martha Gillette, University of Illinois at Urbana-Champaign

2:00	Martha Gillette
2:10	Anna Wirz-Justice, University of Basel, *Co-chair*
2:20	**Melatonin and Neuroendocrine Transduction in the Chronopaleolithic** • Marty Zatz, Editor, *Journal of Biological Rhythms*
2:40	**Melatonin Synthesis: The Ticks and Tocks** • David C. Klein, National Institutes of Health
3:00	**Pineal Melatonin Rhythm as a Code for Photoperiod** • Bruce Goldman, University of Connecticut
3:20	**Refreshment Break**
3:50	Stuart Armstrong, Swinburne University, *Co-chair*
4:00	**Melatonin: Clock, Calendar, Chronobiotic** • Josephine Arendt, University of Surrey
4:20	**What We Have Learned about Melatonin Receptors since Aaron Lerner** • Margarita L. Dubocovich, Northwestern University School of Medicine
4:40	**The Legacy of Aaron Lerner in Ten Years' Time** • Alfred Lewy, Oregon Health and Science University

Program Overview

Saturday, May 17

7:00-9:00 PM **Opening Reception** • *Grand Lawn*

Sunday, May 18

8:00-11:00 AM **Poster Session Setup** • *Magnolia ABC*

8:30–10:30 AM **Symposium 1: Molecular Clocks I** • *Azalea Ballroom*

Chair: Hiroki Ueda, RIKEN

8:30 **The Transcription-Translation Loop In Drosophila** • Michael Rosbash, Brandeis University

9:00 **Transcription and Drosophila Clocks** • Paul Hardin, Texas A&M University

9:30 **A Systems Approach to Elucidate Network Structures of the Circadian Clock** • John Hogenesch, University of Pennsylvania School of Medicine

10:00 **Signaling to Chromatin and the Circadian Clock** • Paolo Sassone-Corsi, School of Medicine, University of California–Irvine

Symposium 2: Circadian Neural Networks • *Camellia I & II*

Chair: Michael Nitabach, Yale University School of Medicine

8:30 **Setting Clock Speed** • Elizabeth Maywood, MRC-Laboratory of Molecular Biology

9:00 **Neuronal Synchronization and Circadian Function** • Johanna Meijer, University of Leiden

9:30 **Precision from Networked, Sloppy, Circadian Cells** • Erik D. Herzog, Washington University, St. Louis

10:00 **Molecular and Neural Control of Drosophila Circadian Behavior** • Patrick Emery, University of Massachusetts Medical School

10:30–11:00 AM **Refreshment Break**

11:00 AM–12:30 PM **Slide Session A: Molecular Clocks I** • *Azalea I & II*

11:00 **1 • Genetic and Temporal Requirements for the Ectopic Induction of the Circadian Clock Program Reveal a Unique Role for the Clock Gene** • Valerie L. Kilman, Northwestern University

11:15 **2 • Linking CLK/CYC Activity to Phosphorylation and In Vivo Phenotypes** • Jerome S. Menet, Brandeis University

11:30 **3 • Rhythmic SAF-A Binding Underlies Circadian Transcription of the Bmal1 Gene** • Yoshiaki Onishi, University of Tsukuba

11:45 **4 • Regulation of Per1 by Aryl Hydrocarbon Receptor** • Shelley A. Tischkau, Southern Illinois University School of Medicine

12:00 **5 • Bmal1 Is a Direct Transcriptional Target of the Orphan Nuclear Receptor, NR2F1** • Fred Pereira, Baylor College of Medicine

12:15 **6 • An Sv-40 Poly Adenylation Signal Sequence in the 3'utr of Per2::Luciferase(Sv) Mice Lengthens Free-Running Period and Increases Per2** • Seung-Hee Yoo, Howard Hughes Medical Institute, Northwestern University

Slide Session B: Feeding and Metabolism • *Camellia I & II*

11:00 **7 • Restricting Food Intake to the Light Phase Results in Weight Gain in C57BL/6J Mice** • Deanna M. Arble, Northwestern University

11:15 **8 • Role of Cardiomyocyte Circadian Clock in Myocardial Metabolic Adaptation** • Ju-Yun Tsai, Baylor College of Medicine

11:30 **9 • Clock Gene Is Required for the Circadian Regulation of Plasma Lipids, Lipoproteins and Microsomal Triglyceride Transfer Protein (MTP)** • Xiaoyue Pan, SUNY Downstate Medical Center

11:45 **10 • Orexin: the Molecular Link between Plasma Glucose and Sleep/Wake Rhythms?** • C.X. Yi, Netherlands Institute for Neurosciences,

12:00 **11 • Linking the Development of Adiposity to Altered Circadian Rhythms** • Molly Bray, Baylor College of Medicine

12:15 **12 • Polymorphisms in the Clock Gene Are Associated with the Metabolic Syndrome and Dyslipidaemia in Man** • E.M. Scott, University of Leeds

Slide Session C: Sleep • *Azalea III*

11:00 **13 • Circadian Changes in the Sleep Electroencephalogram (EEG) under Constant Sleep Pressure in the Rat** • Tom Deboer, Leiden University Medical Center

11:15 **14 • Light-Dark Differences in Heritability of Sleep-Wake Behaviors in the Inbred Mouse** • D. Joseph Owens-Ream, Northwestern University

11:30 **15 • Long-term Effects of Artificial Dawn on Sleep Inertia: Does Melatonin Play a Role?** • M.C. Gimenez, University of Groningen

11:45 **16 • Sleep, Performance, and Error Rates in Anaesthetists Working Day and Night Shifts** • J.F. Cheeseman, University of Auckland

12:00 **17 • Habitual Sleep Length and Subjective Perception of Seasonality are Associated with Melatonin Deficit** • Dieter Kunz, Universitätsmedizin Berlin

12:15 **18 • Effects of Ramelteon on Insomnia Symptoms Induced by Rapid, Eastward Travel** • Phyllis Zee, Northwestern University Medical School

4:30–6:30 PM **Symposium 3: Technological Advances in Circadian Biology** • *Azalea III*

Chair: Charles Allen, Oregon Health and Science University

4:30 **Quantitative Proteomics and Its Applications to Study Proteins under Circadian Control** • Kathryn Lilley, Cambridge University

5:00 **Bioluminescence Imaging of Circadian Clock Gene Expression in Single Cells** • David K. Welsh, University California–San Diego

5:30 **Mapping PDF Receptor Activation and Expression in the Drosophila Brain** • Paul Taghert, Washington University, St. Louis

6:00 **Cellular Dissection of Neuropeptide Signaling** • Mike Nitabach, Yale University School of Medicine

Symposium 4: Comparative Clocks • *Azalea I & II*

Chair: Eran Tauber, University of Leicester

4:30 **The Circadian Clock of the Monarch Butterfly** • Steven M. Reppert, University of Massachusetts Medical School

5:00 **What Season Is It Anyway—With Climate Warming, You Snooze You Lose** • William E. Bradshaw, University of Oregon

5:30 **Biological Rhythms in a Tidal Crustacean** • Michael H. Hastings, MRC Laboratory of Molecular Biology

6:00 **Natural Variation and Evolution of Plant Clock Genes** • C. Robertson McClung, Dartmouth College

Symposium 5: Circadian and Homeostatic Regulation of Human Sleep • *Camellia I & II*

Co-chairs: Shantha Rajaratnam, Monash University and Derk-Jan Dijk, University of Surrey

4:30 **Circadian and Homeostatic Regulation of Sleep in Adolescent Humans** • Mary A. Carskadon, Alpert Medical School of Brown University

5:00 **Homeostatic Sleep Regulation in Morning and Evening Types** • Marie Dumont, Sacré-Cœur Hospital & University of Montréal

5:30 **Age-Related Changes in Homeostatic and Circadian Regulation of Sleep** • Christian Cajochen, Psychiatric University Clinics, Basel

6:00 **Circadian and Sleep-wakefulness Regulation of Cognitive Function in Humans Sleep and Circadian Systems Interact to Regulate Daily Patterns of Cognitive Function** • Kenneth P. Wright Jr., University of Colorado at Boulder

8:00–10:30 PM **Poster Sessions I** • *Magnolia ABC*

Monday, May 19

8:30–10:30 AM **Symposium 6: Interplay between Circadian Clocks and Metabolism** • *Azalea Ballroom*

Chair: Joseph Bass, Northwestern University

8:30 **Tissue Specific Roles of BMAL1 in Lipid Metabolism** • Shigeki Shimba, Nihon University

9:00 **Circadian Control of Metabolism Is Regulated Post-transcriptionally** • Carla B. Green, University of Virginia

9:30 **Analysis of BMAL1 Multi-Protein Complexes** • Charles J. Weitz, Harvard Medical School

10:00 **Clocks and Metabolism in Mice** • Masashi Yanagisawa, University of Texas Southwestern Medical School

Symposium 7: Disordered Human Circadian Clocks • *Camellia I & II*

Chair: Phyllis Zee, Northwestern University

8:30 **Mutations for Human Sleep Phenotype** • Ying-Hui Fu, University of California–San Francisco

9:00 **Human Circadian Rhythms** • Charles Czeisler, Brigham and Women's Hospital, Harvard Medical School

9:30 **Peripheral Circadian Oscillators in Blood and Non-SCN Brain Tissues in Humans** • D.B. Boivin, Douglas Mental Health University Institute

10:00 **Analysis of PER3 Polymorphisms and Haplotypes That Associate with Diurnal Preference and Delayed Sleep Phase Disorder** • Simon N. Archer, University of Surrey

10:30–11:00 AM **Refreshment Break**

11:00 AM–12:30 PM **Slide Session D: Light Signaling** • *Azalea III*

11:00 **19 • Light Resetting and Entrainment in CLOCK-Deficient Mice** • Robert Dallmann, University of Massachusetts Medical School

11:15 **20 • Mice Lacking the PACAP type I Receptor Have Impaired Photic Entrainment and Negative Masking** • Jens Hannibal, Bispebjerg Hospital

11:30 **21 • CDK5—A Modulator of Glutamate Signaling–Determines Amplitude of Phase Shifts** • J. M. Ding, East Carolina University Medical School

11:45 **22 • A Role for PRMT5 in the Regulation of Light Signaling and Clock Function in Arabidopsis** • Marcelo Yanovsky, Universidad de Buenos Aires y CONICET

12:00 **23 • Photoperiodic Flowering Occurs under the External and Internal Coincidence Mechanisms in Arabidopsis** • Takato Imaizumi, University of California–San Diego

12:15 **24 • XAP5 Circadian Timekeeper Coordinates Light Signals to Properly Time the Circadian Clock and Photomorphogenesis in Arabidopsis** • Stacy Harmer, University of California–Davis

Slide Session E: Neuronal Networks • *Azalea I & II*

11:00 **25 • The Functional Neuroanatomy of the PDF Receptor Reveals Output Circuits in the Circadian Pacemaker Network** • Luoying Zhang, Northwestern University

11:15 **26 • Entrainment and Phasing of the Morning and Evening Oscillators in Drosophila : A New Function for the PDF Neuropeptide** • F. Rouyer, Institut de Neurobiologie Alfred Fessard, CNRS

11:30 **27 • Lateral Ventral Neurons Determine Relative Diurnal/Nocturnal Behavior in Drosophila** • Todd C. Holmes, University of California–Irvine

11:45 **28 • A Function for the Large PDF Cells in Drosophila Arousal** • Yuhua Shang, Howard Hughes Medical Institute and National Center for Behavioral Genomics, Brandeis University

12:00 **29 • VIP Synchronizes Circadian Rhythms in Astrocytes** • Luciano "Flaco" Marpegan, T.J. Kral, Washington University, St. Louis

12:15 **30 • An Ensemble of Novel Circadian Oscillators in the Mediobasal Hypothalamus** • Clare Guilding, University of Manchester

Slide Session F: Peripheral Clocks I • *Camellia I & II*

11:00 **31 • Clock Control** • Loning Fu, Baylor College of Medicine

11:15 **32 • Oscillator-Independent Control of Cell Cycle Progression by Circadian Clock Proteins** • Eugin Destici, Erasmus University Medical Center

11:30 **33 • Opposite Actions of Hypothalamic Vasopressin on Circadian Corticosterone Rhythm in Nocturnal Versus Diurnal Species** • A. Kalsbeek, Netherlands Institute of Neuroscience

11:45 **34 • Robust Adverse Effects of Chronic Circadian Desynchronization in Animals under a Physiological "Challenge"** • Fabian Preuss, Northwestern University

12:00 **35 • Scheduled Exposures to a Novel Environment with a Running-Wheel Differentially Accelerate Re-Entrainment of Mice Peripheral Clocks to New Light-Dark Cycles** • Yujiro Yamanaka, Hokkaido University Graduate School of Medicine

12:15 **36 • Chemosensory Rhythms in Drosophila** • Abhishek Chatterjee, Texas A&M University

4:30–6:00 PM **Presidential Symposium: Is It Time for a New View of Circadian Clocks?** • *Azalea Ballroom*

Chair: Martha Gillette, University of Illinois at Urbana-Champaign

Can a Biologist Fix a Radio? A Clock? • Yuri Lazebnik, Cold Spring Harbor Laboratory

Nudging Nature's Networks: High Throughput Biology and the Use of Perturbagens • Steve A. Kay, University of California–San Diego

Diurnal Rhythms: Unrecognized Critical Determinants of Cardiovascular and Renal Health and Disease • Michael J. Sole, University of Toronto

8:30–10:30 PM **Poster Session II** • *Magnolia ABC*

Tuesday, May 20

8:30–10:30 AM **Symposium 8: Post-translational Regulation of Circadian Clocks** • *Azalea Ballroom*

Chair: Carla Green, University of Virginia

8:30 **Circadian System of Cyanobacteria Timed by a Clock Protein KaiC** • Takao Kondo, Nagoya University

9:00 **Transcriptional and Post-transcriptional Feedback Loops of the Circadian Clock** • Michael Brunner, Heidelberg University

9:30 **Post-translational Processes in the Mammalian Circadian Oscillator** • Achim Kramer, Charité Universitätsmedizin, Berlin

10:00 **Post-translational Regulation in Mammals** • Joseph Takahashi, Howard Hughes Medical Institute, Northwestern University

Symposium 9: Circadian Clocks and Sleep • *Camellia I & II*

Chair: Fred Turek, Northwestern University

8:30 **Molecular Analysis of Sleep In Drosophila** • Amita Sehgal, Howard Hughes Medical Institute, University of Pennsylvania Medical School

9:00 **A Non-circadian Role for Clock Genes in Sleep Homeostasis** • Paul Franken, University of Lausanne

9:30 **Finding the Light—Depression, Sleep and Circadian Networks** • Christopher J. Winrow, Merck Research Laboratories

10:00 **Period3 and the Circadian and Homeostatic Regulation of Sleep Physiology and Waking Performance in Humans** • Derk-Jan Dijk, University of Surrey

10:30-11:00 AM **Refreshment Break**

11:00 AM–12:30 PM **Slide Session G: Molecular Clocks II** • *Azalea I & II*

11:00 **37 • Evolutionarily Conserved Features of Vertebrate Cki Delta and Drosophila Dbt in the Circadian Mechanism** • Jeffrey L. Price, University of Missouri

11:15 **38 • DBT-Directed Phosphorylation of the Drosophila Period Protein: Mapping and Functional Analysis** • Lino Saez, The Rockefeller University

11:30 **39 • The Role of Casein Kinase 1 in Circadian Timing in Mammals** • *Andrew Loudon*, University of Manchester

11:45 **40 • Identification of Chemical Compounds Capable of Tuning the Mammalian Circadian Clock** • Zheng Chen, University of Texas Southwestern Medical Center

12:00 **41 • Post-transcriptional and Post-translational Regulation of the Neurospora Circadian Clock** • Yi Liu, University of Texas Southwestern Medical Center

12:15 **42 • Saturation Mutagenesis Screen of CRYPTOCHROME Proteins Reveals Novel PER Binding Domain Common to Both Crys and a CRY2-Specific Repression Domain** • Ellena A. van der Schalie, University of Virginia

Slide Session H: Peripheral Clocks II • *Camellia I & II*

11:00 **43 • Importance of the Transcription Factors CLOCK and BMAL1 in Central and Peripheral Circadian Oscillators** • Caroline H. Ko, Howard Hughes Medical Institute, Northwestern University and University of Toronto

11:15 **44 • Diurnal Variation in Myocardial Ischemia/Reperfusion Tolerance; Mediation by the Circadian Clock within the Cardiomyocyte** • David J. Durgan, Baylor College of Medicine

11:30 **45 • Circadian Activation of the Calcium-Activated Protein Phosphatase Calcineurin in the Heart and Beyond** • Beverly A. Rothermel, University of Texas Southwestern Medical Center

11:45 **46 • Differences in Liver Metabolism between Males and Females Depend on Circadian Timekeeping** • Xavier Bonnefont, Institut de Génomique Fonctionnelle, Montpellier

12:00 **47 • Tissue-Specific Disruption of Mouse Casein Kinase 1 Delta Affects Circadian Function** • Jean-Pierre Etchegaray, University of Massachusetts Medical School

12:15 **48 • Rhythmic Orchestration of the Unfolded Protein Response in the Liver Endoplasmic Reticulum** • Frédéric Gachon, Inserm, Montpellier

Slide Session I: Human Clocks • *Azalea III*

11:00 **49 • Circadian Gene Polymorphisms in Delayed Sleep Phase Disorder** • Daniel F. Kripke, University of California–San Diego

11:15 **50 • Age and Peripheral Human Molecular Circadian Rhythms** • Lucia Pagani, University of Basel

11:30 **51 • Physiological Responses to Circadian Misalignment; Health Implications for Shift Workers** • Frank A.J.L. Scheer, Brigham and Women's Hospital and Harvard Medical School

11:45 **52 • New Insights and Techniques for Understanding the Health Impact of Circadian Disruption** • Mark S. Rea, Rensselaer Polytechnic Institute

12:00 **53 • A Survey of Circadian-Related Sleep Disorders and Melatonin Use in the New Zealand Blind Population** • G.R. Warman, University of Auckland

12:15 **54 • Practical Weekend "Catch-Up" Sleep Interventions to Stabilize Rhythms and Vigilance in Teens** • Stephanie J. Crowley, Brown University

4:30-6:30 PM **Symposium 10: Non-image Forming Photoreception** • *Camellia I & II*

Chair: Ignacio Provencio, University of Virginia

4:30 **The Role of Arrestin-Melanopsin Interaction in Melanopsin Function** • Satchin Panda, Salk Institute for Biological Studies

5:00 **Targeting Killing of Melanopsin-Expressing Retinal Ganglion Cells in the Fully Developed Adult Retina** • Ignacio Provencio, University of Virginia

5:30 **Melanopsin-Containing IpRGCs Are the Main Conduit for Rod/Cone Light Input to Non-imageforming Visual Functions** • Samer Hattar, Johns Hopkins University

6:00 **Intensity- and Duration-Dependent Changes in the Spectral Sensitivity of Human Circadian Photoreception** • Steven Lockley, Harvard Medical School

Symposium 11: Systems Biology and Modeling of Circadian Rhythms • *Azalea I & II*

Chair: John Hogenesch, University of Pennsylvania School of Medicine

4:30 **Models to Understand, Not To Believe** • Andrew J. Millar, University of Edinburgh

5:00 **From Mathematical Models to Molecular Mechanisms in the Neurospora Circadian System** • Jay Dunlap, Dartmouth Medical School

5:30 **Ordered Phosphorylation Governs Oscillation of a Three-Protein Circadian Clock** • Michael J. Rust, Harvard University / HHMI

6:00 **Systems Biology of Mammalian Circadian Clocks** • Hiroki R. Ueda, RIKEN

Symposium 12: Seasonal and Circannual Rhythms • *Azalea III*

Chair: Andrew Loudon, Manchester University

4:30 **Wild Surprises in the Fly** • Charalambos P. Kyriacou, University of Leicester

5:00 **An External Coincidence Model for Control of Plant Growth** • Julin N. Maloof, University of California–Davis

5:30 **Control of Seasonal Rhythms in Mammals** • Francis Ebling, University of Nottingham, Queen's Medical Centre

6:00 **Functional Genomics Analysis of Photoperiodic Time Measurement** • Takashi Yoshimura, Nagoya University

8:00–10:30 PM **Poster Session II** • *Magnolia ABC*

Wednesday, May 21

8:30–10:30 AM **Symposium 13: Interplay between Circadian and Social Behavior** • *Azalea I & II*

Chair: Joel Levine, University of Toronto

8:30 **Identification of a Regulator and Characterization of the Cellular Network Necessary for Male Sex Drive Rhythm in Drosophila** • Hubert Amrein, Duke University Medical Center

9:00 **The Social Clock of the Honeybee—From Social Organization to Plasticity in Clock Gene Expression** • Guy Bloch, The Hebrew University of Jerusalem

9:30 **Fight or Flight (to a New Phase?): On the Timing of Social Repulsion** • William J. Schwartz University Massachusetts Medical School

10:00 **Circadian Food Anticipatory Activity, Social Enhancement, and Leadership in Shoals of Golden Shiners** • Stéphan Reebs, Université de Moncton

Symposium 14: Clocks, Cell Cycle, and Cancer • *Azalea III*

Chair: Marina Antoch, Roswell Park Cancer Institute

8:30 **Circadian Clocks and Cell Cycle, Rhythms in Zebrafish** • Nicholas S. Foulkes, Forschungszentrum Karlsruhe

9:00 **Pharmacological Modulators of the Molecular Clock and Their Potential Therapeutic Applications** • Marina Antoch, Roswell Park Cancer Institute

9:30 **Circadian Release of Hematopoietic Stem Cells** • Paul S. Frenette, Mount Sinai School of Medicine and Black Family Stem Cell Institute

10:00 **Cell Cycle, DNA Damage, and Neurospora Clocks** • Jennifer Loros, Dartmouth Medical School

Symposium 15: Clocks, Brain Function, and Dysfunction • *Camellia I & II*

Chair: Erik Herzog, Washington University, St. Louis

8:30 **Circadian Modulation of Associative Learning** • Lisa C. Lyons, Florida State University

9:00 **A Role for the Circadian Clock Gene Period2 in Synaptic Plasticity and Learned Behavior** • Christopher Colwell, University of California–Los Angeles

9:30 **Regulation of the Murine Monoamine Oxidase a Gene by Circadian Clock Components Implies Clock Influence on Mood** • Urs Albrecht, University of Fribourg

10:00 **A Cell-Based Approach to the Study of Daily Behavior** • Steven Brown, University of Zurich

10:30–11:00 AM **Refreshment Break**

11:00 AM–12:30 PM **Slide Session J: Systems Biology and Modeling** • *Azalea I & II*

11:00 **55 • Quantification of Single-Cell Bioluminescence Data** • Pal O. Westermark, Humboldt University, Berlin

11:15 **56 • Visualization of Frq Message and Protein Dynamics through Luciferase Reporters in the Neurospora Crassa Circadian System** • Luis F. Larrondo, Dartmouth Medical School

11:30 **57 • A Mathematical Model of the Decoupled Mouse Circadian Clock** • Henry P. Mirsky, University of California–Santa Barbara

11:45 **58 • Regulation of Clock Controlled Genes in Mammals** • Hanspeter Herzel, Humboldt University, Berlin

12:00 **59 • Predicting the Electrical Activity of the SCN** • Casey O. Diekman, University of Michigan

12:15 **60 • The Endogenous Circadian Pacemaker Imparts a Scale-Invariant Pattern of Heart Rate Fluctuations Across Time Scales Spanning Minutes to 24 Hours** • Kun Hu, Brigham and Women's Hospital

Slide Session K: Entrainment and Photoreception • *Azalea III*

11:00 **61 • Identification of Novel Genes Involved in Light Dependent CRY Degradation through a Genome-Wide Rnai Screen** • Shailesh Kumar, University of Pennsylvania

11:15 **62 • Fundamental Differences between Light and Temperature Entrainment Mechanisms in Drosophila** • Carla Gentile, Queen Mary University of London

11:30 **63 • A Circadian Clock in the Inner Retina Regulated by Dopamine** • Douglas G. McMahon, Vanderbilt University

11:45 **64 • Melatonin Modulates Visual Processing in the Mouse Retina** • Gianluca Tosini, Morehouse School of Medicine

12:00 **65 • The Spectral Sensitivity of Human Circadian Photoreception Is Dynamic and Changes Depending on the Irradiance and Duration of Light** • Joshua J. Gooley, Brigham and Women's Hospital

12:15 **66 • The Spectral Quality of Light Affects Brain Responses to Emotional Stimuli in Humans** • Vandewalle Gilles, University of Liège

Slide Session L: Neural Clocks and Output • *Camellia I & II*

11:00 **67 • Ca2+ Dependent Protein Kinase 9 (CDPK9) Regulates a Subset of Circadian Output Pathways** • Rachel Green, Hebrew University

11:15 **68 • Diurnal and Nocturnal Temporal Silencing of Period1::D2gfp Neurons in the Suprachiasmatic (SCN) Nucleus of Mice** • Mino D. Belle, University of Manchester

11:30 **69 • Blocking the Fast Delayed Rectifier Current at Late Night Phase Shifts or Abolishes the SCN Electrical and Molecular Rhythm** • Stephan Michel, Leiden University Medical Center

11:45 **70 • A Cellular Analysis of Circadian Rhythms in Mesencephalic Trigeminal Nuclei** • D.J. Hiler, Bowling Green State University

12:00 **71 • Dissecting the Logic of a Circadian Neural Circuit** • Ben Collins, New York University

12:15 **72 • Functional Genomics on Drosophila Pacemaker Neurons Identifies a Rhythmically Expressed K+ Channel as a Major Contributor to Pacemaker Neuron Excitability** • Marc Ruben, New York University

4:00–5:00 PM **Business Meeting** • *Azalea Ballroom*

5:30–6:30 PM **Pittendrigh/Aschoff Lecture: Ueli Schibler, University of Geneva** • *Azalea Ballroom*
Chair: Ravi Allada, Northwestern University

The Mammalian Circadian Timing System: From Gene Expression to Metabolism • Ueli Schibler, University of Geneva

8:00–11:00 PM **Banquet** • *Magnolia ABC*

Poster Titles

Molecular Clocks

P1 • **Post-transcriptional Regulation of the Neurospora Circadian Clock by the FRQ-FRH Complex** • Jinhu Guo, Ping Cheng, Yi Liu, Department of Physiology, University of Texas Southwestern Medical Center at Dallas

P2 • **The Role of the Negative Feedback Loop Genes, Cryptochrome and Period, in Circadian Rhythmicity** • Aaron Schirmer[1*], Vivek Kumar[2], Michael Marshall[1], Eun-Joo Song[2], Andrew Schook[2], Joseph Takahashi[1,2], [1]Dept. of Neurobiology and Physiology, Northwestern University; [2]Howard Hughes Medical Institute, Northwestern University

P3 • **A Novel REV-ERBα Ligand Resets the Peripheral Clock in a Phasic Manner—Insight into the Role of REV-ERBα in the Peripheral Clock** • Qing Jun Meng[1], Weiqun Lu[1*], Stephen Beesley[1], Julie Gibbs[1], David Ray[2], and Andrew Loudon[1], [1]Faculty of Life Sciences, and [2]Faculty of Medicine, University of Manchester, United Kingdom. M13 9PT

P4 • **PAS-Domain Interactions of Drosophila and Mouse PERIOD Clock Proteins** • Sven Hennig*, Holger Strauss, Julia Arens, Sabrina Schulze, and Eva Wolf

P5 • **Kinases and Phosphatases Regulate Stability and Nuclear Entry of Drosophila CRYPTOCHROME** • Celia Hansen, Stephane Dissel, Özge Özkaya, Charalambos P. Kyriacou, and Ezio Rosato*, Department of Genetics, University of Leicester

P6 • **Possible Involvement of the Members of P160 Family of Coactivators in the Circadian Oscillation of Clock Genes** • Masaaki Ikeda*, Cheng Piao, Fang Yang, Megumi Kumagai, Yoshihiro Nakajima

P7 • **Molecular and In Vivo Functional Studies on Sequential Phosphorylation of Mcry2** • Nobuhiro Kurabayashi*, Arisa Hirano, Tsuyoshi Hirota, and Yoshitaka Fukada, Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Japan

P8 • **Feedback Loops Regulating Transcription of the Neurospora Clock Gene Wc-2** • Andrea Neiss, Tobias Schafmeier*, Michael Brunner

P9 • **Towards the Beginning of Time: Rhythms in Embryonic Stem Cells** • Jiffin K. Paulose*, Edmund B. Rucker, Vincent M. Cassone

P10 • **MicroRNA Rhythms in the Chick Pineal Gland** • Vikram R. Shende*, Philip D. Beremand, and Vincent M. Cassone , Texas A&M University

P11 • **Circadian Protein Interaction Networks** • Thomas Wallach[1*], Pablo Porras Millan[2], Astrid Grudziecki[1], Christian Haenig[2], Erich E. Wanker[2], and Achim Kramer[1], [1]Charité—Universitätsmedizin Berlin, Laboratory of Chronobiology, Germany; [2]Max Delbrück Center for Molecular Medicine, Neuroproteomics, Berlin, Germany

P12 • **Proteomic Analysis of Per1 and Per2 Multirotein Complexes In Vivo** • Charo Robles*, Darko Knutti, Hao Anh Duong, and Charles J. Weitz, Harvard Medical School

P13 • **Analysis of Protein Interactions in the Neurospora Crassa Circadian Clock** • Christopher L. Baker*, Arminja Kettenbach, Mi-Shi, Scott A. Gerber, Jennifer J. Loros, and Jay C. Dunlap, Department of Genetics, Dartmouth Medical School

P14 • **Proteomics Analysis of Bmal1 Multiprotein Complexes** • Charo Robles, Darko Knutti, Cyril Boyault*, Charles J. Weitz, Harvard Medical School

P15 • **Functional Conservation of Exon 19 between CLOCK and NPAS2** • Saurabh Sahar*, Kevin C. Pham, and Paolo Sassone-Corsi

P16 • **Nocturnin Expression Is Regulated Post-transcriptionally by miR-122** • Shihoko Kojima[1*], David Gatfield[2], Carla Green[1], [1]University of Virginia, [2]University of Geneva

P17 • **The Jumonji Protein Jarid1a/RBP2 Modulates Circadian Rhythms** • Luciano De Haro, Sandhya Pulivarthy, and Satchidananda Panda, The Salk Institute

P18 • **Biochemical Analysis of PER and BMAL1 Protein Complexes** • Kiran Padmanabhan, Cyril Boyault, Charo Robles, Darko Knutti, and Charles J. Weitz, Department of Neurobiology, Harvard Medical School

P19 • **Mass-Spectrometric Mapping of PER2 Phosphorylation Sites and Analysis of Their Functional Relevance** • Jens T. Vanselow*, Andreas Schlosser, and Achim Kramer, Charité, Universitaetsmedizin Berlin

P20 • **Investigating a Role for the Circadian De-Ubiquitinating Enzyme, USP2** • Heather Scoma, Geetha Yadav, Joseph Fogerty, Monica Humbly, Qingjiong Zhang, and Joseph C. Besharse*, Medical College of Wisconsin

P21 • **Subcellular Localization of the Circadian Deadenylase Nocturnin** • E. Garbarino-Pico* and C.B. Green. Department of Biology, University of Virginia

P22 • **Casein Kinase I E/D Are Essential Kinases for PERIOD Phosphorylation and the Circadian Clock** • Hyeong-Min Lee* and Choogon Lee*, College of Medicine, Florida State University

P23 • **Role of Dbt's Kinase Activity in the Circadian Mechanism of Drosophila** • Michael J. Muskus*, Fabian Preuss, Jin-Yuan Fan, Edward S. Bjes, and Jeffrey L. Price, Division of Molecular Biology, School of Biological Sciences, University of Missouri

P24 • **A Role for DOUBLETIME in Bridging CLK Phosphorylation by Other Kinases** • Wangjie Yu[1*], Hao Zheng[1], Jeffery L. Price[2] and Paul E. Hardin[1], [1]Department of Biology and Center for Research on Biological Clocks, Texas A&M University, [2]School of Biological Sciences, University of Missouri

P25 • **Tissue-Specific Circadian Regulation of the Two Isoforms of the Orphan Nuclear Receptor RORgamma** • Valérie Mongrain*, Xuan Ruan, Hugues Dardente, and Nicolas Cermakian, Laboratory of Molecular Chronobiology, Douglas Mental Health University Institute, McGill University, Montreal

P26 • **Rhythmic SAF-A Binding Underlies Circadian Transcription of the Bmal1 Gene** • Yoshiaki Onishi1*, Syuji Hanai[1], Tomoya Ohno[1,2], Yasuhiro Hara[1,2], Norio Ishida[1,2], 1Institute for Biological Resources and Functions, AIST, [2]Graduate School of Life and Environmental Sciences, University of Tsukuba

Genetics

P27 • **Temperature Compensation in the Neurospora Clock** • Arun Mehra[1*], Mi Shi[1], Hildur V. Colot[1], Jennifer J. Loros[1,2], Jay C. Dunlap[1,2], Dartmouth Medical School, [1]Departments of Genetics and [2] Biochemistry

P28 • **Perturbations in the Nonsense-Mediated Decay Pathway Affect the Neurospora Clock** • Arun Mehra[1*], Patrick D. Collopy[1], Jennifer J. Loros[1,2], Jay C. Dunlap[1,2], Dartmouth Medical School, [1]Departments of Genetics and [2] Biochemistry

P29 • **The Neurospora Protein Phosphatase 4 Homologue Is an Important Circadian Clock Component by Regulating the Phosphorylation States of the Clock Proteins** • Joonseok Cha*, Shwu-Shin Chang, and Yi Liu, University of Texas Southwestern Medical Center

P30 • **Neurospora CHD-2 Remodels Chromatin at the Clock Gene Frequency and Is Needed for the Epigenetic Transfer of Time** • William J. Belden*, Jennifer J. Loros, and Jay C. Dunlap, Dartmouth Medical School

P31 • **Circadian Clock Genes in a Tidal Animal** • D.C. Wilcockson[1]*, L. Zhang[2]*, S.G. Webster[1], M.H. Hastings[3], C.P. Kyriacou[2], [1]School of Biological Sciences, Bangor University, [2]Department of Genetics, Leicester University, [3]MRC Laboratory of Molecular Biology, Division of Neurobiology

P32 • **Characterizing a Novel Clock Gene in Neurospora** • Randy Lambreghts[1]*, Jay Dunlap[1], Jennifer Loros1[2], Departments of [1]Genetics and [2]Biochemistry, Dartmouth Medical School

P33 • **Transgenic Drosophila with Conditional Circadian Clock Function** • Tadahiro Goda*, S. Jake Currie, and Herman Wijnen, University of Virginia

P34 • **Twenty-Four, a Novel Drosophila Circadian Clock Gene, Is Required for Robust Behavioral Rhythms and Wild-Type PERIOD Expression in Pacemaker Neurons** • Chunghun Lim[1]*, Jongbin Lee[2], Joonho Choe[2], and Ravi Allada[1], [1]Department of Neurobiology and Physiology, Northwestern University, [2]Department of Biological Sciences, KAIST

P35 • **Regulation of Narrow Abdomen, a Unique Ion Channel That Functions in Circadian Neural Output in Drosophila** • Bridget C. Lear* and Ravi Allada, Northwestern University

P36 • **Extreme Early-Running Behavior in Cast/Eij Inbred Mice** • P. Jiang[1], M. Striz[1], K. Shimomura[2,3], J.S. Takahashi[2,3], J.P. Wisor[4], B.F. O'Hara[1], [1]Dept. of Biology, University of Kentucky; [2]Howard Hughes Medical Institute; [3]Center for Functional Genomics and Dept. of Neurobiology and Physiology, Northwestern University; [4]Molecular Neurobiology Lab, Biosciences Division, SRI International

P37 • **Functional Analysis of Mcry1: In Vitro Versus In Vivo** • Inês Chaves*, Monika Bajek, Sander Barnhoorn and Bert van der Horst, Erasmus University Medical Center

P38 • **Quantitative Genetic and QTL Analysis of Entrained Circadian Activity Rhythms in Drosophila** • Bernard Possidente[1]*, Debra Possidente[2], Douglas Ruden[3] and Helmut V.B. Hirsch[2], [1]Skidmore College, Biology Department; [2]SUNY Albany, Biology Department, [3]Wayne State University, Inst. of Env. Health Science

P39 • **Genetic Components on Chromosome 13 Regulate Daily Physical Activity Pattern in Mice** • He S. Yang; Kazuhiro Shimomura, Aaron D. Laposky, and Fred W. Turek, Center for Sleep and Circadian Biology, Northwestern University

P40 • **Behavioral Activity Phenotypes in Mice Deficient in the Circadian De-ubiquitinating Enzyme, USP2** • Geetha Yadav*+, Heather Scoma, Qingjiong Zhang, and Joseph C. Besharse, Medical College of Wisconsin; + Present address: Bio-Rad Laboratories, Hercules, CA.

P41 • **High Resolution Mapping of Frp-1 Using Multiple Strain Advanced Intercross Lines** • Kazuhiro Shimomura[2,3]* Chiaki Omura[2,3], Tim Wiltshire[4], Mathew Pletcher[5], and Joseph S. Takahashi[1,2,3], [1]Howard Hughes Medical Institute, [2]Center for Functional Genomics; [3] Department of Neurobiology and Physiology Northwestern University, [4]Genomics Institute of the Novartis Research Foundation, [5]The Scripps Research Institute of Florida

P42 • **Circadian Period in Chromosome Substitution Strain Mice** • John Hofstetter*, Aimee Mayeda, Indiana University, Veterans Administration

P43 • **Super-Duper Short: Aa New Circadian Mutation in Syrian Hamsters** • Eric L. Bittman*, Alexander Bois, and Stefanie Monecke, Department of Biology, University of Massachusetts

P44 • **Natural Allelic Variation in Circadian Clock Function in Brassica Rapa** • Xiaodong Xu[1], Qiguang Xie[1], Ping Lou[1], Marc Brock[2], Cynthia Weinig[2], and C. Robertson McClung[1], [1]Department of Biological Sciences, Dartmouth College, [2]Department of Botany, University of Wyoming

P45 • **Length Variation in the Chinook Salmon (Oncorhynchus Tshawytscha) Clock Polyq: Does It Contribute to Seasonal Differences in Migration Timing?** • Kathleen G. O'Malley*, National Research Council, Northwest Fisheries Science Center, Seattle, Washington

P46 • **Evolution of Teleost Fish Circadian Clock Genes: Preservation of Different Ancient Duplicate Circadian Clock Genes in Different Teleost Fishes** • Han Wang, Department of Zoology and Stephenson Research & Technology Center, University of Oklahoma

Cellular and Developmental

P47 • **Increased Coherence of Cellular Rhythms in Mature NIH 3T3 Fibroblast Cultures** • John S. O'Neill*, Liz Maywood, and Michael Hastings, Medical Research Council

P48 • **Actin's Role in Signaling Early Night Phase Shifts in Rodent SCN** • Jennifer M. Arnold[1]*, Sheue-Houy Tyan[2], Jennifer W. Mitchell[2], and Martha U. Gillette[1,2], [1]Department of Molecular & Integrative Physiology and [2]Department of Cell & Developmental Biology, University of Illinois at Urbana-Champaign

P49 • **Atypical PKC Signaling in Circadian Plasticity in Rat Suprachiasmatic Nucleus** • Sufang Huang* and Martha U. Gillette, Department of Cell & Developmental Biology, University of Illinois at Urbana-Champaign

P50 • **Calcium Signaling Induced by Glutamate in Rat SCN Neurons** • Tongfei A. Wang[1]*, Martha U. Gillette[1,2,3], [1]Department of Molecular and Integrative Physiology, [2]Department of Cell and Developmental Biology, [3]Neuroscience Program, University of Illinois at Urbana-Champaign

P51 • **Getting There on Time: NO/cGMP Signal Transduction and Circadian Entrainment** • Plano S.A., Agostino P.V., Chiesa J.J. and Golombek D.A., Universidad Nacional de Quilmes, Buenos Aires, Argentina

P52 • **Forskolin Triggers the Circadian Oscillation in the Mouse Immortal Hepatocytes** • Urula Prosenc[1]*, Jean Marc Pascussi[2], Damjana Rozman[1], [1]Center for Functional Genomics and Bio-Chips, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Zaloska, SI-1000 Ljubljana, Slovenia¸ [2]INSERM, Montpellier, France

P53 • **Synchronization Pathways in the Fibroblast Clocks** • Mariko Izumo[1]*, Jason Chong[1], and Joseph Takahashi[1, 2,], [1]Center for Functional Genomics, Northwestern University, [2]Howard Hughes Medical Institute

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P267 • **Effects of Glaucoma on the Circadian Timing System in Mice and Men** • H.M. Cooper[1,2], E. Drouyer[1, 2], O. Dkhissi-Benyahya[1, 2], C. Gronfier[1, 2], C. Chiquet[1,3], E. WoldeMussie[4], G. Ruiz[5], L.A. Wheeler[5], and P. Denis[1,6], [1]INSERM, U846, Stem Cell and Brain Research Institute, Department of Chronobiology, F-69500, Bron, France; [2]University of Lyon, France; [3]Department of Ophthalmology, CHU de Grenoble- Faculté de Médecine, Grenoble, France; [4]Pfizer, Inc, San Diego, CA; [5]Allergan Inc., Irvine, California; [6]Department of Ophthalmology, CHU de Lyon-Hopital Edouard Herriot, Lyon, France

P268 • **Long-term Effects of Artificial Dawn on Sleep Inertia: Does Melatonin Play a Role?** • M.C. Gimenez[1]*, M. Hessels[2], M. van de Werken[1], B. de Vries[1], D.G.M. Beersma[1], M.C.M. Gordijn[1]. [1]Department of Chronobiology, Center for Life Sciences, University of Groningen, The Netherlands. [2]Medical Design Engineering, Amsterdam, The Netherlands

P269 • **Effects of Diurnal and Nocturnal Bright Light Exposure on Human Performance and Wake EEG** • Melanie Rüger[1,2]*, Marijke C.M. Gordijn[1], Bonnie de Vries[1], Domien G.M. Beersma[1], [1]Department of Chronobiology, University of Groningen, The Netherlands; [2]Division of Sleep Medicine, Harvard Medical School, Brigham and Women's Hospital, Boston.

P270 • **The Effect of Incremental Changes in Light Onset on Reentrainment Rate of Octodon degus Following a 12-Hour Phase Advance** • T.J. Jechura* and D.D. Shuboni, Department of Psychology, Albion College

P271 • **Non-visual Sensitivity to Light in Humans** • C. Gronfier[1]*, C. Chiquet[1,2], B. Claustrat[1,3], P. Denis[4], and H.M. Cooper[1], [1]INSERM, U846, Stem Cell and Brain Research Institute, Department of Chronobiology, F-69500, Bron, France; University of Lyon, 69003, Lyon, France; [2]Department of Ophthalmology, CHU de Grenoble- Faculté de Médecine, Grenoble, France; [3]Department of Radioanalysis, Hôpital Neurologique, Lyon, France; [4]Department of Ophthalmology, CHU de Lyon-Hopital Edouard Herriot, Lyon, France

P272 • **Differences in Breast Cancer Risk in Blind Women with and without Light Perception** • Erin E. Evans*, Richard G. Stevens, and Steven W. Lockley, Brigham and Women's Hospital/University of Surrey

P273 • **Room Light Alters the Timing of Melatonin Synthesis** • Joshua J. Gooley*, Kyle Chamberlain, Charles A. Czeisler, and Steven W. Lockley, Brigham and Women's Hospital

P274 • **Subjective Tiredness, Wrist Activity, and Sleep Fragmentation Score under the Influence of Daytime Low UV Light Radiation in Humans** • Nana N. Takasu[1]*, Tomoko Wakamura[1], Tadashi Oishi[2], Morihiro Yoneda[3], Kanako Muramatsu[3], Kaori Komatsu[3], Masayuki Kondo[4], Hiromi Tokura[5], [1]Graduate School of Medicine Kyoto University, Kyoto, Japan, [2]Nara Saho College, Nara, Japan, [3]Nara Womens University, Nara, Japan, [4]Sekisui House, Ltd., Kyoto, Japan, [5]The Hong Kong Polytechnic University, Hong Kong, China

P275 • **Correlations among Inter-Individual Differences in Non-image Forming Effects of Light at Night** • Shigekazu Higuchi*, Sayaka Aritake, Minori Enomoto, Hiroyuki Suzuki, Akiko Hida, Miyuki Tamura, Masaya Takahashi, and Kazuo Mishima

P276 • **Acute Effects of a One-Hour 10,000 Lux Light Exposure on Subjective Sleepiness Ratings** • Melanie Rueger*, Catherine J. Hanley, Jessica Ricker, Charles A. Czeisler, and Steven W. Lockley, Division of Sleep Medicine, Brigham and Women's Hospital, Harvard Medical School Boston, MA

P277 • **Total Daily Excretion of Urinary 6-Sulphatoxymelatonin during Three Consecutive Days of Simulated Night Work** • Marie Dumont*, Zoé Morin, and Hélène Blais, Chronobiology Laboratory, Sacré-Coeur Hospital and University of Montréal, Montréal, Canada

P278 • **The Association between Sleep and Rate of Change in Body Temperature across Circadian Phase and the Menstrual Cycle** • Ari Shechter*, Philippe Boudreau, and Diane B. Boivin, Centre for Study and Treatment of Circadian Rhythms, Douglas Mental Health University Institute, McGill University

P279 • **Circadian Rhythms in Behavioral Inhibition and Flexibility** • Candelaria Ramirez*, Aida Garcia, Javier Talamantes, Pablo Valdez, Universidad Autonoma de Nuevo Leon

P280 • **The Effects of Sleep Deprivation on Pheromone Attraction** • I.B. Mondrow* and T.J. Jechura, Department of Psychology, Albion College

P281 • **Circadian Rhythms of Functional Brain Latealization in Humans** • Constanze S. Hahn*, Markus Hausmann*, and Onur Güntürkün*; University of Bochum, Germany

P282 • **A Circadian Phase-Dependent Acute Effect of Moderate-Intensity Exercise on Melatonin Levels in Humans** • Sean W. Cain*, Joseph Cohen, Jeanne F. Duffy, and Charles A. Czeisler, Harvard Medical School

P283 • **At-Sea Testing of Alternate Watchstanding Schedules on U.S. Navy Submarines** • LT Christopher T. Steele*, Ph.D., James Whanger, LT Jon Vanderweele, D.O., Wayne Horn, M.D., Naval Submarine Medical Research Laboratory

P284 • **Sleep-Activity Circadian Rhythm Abnormalities Reflect Self-Assessed Depression and Anxiety in Patients with Advanced Non-small Cell Lung Cancer** • Dinah Faith Quiton*, Jovelyn Du-Quiton, Patricia A Wood, James F. Grutsch, Digant Gupta, Kevin Tyer, Christopher G. Lis, Robert D. Levin, James B. Burch, and William JM Hrushesky, Dorn VA Medical Center

P285 • **Sleep Quality and Circadian Function in Patients with Advanced Lung Cancer** • J. Reynolds[2]*, J. Grutsch[1], P. Wood[2,3], D. Gupta[1], C. Lis[1], R. Levin[1], J. Du-Quiton[2,3], M. Daehler[1], D. Quiton[2,3], W. Hrushesky[2,3,4], [1]Cancer Treatment Centers of America at Midwestern Regional Medical Center, Zion, IL; [2]Medical Chronobiological Laboratory, Dorn Research Institute, WJB Dorn VA Medical Center, School of Medicine and [3]School of Public Health, 4School of Engineering and Information Technology, University of South Carolina

P286 • **Circadian Rhythm Analysis: A Pilot Study of Single vs. Serial Section Cosinor Analysis** • M.A. Vincent*, S.K. Hanneman, and N.S. Padhye, The University of Texas Health Science Center at Houston School of Nursing

P287 • **Chronic Deterioration in Psychomotor Vigilance Performance during Resident Work Schedules** • Jason P. Sullivan, Rosa C. Chidgey, Erin E. Evans, Charles. A. Czeisler, Steven W. Lockley*, Brigham and Women's Hospital

P288 • **Predicting Risk of Cognitive Performance Decrements during Resident Work Schedules** • Jason P. Sullivan, Erin E. Evans, Brian E. Cade, Christopher P. Landrigan, and Steven W. Lockley*, Brigham and Women's Hospital

P289 • **Efficacy of Blue-Enriched Fluorescent Light for Melatonin Suppression and Circadian Phase Resetting** • J. Hanifin[1]*, K. Cecil[1], K. West[1], M. Jablonski[1], B. Warfield[1], M. James[1], M. Thiessen[1], S. Jasser[1], M. Stone[1], R. Fucci[1], E. Martin[1], B. Byrne[1], E. Gerner[1], M. Rollag[1], S. Lockley[2], and G. Brainard[1],

[1]Department of Neurology Thomas Jefferson University; [2]Division of Sleep Medicine, Brigham and Women's Hospital, Harvard Medical School

P290 • **Interaction between Endogenous Circadian System and Behavioral Stressors on Cardiovascular Risk Factors in Humans** • Frank A.J.L. Scheer[1,2],* Kun Hu[1,2], Heather Evoniuk[1], Erin E. Kelly [1], Atul Malhotra[1,2], Mary Hahn[1], Lauren A. Doamekpor[1], Michael D. Laker[1], Jignasha Patel[1], Carolina Smales[1], and Steven A. Shea[1,2], [1]Medical Chronobiology Program, Division of Sleep Medicine, Brigham and Women's Hospital, Boston; [2]Division of Sleep Medicine, Harvard Medical School, Boston

P291 • **Using Circadian Time To Optimize Hematopoietic Stem Cell Harvest for Clinical Transplantation** • Daniel Lucas*, Michela Battista, Patricia Shi, and Paul S. Frenette, Mount Sinai School of Medicine

P292 • **Does the Timing of "Morning" Alcohol Administration Differentially Affect Sleepiness?** • Eliza Van Reen* and Mary A. Carskadon, Mount Sinai School of Medicine

Sleep

P293 • **Basal Forebrain Activation in Day-Active and Night-Active Grass Rats** • Alexandra Castillo-Ruiz[1*], Joshua P. Nixon[2], Laura Smale[1], and Antonio A. Nunez[1], [1]Department of Psychology, Michigan State University; [2]Minnesota Craniofacial Research Training Program, University of Minnesota

P294 • **Sleep Responses to 6-H Deprivation in Male Juvenile Octodon degus** • Jamie Perryman[1*], Mark R. Opp[1,2], Theresa Lee[1,3], [1]Neuroscience Graduate Program; [2]Department of Anesthesiology; [3]Department of Psychology, University of Michigan

P295 • **Subregional Suprachiasmatic Control of Circadian Outputs** • M.L. Lee*, and B. Swanson, de la Iglesia, H.O., University of Washington

P296 • **Does Disruption of Circadian Rhythms Mediate the Inhibitory Effects of Sleep Deprivation on Hippocampal Neurogenesis in the Adult Rat?** • R.J. Mear*, A. Mueller, R.E. Mistlberger, Department of Psychology, Simon Fraser University

P297 • **Unihemispheric Sleep in the Bearded Dragon throughout the 24-Hour Period and under Threat Conditions** • M.M. Roberts*, A.D. McKelvy, and T.J. Jechura, Department of Psychology, Albion College

P298 • **Per1 and Per2 Expression in the Forebrain of SCN Lesioned and Intact Mice in Response to Sleep Deprivation** • L. Liu[1*], P. Jiang[1], K. Franklin[2], G. Hagiwara[3], J. Tsai[3], K. Stephenson[3], R. Vilms[3], H.C. Heller[3], P. Franken[4], P. Bourgin[3,5], M.J. Duncan[2], and B.F. OHara[1], [1] Department of Biology, [2]Anatomy & Neurobiology, University of Kentucky, Lexington, KY; [3]Dept.Biol Sci., Stanford University, Palo Alto, CA; [4]Ctr. for Integrative Genomics, University of Lausanne, Switzerland; [5]Neurology, University of Strasbourg, France

P299 • **Glutamatergic Neurons in the Doromedial Hypothalamic Nucleus Regulate Rhythms of Locomotor Activity** • N. Vujovic*, P.M. Fuller, J. Lu, Q. Tong, B.B. Lowell, and C.B. Saper, Beth Israel Deaconess Medical Center

P300 • **Differences in Sleep Patterns Affect Recovery in a Segregating Population of Sleep-Deprived Mice** • Daniel P. Radzicki *, Deanna L. Williams, Karrie Mrazek, Martha Hotz Vitaterna, Aaron D. Laposky, Christopher J. Winrow, John Renger, and Fred W. Turek, Northwestern University

P301 • **Diurnal Differences in Correlations of Sleep-Wake Phenotypes and Depressive-Like Behaviors** • Karrie Mrazek*, Deanna L. Williams, Daniel P. Radzicki, Martha Hotz Vitaterna, Aaron D. Laposky, Christopher J. Winrow, John Renger, and Fred W. Turek, Center for Sleep and Circadian Biology

Sunday, May 18

Symposium 1: Molecular Clocks I

Signaling to Chromatin and the Circadian Clock

Paolo Sassone-Corsi, Department of Pharmacology, School of Medicine,University of California–Irvine

A large array of endocrine, metabolic and physiological functions are governed by the circadian clock. The molecular mechanisms underlying the circadian clock comprise interconnected transcriptional-translational feedback loops. The protein CLOCK is an essential transcriptional activator in mammalian feedback loop. We have demonstrated that CLOCK possesses intrinsic enzymatic histone acetyl transferase (HAT) activity and that the acetylation of histones by CLOCK stimulates transcription of clock and clock-controlled genes (CCGs), acting as the positive limb of the loop. (Cell 125: 497-508; 2006). Importantly, we have recently shown that CLOCK possesses the capacity to acetylate non-histone substrates. Specifically, CLOCK acetylates its own partner BMAL1 at a single lysine K537, an event that controls circadian function. Indeed, CLOCK-mediated acetylation of BMAL1, hence the enzymatic interplay between two core components of the clock machinery, is essential for circadian function (Nature 450: 1086-1090; 2007). Our recent studies have been centered on how the enzymatic activity of CLOCK is influenced by a number of signaling pathways. The results of these analyses have intriguing implications as they identify some molecular links between the circadian clock and the regulation of sleep.

Symposium 2: Circadian Neural Networks

Setting Clock Speed

Elizabeth Maywood*, Johanna Chesham, Pat Nolan, Andrew Loudon, and Michael Hastings
Neurobiology Division, MRC-Laboratory of Molecular Biology, Cambridge, CB20QH

At the cellular level, the mammalian clockwork of the suprachiasmatic nuclei is viewed as a series of interlocked transcriptional/translational feedback loops, synchronized and sustained by neuropeptidergic signaling. How this whole process takes ca. 24h is not fully understood; but for the clock to run effectively changes in protein stability, phosphorylation state and subcellular localization must be tightly regulated. A recently identified mutation in an F-box protein Fbxl3 (*Afterhours*; Godhino et al Science 2007) revealed that the period lengthening effect (28h in the homozygous mutant) of this point mutation resulted from a selective preservation of CRY proteins. A second mutation, the *tau* allele of casein kinase 1 epsilon (CSK1 ; Meng et al Neuron 2008), had the effect of speeding up the clock from 24h in the wild-type to 20h in the homozygous mutant. This mutation was found to facilitate the degradation of endogenous PER proteins. By crossing these two lines of mutant mice with independent biochemical substrates for degradation, we predicted that within the genetic network the *Afterhours* phenotype would be dominant, the whole cycle running as fast as the slowest component. This was not the case as the *tau* mutation reversed the period lengthening effect of the *Afterhours* mutation in a dose-dependent manner. This demonstrates the complexity of the genetic network in regulating clock speed and that these two mutations have independent substrate targets for degradation. These results will help inform on the precise timing and order of biochemical events in the molecular loop. Supported by MRC, EUCLOCK, BBSRC

4:30–6:00 PM

Symposium 3: Technological Advances in Circadian Biology

Bioluminescence Imaging of Circadian Clock Gene Expression in Single Cells

David K. Welsh[1,2]* and Steve A. Kay[2], [1]Dept. Psychiatry, and [2]Dept. Cell & Developmental Biology, University California–San Diego

Since pioneering studies over 50 years ago in the bioluminescent marine dinoflagellate Gonyaulax (Hastings & Sweeney, Proc Natl Acad Sci USA 43:804, 1957), bioluminescence has been used to monitor circadian clock function. In recent years, exogenous luciferase genes have been introduced into a wide variety of organisms, including cyanobacteria (Synechococcus), plants (Arabidopsis), insects (Drosophila), fish (zebra fish), and rodents. Luciferase enzymes can catalyze the emission of light from a substrate to report clock gene expression in vivo, in cultured tissues, or in cultured cells or cell lines. Luminescent reporters are generally more sensitive and less toxic than fluorescent reporters such as GFP, making them ideal for long-term, longitudinal studies of circadian clock function. However, they are also exceedingly dim, and so most studies have used photomultiplier tubes to collect photons from many cells at once. Because many important questions in circadian biology require monitoring clock function with single cell resolution, we have worked to develop and optimize bioluminescence imaging of circadian clock gene expression in mammalian cells. Important elements of this methodology to be covered in this presentation include reporter design, microscope setup, optics and choice of objective lens, selection of a CCD camera, optimizing camera settings for data acquisition, and data analysis. [Supported in part by K08 MH067657 (DKW).]

Mapping PDF Receptor Activation and Expression in the Drosophila Brain

P.H. Taghert, O.T. Shafer, S.H. Im, D.J. Park, R. Dunbar-Yaffe, V. Nikolaev, and M. Lohse

We have studied PDF signaling that underlies adult locomotor rhythms by introducing a realtime genetic reporter to detect cAMP concentration changes in neurons of the intact fly brain. The FRET-based assay produces signals that are robust and relatively rapid. We report on PDF receptor (PDFr) activation by PDF among the different circadian pacemaker neuron groups. Our genetic evidence suggests PDFr is expressed among most but not all such groups. Previous observations on PDFr expression using antibodies lack genetic specificity. Therefore, we have used recombineering methods to generate a large transgene encompassing the pdfr locus and which includes an epitope tag. We will report on the the pattern of epitope-tagged receptor and its ability to rescue the pdfr mutant phenotype.

Symposium 4: Comparative Clocks

The Circadian Clock of the Monarch Butterfly

Steven M. Reppert, Department of Neurobiology, University of Massachusetts Medical School

The circadian clock plays a vital role in monarch butterfly (*Danaus plexippus*) migration by providing the timing component of time-compensated sun compass orientation, which contributes to navigation to the overwintering grounds. The location of circadian clock cells in monarch brain has been identified in the dorsolateral protocerebrum (pars lateralis); these cells express PERIOD, TIMELESS, and a *Drosophila*-like cryptochrome (designated CRY1). Monarch butterflies, like all other non-drosophilid insects so far examined, express a second *cry* gene (designated insect CRY2) that encodes a vertebrate-like CRY that is also expressed in pars lateralis. An ancestral circadian clock mechanism has been defined in monarchs, in which CRY1 functions as a blue-light photoreceptor for photic entrainment, while CRY2 functions within

the clockwork as the major transcriptional repressor of an intracellular negative transcriptional feedback loop. A CRY1-staining neural pathway has been identified that may connect the circadian (navigational) clock to polarized light input important for sun compass navigation, and a CRY2-positive neural pathway has been discovered that may communicate circadian information directly from the circadian clock to the central complex, the likely site of the sun compass. The monarch butterfly may thus use the CRY proteins as both clock components and as outputs that connect the clock to various aspects of the sun compass apparatus.

What Season Is It Anyway—with Climate Warming, You Snooze You Lose

William E. Bradshaw* and Christina M. Holzapfel, CEEB, 5289 University of Oregon

Animals from rotifers to rodents use the length of day (photoperiodism) to tell them when to take their winter nap (dormancy or migration) and often when to begin development and reproduction in the spring. Exactly how animals use day length to determine life-altering transitions in their seasonal life histories has been at the center of much research and debate for more than half a century. Herein, we discuss the underlying genetic architecture of the seasonal photoperiodic timer. We present the first QTL map of photoperiodic response in any animal and show how the QTL for the evolution of photoperiodic response interface with QTL for dormancy. We conclude with a discussion of how genetic shifts in photoperiodic response contribute to the evolution of animal populations confronted with rapid climate change. This research was supported by NSF grants DEB-0412573 and IOB-0445710.

Natural Variation and Evolution of Plant Clock Genes

C. Robertson McClung, Dartmouth College

It is abundantly clear that there is considerable natural variation in clock function within flies, fungi and plants. We have identified quantitative trait loci (QTL) contributing to clock function in Arabidopsis thaliana and Brassica rapa. One of the central premises of the study of circadian rhythms is that a functional circadian clock enhances organismal fitness and recent studies have provided experimental proof that this is so. We have tested the hypothesis that mutational differences in clock genes among accessions are subject to selection through sequence analysis of PSEUDO-RESPONSE REGULATOR 7 (PRR7; At5g02810) from >100 Arabidopsis accessions. There is a significant excess of replacement mutations relative to synonymous mutations (20 and 5, respectively; $p<0.005$), consistent with diversifying selection at the PRR7 locus. This cannot be attributed to selection occurring at adjacent loci, as one flanking gene shows excess synonymous mutations (6 replacement and 31 synonymous; $p<0.001$) and the other flanking gene shows roughly equal numbers of replacement and synonymous mutations (10 and 14, respectively; $p>0.5$), consistent with purifying and neutral selection, respectively. This suggests selection for multiple allelic variants at the PRR7 locus, which is consistent with distinct PRR7 alleles contributing differentially to organismal fitness in different environments. We are currently testing whether these different alleles are functionally equivalent through complementation of mutants defective in PRR7 activity. Work on circadian rhythms in my lab is supported by grants from the National Science Foundation (MCB-0343887 and IOB-0517111) and from the United States-Israel Binational Science Foundation (2005223).

Symposium 5: Circadian and Homeostatic Regulation of Human Sleep

Circadian and Homeostatic Regulation of Sleep in Adolescent Humans

Mary A. Carskadon, Ph.D., Sleep and Chronobiology Research Lab, E.P. Bradley Hospital
Department of Psychiatry and Human Behavior, Alpert Medical School of Brown University

Human adolescents undergo a marked phase delay of sleep patterns that—in 21st century US—results in a pattern of short sleep on school days in comparison to longer and later sleep on weekends. Our data

have shone an association of pubertal development with changes in the circadian timing system as well as changes in sleep homeostasis. To wit, melatonin phase is correlated with pubertal stage under self-selected conditions as well as following constraints to sleep-wake (and thereby light-dark) exposure. Indications that period may be longer in adolescents than adults begs the question whether intrinsic period undergoes developmentally 'programmed' changes across adolescence and into adulthood that could underlie the phase changes. We also have shown that sleep homeostasis differs in pre- and post-pubertal adolescents; specifically, the accumulation rate of 'Process S' (i.e., 'sleep pressure') across waking is slower in the more mature adolescents. This developmental trend may also contribute to a delay in the timing of sleep onset in older adolescents. When adolescents' lives are filled with days that begin early for school and activities and opportunities that extend the waking day (with the assistance of clock-dependent alerting and diminished sleep pressure), the outcome is a limitation of sleep length that can lead to poor outcomes. For example, we have shown in phase delayed teens who have an early school start time that 'pathologically' short sleep latencies and short REM sleep latencies when morning naps are provided, manifesting a pattern that does not foster school performance and indeed resembles narcolepsy.

Homeostatic Sleep Regulation in Morning and Evening Types

Marie Dumont* and Valérie Mongrain, Chronobiology Laboratory, Sacré-Cœur Hospital and University of Montréal, Montréal, Canada

Morning and evening chronotypes, as identified with the Horne and Ostberg's Morningness-Eveningness Questionnaire (MEQ), differ in the timing of their spontaneous main sleep episode. They usually show an early and late endogenous circadian phase, respectively, consistent with early and late circadian sleep propensity. In our recent study, we identified a sub-group of morning and evening types having normal, intermediate circadian phases, in spite of spontaneous early and late sleep schedules. In this presentation, we will review the characteristics of homeostatic markers of sleep regulation in these subjects during baseline sleep and in response to increased sleep pressure. These exploratory results suggest that differences in the dynamics of homeostatic sleep regulation may underlie morning or evening diurnal preference in some individuals. The significance of these observations for the understanding of the pathophysiology of circadian rhythm sleep disorders will be discussed.

Circadian and Sleep-Wakefulness Regulation of Cognitive Function in Humans Sleep and Circadian Systems Interact To Regulate Daily Patterns of Cognitive Function

Kenneth P. Wright Jr., Ph.D., Department of Integrative Physiology Director; Sleep and Chronobiology Laboratory, Centers for Neuroscience and the Integrative Study of Work, University of Colorado at Boulder

Under entrained conditions, these fundamental central nervous systems processes interact to promote and maintain alert wakefulness across the biological day. This talk will describe findings from research studies aimed at investigating the interaction between the sleep homeostatic and circadian systems on cognition as well as discuss individual differences in cognitive performance impairment with increased homeostatic sleep drive and wakefulness during the biological night.

Symposium 6: Interplay between Circadian Clocks and Metabolism

Tissue Specific Roles of BMAL1 in Lipid Metabolism

Shigeki Shimba*, and Masakatsu Tezuka, Department of Health Science, College of Pharmacy, Nihon University, Funabashi, Chiba, Japan

BMAL1 is a transcription factor that regulates circadian rhythm. In addition to the functions as a molecular clock, we previously reported that BMAL1 plays role in the regulation of adipogenesis (PNAS 2005). To understand more precise roles of BMAL1 in adipocytes, we established BMAL1 flox mice (C57BL6 background) and generated the conventional- and the tissue (adipocytes, hepatocytes, or muscle) specific—BMAL1 KO mice. Similar to the Mop3 KO mice, the conventional B6-BMAL1 KO mice exhibit less food intake and smaller body weight, although these phenotypes were more profound in the B6-BMAL1 KO mice. Interestingly, the BMAL1 KO mice gained more body weight than the control mice under the high fat diet challenges. After only 2day of high fat feeding, a great amount of sebum was secreted from the BMAL1 KO mice. After 4 weeks of feeding with high fat diet, liver in the BMAL1 KO mice accumulated more lipids and was enlarged compared to that in the control mice. Gene Chip analysis showed that BMAL1 regulates fatty acids synthesis positively in adipocytes but negatively in muscle and liver. Consequently, these results indicate that BMAL1 plays tissue specific roles in lipid metabolism. Also, disruption of BMAL1 functions may lead to onset of obesity and metabolic syndrome.

Circadian Control of Metabolism Is Regulated Post-transcriptionally

Carla B. Green, Department of Biology, University of Virginia

Circadian clocks coordinate many aspects of physiology and behavior with respect to time of day. The mammalian circadian system consists of a central oscillator in the suprachiasmatic nucleus of the hypothalamus, which coordinates peripheral clocks in organs throughout the body. Recent evidence has demonstrated that circadian clocks control the rhythmic expression of a large number of genes involved in metabolism and other aspects of circadian physiology. However, the consequences of genetic disruption of circadian controlled pathways remain poorly defined. Our studies have focused on a rhythmically expressed gene called Nocturnin (Ccrn4l) which encodes a deadenylase—an enzyme that specifically removes the poly(A) tails from mRNAs and has been implicated in the regulation of mRNA stability or translatability. This gene is not part of the central clock mechanism, but instead is a highly rhythmic output of the clock with peak expression in the early night in a wide range of metabolically-relevant tissues. The targeted disruption of the Nocturnin gene in mice confers resistance to diet-induced obesity. We found that mice lacking Nocturnin remain lean on high fat diets, with lower body weight and reduced visceral fat. However, unlike lean lipodystrophic mouse models, these mice do not have fatty livers. The mutant mice do not exhibit increased activity or reduced food intake. However, gene expression data suggest that the Nocturnin knockout mice have deficits in lipid metabolism or uptake, in addition to changes in glucose and insulin sensitivity. Our data support a pivotal role for Nocturnin downstream of the circadian clockwork in the post-transcriptional regulation of genes necessary for nutrient uptake, metabolism, and storage.

Symposium 7: Disordered Human Circadian Clocks

Mutations for Human Sleep Phenotype

Ying He, Jimmy Holder, Ying-Hui Fu*, University of California San Francisco

Over the last decade, we have collected genetic material from individuals and families with circadian rhythm disturbances including familial advanced sleep phase syndrome (FASPS) and familial delayed sleep phase syndrome. To date, two genetic causes of FASPS have been reported. One is due to a mutation in *PER2* and the other is due to a mutation in a gene encoding a protein kinase called *Casein Kinase I delta* (*CKI*δ). Recently, we have identified a mutation which leads to a short sleep requirement phenotype in human. This represents a new opportunity for us to begin probing the mechanisms of human sleep homeostasis. Sleep and circadian function are distinct processes that interact in living organisms. Although these two systems can operate independently, recent studies indicate a more intimate relationship. Despite the exciting developments in the last decade on the neuronal pathways involved in wakefulness, sleep induction/maintenance, and molecular characterization of the circadian clock, the mechanism that controls how much sleep we need is entirely unknown. The approach of using Human Genetics to identify genes that are important in human behavioral phenotypes has proven fruitful in FASPS. Identification of mutations that give rise to alterations in sleep requirement represents a unique opportunity for delving into the molecular regulatory mechanisms of sleep homeostasis. Understanding of human sleep homeostasis has the potential to produce an enormous impact on our understanding of many biological pathways including brain functions (vigilance, short-term and long-term memory, executive function, math processing, cognitive speed, and spatial orientation), behavior, health, and longevity.

Peripheral Circadian Oscillators in Blood and Non-SCN Brain Tissues in Humans

D. B. Boivin[1] , F.O. James[1], E. Waddington-Lamont[1], and N. Cermakian[2], [1]Centre for Study and Treatment of Circadian Rhythms, Douglas Mental Health University Institute, Montréal, Québec, Canada; [2]Laboratory of Molecular Chronobiology, Douglas Mental Health University Institute, Montréal, Québec, Canada

Recent evidence demonstrated the existence of functional circadian clocks outside the central circadian pacemaker. We investigated the existence of these peripheral clocks in human subjects. In a first study, bi-hourly blood sampling over three consecutive days in 6 subjects revealed a significant HPER1 and HPER2 oscillation in peripheral blood mononuclear cells (PBMCs) that peaked early after waketime and was comparable under LD and CR conditions. In a second study, 5 healthy young subjects were placed on a 10-hour delayed sleep/wake schedule simulating night-time work. An 8-hour bright light stimulus during night shifts was used to reset the circadian pacemaker to the delayed schedule. Serial 24-hour blood sampling sessions were performed to assess the circadian variation of plasma melatonin, plasma cortisol, and clock genes expression in PBMCs. Following 9 days on the night schedule, hormonal rhythms were adapted to the shifted schedule. HPER1 and HPER2 expression in PBMCs displayed significant circadian rhythmicity which was in a conventional relationship with the shifted sleep/wake schedule. In a third study, we explored the presence of peripheral oscillators in non-SCN brain tissue by looking at *post mortem* brain tissue from Alzheimer's Disease (AD) patients and controls obtained from the Douglas Mental Health University Institute Brain Bank. We were able to detect a significant effect of time of death for *PER1* in the cingulate cortex and pineal gland. Altogether, these studies confirm the existence of peripheral circadian oscillators in human non-SCN tissue and their implications for shifted sleep schedules.

Analysis of PER3 Polymorphisms and Haplotypes That Associate with Diurnal Preference and Delayed Sleep Phase Disorder

Simon N. Archer, Jayshan Carpen, Mark Gibson, Gim Hui Lim, Jonathan Johnston, Debra J. Skene, and Malcolm von Schantz, Surrey Sleep Research Centre, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom

Diurnal preference and DSPD have been associated with polymorphisms in *PER3*. *PER3* promoter and coding region polymorphisms were genotyped and haplotype associations were characterized in subjects with extreme diurnal preference and DSPD (*n*=80 for extreme morning and evening groups, n=23 for DSPD). The functional significance of polymorphisms and potential enhancer and repressor elements within the *PER3* promoter was investigated with luciferase reporter constructs comprising different haplotype combinations and sequential deletions of the promoter. Three promoter region SNPs (G-320T, C-319A, G-294A) and five coding-region SNPs (T2115G, C2765T, 3206 4/5 VNTR, T3285C, A3648G) were identified in our samples. In addition, a novel 2-nucleotide-repeat VNTR polymorphism was found in the promoter (-318 1/2 VNTR). In the promoter region, the -320T and -319A alleles occurred more frequently in DSPD compared to morning (P=0.042 for each) or evening types (P=0.006 and 0.033, respectively). Genotype frequency analysis for the nine polymorphisms predicted the existence of 16 haplotypes. Haplotypes containing the promoter allele combination TA2G were more prevalent in DSPD compared to morning (P=0.033) or evening types (P=0.002). Luciferase-driven expression was significantly reduced in the GC2A (P< 0.05) and the rare TA1G (P<0.001) haplotypes, compared to the TAG haplotype. Promoter deletion reporter constructs defined two regions between -703 and -605, and between -283 and -80 that significantly enhanced reporter gene expression. Specific haplotypes predicted from the combined promoter and coding-region polymorphisms show significant associations with diurnal preference and DSPD. These data show that combined polymorphisms in *PER3* could affect gene expression and function, leading to phenotypic differences.

4:30–6:00 PM

Presidential Symposium: Is It Time for a New View of Circadian Clocks?

Chair: Martha Gillette, University of Illinois at Urbana-Champaign

Can a Biologist Fix a Radio? A Clock?

Yuri Lazebnik, Cold Spring Harbor Labs

By trying to answer the question posed in the title I will argue that the existing disparity between the effort of biological research and its meaningful outcome can be explained by a similar disparity between the complexity of the processes that we study and the tools with which most of us communicate. This argument will be intended to support the view that creating commonly accepted tools of communication that are sufficiently sophisticated to both adequately reflect biological processes and, importantly, be useful to an average biologist can be of significant practical benefit.

Nudging Nature's Networks: High Throughput Biology and the Use of Perturbagens

Steve A. Kay, Section of Cell and Developmental Biology, Division of Biological Sciences, University of California–San Diego

Our views of biological regulatory networks are changing quickly as our ability to collect biological data *en masse* continues to expand, and is coupled with computational resources for data visualization and network reconstruction. This is no less true for the circadian clocks field, where the canonical ideas of

"input", "clock component" and "output" are now somewhat blurred. This makes it necessary to abandon some of this old syntax for a more comprehensive view of the circuitry underlying rhythm generation and maintenance. This is particularly true where we are defining the interfaces between the networks that are associated with rhythm generation and those that are required for physiological responses, such as glucose and lipid metabolism. We also need to abandon the old idea of the "Transcription-Translation Oscillator (TTO)" for a view of hierarchical control circuits where the pace of the intracellular oscillator(s) is largely affected by regulated protein turnover, overlaid upon a topology of transcriptional feedback that is necessary to generate rhythms and drive cyclic processes. Multiple loop architecture clock systems are less to do with "core" and "stabilizing" concepts than rhythm generation and primary output nodes. This talk will draw on published and new data to provide examples of how we still have much to discover regarding the architecture and mechanisms of clocks from the organism to the intracellular level. An emphasis will be placed on the utility of high throughput approaches using a variety of "perturbagens" to probe clock networks and identify their constituent components.

Diurnal Rhythms: Unrecognized Critical Determinants of Cardiovascular and Renal Health and Disease

Michael J. Sole*, University Health Network, Heart and Stroke Lewar Centre of Excellence, University of Toronto

Diurnal rhythms influence both normal cardiovascular function and the incidence of acute adverse cardiac events. Shift workers have an increased risk of heart attack, stroke and sudden death. However, there are no data actually demonstrating that circadian disruption can cause cardiovascular disease. In our first studies we demonstrated diurnal cycling in over 16% of genes expressed in the heart and aorta of normal mice. Next we examined a murine model of pressure overload (transverse aortic constriction—TAC) in a rhythm disruptive 10hr light/10 hour dark environment. In contrast to TAC in a normal 24 hour day/night cycle, rhythm disturbed TAC exhibited exacerbated hypertension, disproportionate, perivascular and myocardial fibrosis but only minimal myocyte hypertrophy. Key genes in the cardiomyocyte hypertrophic pathway were paradoxically down-regulated and the rhythm of clock related genes was disrupted. Phenotypic and molecular rescue only occurred when the external rhythm was allowed to synchronize with the animals innate 24-hour internal rhythm. Finally, we examined cardiovascular and renal integrity using a natural model of early circadian entrainment, the +/*tau* hamster; this hamster genotype exhibits 22h cyclic behaviour patterns. In a 24 hour world +/*tau* hamsters exhibit significant fragmentation of diurnal activity and die prematurely; pathology demonstrates severe dilated cardiomyopathy and renal failure with renal tubular disease. On 22h light cycles appropriate for their genotype, cyclic behaviour patterns are normalized and hearts, kidneys and life expectancy are normal. In conclusion: synchrony between external and internal circadian clocks is critical to the integrity of the heart, blood vessels and kidney in both health and disease.

Tuesday, May 20

8:30–10:30 AM

Symposium 8: Post-translational Regulation of Circadian Clocks

Transcriptional and Post-transcriptional Feedback Loops of the Circadian Clock of Neurospora

Tobias Schafmeier, Andrea Neiss, Orfeas Dintsis, and Michael Brunner; University of Heidelberg Biochemistry Center

FREQUENCY (FRQ) and the White Collar Complex (WCC), consisting of the subunits WC-1 and WC-2, are central components of positive and negative feedback loops of the circadian clock of

Neurospora crassa. In the positive loop FRQ supports accumulation of WC-1 on a posttranslational level and activates wc-2 transcription. We show, that the same function of FRQ underlies negative and positive feedback. Thus, inactivation and accumulation of the WCC are both due to its FRQ-dependent phosphorylation. Hypophosphorylated WCC is active, binds to DNA and is rapidly degraded. FRQ-dependent phosphorylation of WCC lowers its affinity for DNA, leads to stabilization and accumulation of the WCC in the cytosol. In addition to posttranslational regulation of WCC function and abundance, expression of wc-1 and wc-2 are regulated on the level of transcription in a complex network of interconnected feedback loops. The WCC indirectly activates wc-1 transcription and represses wc-2 transcription by supporting expression of a putative activator of wc-1 and a repressor of wc-2. FRQ acts as an activator of wc-2 transcription by inhibiting the activity of the WCC.

Symposium 9: Circadian Clocks and Sleep

Finding the Light—Depression, Sleep and Circadian Networks

Christopher J. Winrow, Depression and Circadian Disorders Department, Merck Research Laboratories

The development of translational approaches for neuroscience drug discovery poses a particular challenge. By examining preclinical models including genetically inbred/altered mouse strains we can investigate the connections between behavioral traits and underlying genetic mechanisms. Importantly, the association of psychiatric diseases with sleep and circadian dysregulation has been strengthened by both clinical and preclinical observations, and is particularly relevant to depression and bipolar disorder. We have applied genetic and genomic approaches to examine underlying networks and processes contributing to disease pathology in order to discover novel targets for therapeutic intervention. In a collaborative project we used quantitative phenotyping, high-density genome-wide genotyping, and expression profiling to identify pathways affecting sleep, depression and circadian behavior between inbred mouse strains. These studies resulted in the discovery of more than 50 quantitative trait loci (QTL) associated with over 20 distinct phenotypes, and uncovered previously unrecognized relationships including the observation that REM sleep in the active period is under independent genetic control. To understand the mechanistic basis of these relationships we applied new technologies including laser capture microdissection (LCM) to interrogate CNS circuits at the transcriptional and proteomic level. This approach permitted the evaluation of connections between anatomical regions governing circadian regulation and sleep/wake control. We used LCM to examine circadian changes and identify novel arousal pathways responding to environmental manipulation in discrete brain regions. This presentation will describe how integrated genetic, genomic and behavioral approaches can be used effectively for the discovery and development of novel targets for sleep and circadian disorders, depression, anxiety and other psychiatric diseases.

PERIOD3 and the Circadian and Homeostatic Regulation of Sleep Physiology and Waking Performance in Humans

Derk-Jan Dijk, Antoine Viola, John Groeger, Malcolm von Schantz, and Simon Archer

Surrey Sleep Research Centre and Department of Psychology, University of Surrey, Guildford, United Kingdom

A variable number tandem repeat polymorphism in the coding region of *PERIOD3* is linked to extreme diurnal preference such that the prevalence of the PER3$^{5/5}$ genotype is higher among morning types.[1,2] We have characterized the effects of this polymorphism on the circadian and homeostatic regulation of sleep physiology and waking performance in a prospective study in which participants were selected only on the basis of their *PER3* genotype.[3] Endocrine and molecular markers of circadian phase did not differ between the genotypes.[3,4] In contrast, sleep physiology and the observed circadian amplitude of cognitive decline

during the biological all differed markedly between the two homozygous genotypes. *PER3*$^{5/5}$ individuals showed several signs of elevated homeostatic sleep pressure, including altered sympathetic/parasympathetic cardiac control during sleep.[5] *PER3*$^{5/5}$ individuals were also more susceptible to the negative effects of sleep deprivation on performance and in particular on tasks of executive function in the early morning.[6] FMRI studies conducted in the Cyclotron Research Centre of the University of Liege in an independent sample of *PER3*$^{5/5}$ and *PER3*$^{4/4}$ participants have confirmed that this polymorphism is a genetic marker of increased susceptibility to the effects of sleep loss.[7] Computer simulations of the non-additive interaction of homeostatic and circadian processes in the regulation of waking performance show that the data are consistent with the hypothesis that this polymorphism affects parameters of the sleep homeostat. [1]Archer *et a*l. SLEEP 2003; 26:413-415. [2]Jones *et al.* J Sleep Res 2007;16:12-16. [3] Viola *et al.* Curr Biol 2007; 17:613-618. [4] Archer *et al*. SLEEP 2008; 31. [5] Viola *et al.* submitted. [6]Groeger *et al*. Submitted. [7] Vandewalle *et al*, SRBR 2008

4:30-6:00 PM

Symposium 10: Non-image Forming Photoreception

Targeting Killing of Melanopsin-Expressing Retinal Ganglion Cells in the Fully Developed Adult Retina

I. Provencio[1], D. Goz[1], M.D. Rollag[1], D.A. Lappi[2], L.P. Morin[3], [1]Dept. of Biology, University of Virginia; [2]Advanced Targeting Systems, San Diego, CA; [3]Dept. of Psychiatry, Stony Brook University

Intrinsically photosensitive retinal ganglion cells (ipRGCs) in mammals express melanopsin as their photopigment and project to brain areas involved in circadian entrainment. We have developed a saporin-based immunotoxin to target ipRGCs in the mouse retina. Saporin is a type 1 ribosome-inactivating protein extracted from the seeds of the soapwort, *Saponaria officinalis*. UF008, an anti-melanopsin antibody raised against the 15 N-terminal amino acids of mouse melanopsin, has been conjugated to saporin (Advanced Targeting Systems, San Diego, CA). This immunotoxin is a new tool for studying the effects of melanopsin cell loss in the fully developed adult retina on circadian behavior and other forms of non-visual photophysiology.

The Role of Arrestin-Melanopsin Interaction in Melanopsin Function

Satchin Panda[1]*, Megumi Hatori[1], and Victoria Piamonte[2], [1]Salk Institute for Biological Studies, La Jolla, California; [2]Genomics Institute of the Novartis Research Foundation, San Diego, California

Melanopsin bears sequence and functional similarity to opsins from invertebrates. The photochemical properties of invertebrate opsins are modulated by phosphorylation and interaction with arrestin. We have previously shown that arrestin is necessary for the photoisomerase activity and for desensitization of ectopically expressed melanopsin in Xenopus oocytes. To further understand the role of arrestin in melanopsin function, we investigated the interaction mechanism of these two proteins. Photoactivated melanopsin is primarily Ser/Thr phosphorylated at multiple sites. Phosphorylation of these residues regulates the photochemical properties of melanopsin. Typically, phosphorylation of GPCRs enhances its affinity for arrestin. Both beta arrestin-1 and -2 are expressed in the retina and can functionally interact with melanopsin. Results from these studies imply the relative abundance of melanopsin and arrestin may fine-tune ipRGC function.

Melanopsin-Containing IpRGCs Are the Main Conduit dor Rod/Cone Light Input to Non-Imageforming Visual Functions

Samer Hattari*. Ali D. Güler[1], Jennifer L. Ecker[1], Gurprit S. Lall[2], Olivia Dumitrescu[3], Kwoon Y. Wong[3], Cara M. Altimus[1], David M. Berson[3], and Robert J. Lucas[2], [1]Department of Biology, Johns Hopkins University; [2]Faculty of Life Sciences, University of Manchester; [3]Department of Neuroscience, Brown University

Rods, cones, and melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs) are responsible for all light detection in the mammalian retina. The rods and cones relay light information for both image- and non-image-forming functions through a multisynaptic pathway to the brain by means of retinal ganglion cells (RGCs). The ipRGCs, which are photoreceptive and receive synaptic input from rod-cone networks, account for a small fraction of the RGC population and project to brain regions mainly involved in processing nonimage light information. To address how rod-cone information is conveyed to the brain, we genetically ablated the ipRGCs in mice. In the absence of ipRGCs, we demonstrated a severe impairment in light detection for NIF functions, while pattern vision and visuomotor functions were retained. Therefore, light signals for irradiance detection and pattern vision are separated at the retinal ganglion cell level, with ipRGCs acting as the principal conduit for NIF light detection, relaying both intrinsic (melanopsin-derived) and extrinsic (rod/cone-derived) photic signals to the brain. To further understand this unique class of RGCs, we generated mice that express Cre recombinase in ipRGCs. Using reporter lines, we revealed at least three separate classes of ipRGCs, each with distinct morphological properties and intrinsic light response characteristics. We also showed more extensive innervation of target brain regions, including areas previously thought not to be innervated by ipRGCs. Finally, we present preliminary evidence suggesting that different ipRGC subtypes have distinct developmental and axonal targeting programs. These data support that there are physical divisions in NIF functions that are mediated by different classes of ipRGCs.

Intensity- and Duration-Dependent Changes in the Spectral Sensitivity of Human Circadian Photoreception

Steven W. Lockley, Ph.D., Division of Sleep Medicine, Brigham and Women's Hospital, Harvard Medical School

The photoreceptor system that mediates circadian, neuroendocrine and neurobehavioral responses to light in humans has not been fully characterized. We therefore examined the spectral sensitivity of circadian phase resetting and melatonin suppression in subjects exposed to a range of irradiances of monochromatic green (555 nm) (n=24) or blue light (460 nm) (n=24) for 6.5 h during the early biological night. As predicted, the melatonin suppression sensitivity during the entire 6.5-hour duration was greater for 460 nm as compared to 555 nm light exposure. The melatonin suppression response during the first part of the light exposure appeared to be most sensitive to 555 nm light, however, but then demonstrated a short-wavelength shift in sensitivity across time. Similarly, phase delays of the melatonin rhythm also appeared to be more sensitive to 555 nm as compared to 460 nm light at low but not high irradiances, indicating a short-wavelength shift in sensitivity with increasing light intensity.

These results show that human circadian photoreception is a dynamic process where the three-cone photopic system appears to function as the primary circadian photoreceptor system at low irradiances and for short-duration light exposure, whereas the melanopsin-containing retinal ganglion cells function as the predominant circadian photoreceptors at high irradiances and over longer duration light exposure.

4:30-6:00 PM

Symposium 11: Systems Biology and Modeling of Circadian Rhythms

Models To Understand, Not To Believe

Andrew J. Millar[1], Treenut Saithong[1], Ozgur E. Akman[1], Kevin Stratford[1], Carl Troein[2], Kieron D. Edwards[2], Laszlo Kozma-Bognar[2], David A. Rand[3]; Centre for Systems Biology at Edinburgh, University of Edinburgh, Edinburgh; [1]Edinburgh Parallel Computing Centre, University of Edinburgh; [2]Present address: Institute of Plant Biology, Biological Research Center, Szeged, Hungary; [3]Warwick Systems Biology Centre, University of Warwick

Systems biology approaches are helping us to understand the complexity of the clock mechanisms, challenging some intuitive notions and refining others. To develop new mathematical models of the clocks in Arabidopsis and Neurospora, we combine timeseries of molecular data and luciferase (LUC) reporter gene imaging, with analysis of plant clock mutants, statistical parameter estimation and global parameter searches. The resulting differential equation models of the clock and the photoperiod sensor allowed us to predict the properties of an unidentified clock component in Arabidopsis, which was later identified experimentally (Locke et al., *Mol.Syst. Biol.* 2005 and 2006). The models simplify much biological detail in order to aid reasoning about the clock network and the design of experiments. In contrast to earlier, qualitative modelling approaches, we have focussed our Arabidopsis work on models that closely match the growing volumes of molecular timeseries data. Models are now refined by direct reference to the molecular data, and tested for their close match and for their robustness to parameter variations. This prioritises our current experimental approaches, including measuring biochemical parameter values for the LUC reporter and for clock components. Scaling up these approaches favours community effort and requires informatics infrastructure. Analysing the models points towards general principles of clock operation, with specific illustrations from data: 1. how the flexibility of timing favours clocks with multiple feedback loops (Rand et al., *Interface* 2004; *J.Theor. Biol.* 2006), 2. how complexity in both the clock and the light input pathways is required to reconfigure the clock under different photoperiods, 3. how an 'isoform switch' between closely-related clock components can facilitate the control of period over a temperature range, including temperature compensation (Akman et al., *Mol. Syst. Biol.* 2008). We have recently evolved novel clock and non-clock networks in silico, effectively challenging our earlier thinking, and will discuss the results.

From Mathematical Models to Molecular Mechanisms in the Neurospora Circadian System

Christian Hong[1], *, Ingunn Jolma[2], Peter Ruoff[2], Jennifer Loros[1,3], and Jay Dunlap[1],

Departments of [1]Genetics and [3]Biochemistry, Dartmouth Medical School, Hanover, NH, USA; [2]Department of Mathematics and Natural Science, University of Stavanger, Stavanger, Norway

Transcription/translation feedback loops involving *frq* are central to circadian rhythms in *Neurospora*. The transcription factors WC-1 and WC-2 activate frq expression in a circadian and light-dependent manner by binding to the Clock Box in the *frq* promoter. Additional feedback loops are intertwined with this negative feedback loop. Due to the complexity of multiple feedback loops, a mathematical model provides a good way to understand the underlying system, and to identify inconsistencies within line-diagram models. In *Neurospora*, the amount of the clock protein FRQ oscillates with a period of about 22 h, and FRQ inhibits its own synthesis by interacting with WC-1. Importantly, the pertinent events that close this negative feedback loop happen within the nucleus. A mathematical model of this oscillator recapitulates phase differences observed between frq mRNA and FRQ protein, FRQ and WC-1 proteins, and suggested distinctively different total vs. nuclear ratios of FRQ and WC-1, which we have confirmed experimentally. Specifically, nuclear FRQ levels are lower than nuclear WC-1 even though total FRQ levels are greater

than the total WC-1. Due to the limiting amount of FRQ in the nucleus, it's possible to hypothesize that FRQ would act as a direct or indirect catalytic component inactivating WC-1, as opposed to non-catalytic physical binding and inactivation of WC-1. The model, however, demonstrates that a non-catalytic, direct physical interaction of FRQ with WC-1 in the nucleus can generate robust oscillations even though nuclear FRQ levels are significantly lower than the levels of nuclear WC-1.

Ordered Phosphorylation Governs Oscillation of a Three-Protein Circadian Clock

Michael J. Rust*, Joseph S. Markson, William S. Lane, Daniel S. Fisher, and Erin K. OShea, Harvard University / HHMI

The simple circadian oscillator found in the cyanobacterium Synechococcus elongatus can be reconstituted in vitro using three proteins—KaiA, KaiB and KaiC. The total phosphorylation level of KaiC oscillates with a circadian period, but the mechanism underlying its sustained oscillation remains unclear. Here we show that four forms of KaiC differing in their phosphorylation state appear in an ordered pattern arising from the intrinsic autokinase and autophosphatase rates of KaiC and their modulation by KaiA. Kinetic and biochemical data indicate that KaiC phosphorylated only at S431 inhibits the activity of KaiA via interaction with KaiB. We hypothesize that stoichiometric inhibition of KaiA by KaiC provides the crucial nonlinear feedback that sustains oscillation. We convert this hypothesis into a mathematical model where the rates of phosphorylation and dephosphorylation are set by experimental data on partial reactions. This model quantitatively reproduces the circadian period and the distinctive phases and amplitudes of the four KaiC phosphoforms, providing a framework to further explore the mechanism of this circadian clock.

Systems Biology of Mammalian Circadian Clocks

Hiroki R. Ueda, Laboratory for Systems Biology and Functional Genomics Unit, Center for Developmental Biology, RIKEN

The logic of complex and dynamic biological networks is difficult to elucidate without comprehensive identification of network structure1-3, prediction and validation based on quantitative measurement and perturbation of network behavior4 and design and implementation of biological networks driven by the same logic as the original network. Mammalian circadian clock system is such a system consisting of complexly integrated regulatory loops and displaying the various dynamic behaviors including i) endogenous oscillation with about 24-hour period, ii) entrainment to the external environmental changes (temperature and light cycle), and iii) temperature compensation over the wide range of temperature. First, towards the system-level understanding of the entrainment (ii), we performed comprehensive expression profiles of mammalian central clock after the light pulse. We found that central clock tissue can represent both the intensity and change of light in different sets of genes, which can be molecular probes for the parametric and non-parametric responses independently proposed by Aschoff and Pittendrigh, respectively. We will present our current understanding of entrainment. Second, towards the system-level understanding of the period- determining processes in mammalian circadian clocks (i), we performed comprehensive perturbation analysis by using ~1,200 pharmacologically active compounds in human and mouse circadian clock cell lines. We will present our current understanding of period-determination processes. Finally, if I have a time, we will also introduce a current progress in temperature compensation in circadian clocks (iii). Explanations for temperature compensation of circadian clocks are classified roughly into two hypotheses, balanced reaction theory, and robust reaction theory. The difference between the two hypotheses lies in whether temperature compensation is achieved by the balance of molecular networks or by the robustness of clock molecules itself. You may be surprised by our current experimental results. Reference: 1. Ueda, H.R. et al, *Nature* 418, 534-539 (2002); 2. Ueda, H.R. et al, *Nat. Genet.* 37, 187-192 (2005); 3. Sato T K, et al, *Nat Genet.* 38:312-9 (2006) ; 4. Ukai H, et al, *Nat Cell Biol.* 9:1327-34 (2007)

4:30-6:00 PM

Symposium 12: Seasonal and Circannual Rhythms

An External Coincidence Model for Control of Plant Growth

Kazunari Nozue[1], Michael F. Covington[1], Paula D. Duek[2], Séverine Lorrain[2], Christian Fankhauser[2], Stacey L. Harmer[1], and Julin N. Maloof[1], [1]Department of Plant Biology, College of Biological Sciences, University of California–Davis; [2]Center for Integrative Genomics, University of Lausanne, Genopode Building, CH-1015 Lausanne, Switzerland

Most organisms use circadian oscillators to coordinate physiological and developmental processes such as growth with predictable daily environmental changes such as sunrise and sunset. Plant cell growth requires energy and water, factors that oscillate due to diurnal environmental changes. Indeed, two important factors controlling stem growth are the internal circadian oscillator and external light levels. However, most circadian studies have been performed in constant conditions, precluding mechanistic study of interactions between the clock and diurnal variation in the environment. Studies of stem elongation in diurnal conditions have revealed complex growth patterns but no mechanism has been described. Here we show that the growth phase of *Arabidopsis* seedlings in diurnal light conditions is shifted 8-12 hours relative to plants in continuous light, and we describe a mechanism underlying this environmental response. We find that the clock regulates transcript levels of two bHLH genes, *PIF4* and *PIF5*, while light regulates their protein abundance. These genes function as positive growth regulators; the coincidence of high transcript levels (by the clock) and protein accumulation (in the dark) allow them to promote plant growth at the end of the night. Thus these two genes integrate clock and light signalling and their coordinate regulation explains the observed diurnal growth rhythms. This interaction may serve as a paradigm for understanding how endogenous and environmental signals cooperate to control other processes.

Control of Seasonal Rhythms in Mammals

Fran Ebling, University of Nottingham, Queen's Medical Centre

Most mammals inhabiting temperate and arctic habitats display profound seasonal rhythms of behaviour and physiology, including reproduction, fattening, moulting and hibernation. These generally reflect underlying long-term rhythmic processes which are entrained by the change in ambient photoperiod, mediated via changes in the nocturnal duration of melatonin secretion. The Siberian hamster provides a convenient model species for investigating the underlying mechanisms. It gains body weight in the summer and then survives winter by reducing food intake, catabolising fat reserves and undergoing bouts of torpor. Analysis of differential gene expression in the hypothalamus and lesion studies in this species reveal that the long-term changes in energy balance are not simply effected by the brain centres and peptidergic pathways known to underlie short-term homeostatic regulation. The majority of gene expression changes are confined to two restricted areas: the dorsomedial posterior arcuate nucleus, and the ventral ependymal layer of the third ventricle. Functions encoded by these "seasonal" genes include thyroid hormone metabolism, retinoic acid and histaminergic signalling, and VGF and secretogranin production. The changes in thyroid hormone availability that are brought about by differential activity of deiodinase enzymes are of particular importance because experimental manipulation of central thyroid levels can prevent seasonal cyclicity. Given the importance of thyroid hormone in the initial development of the brain it has been hypothesized that thyroid hormone-dependent plasticity of hypothalamic connections and neurogenesis underlie seasonal cycles of food intake and body weight.

Functional Genomics Analysis of Photoperiodic Time Measurement

Takashi Yoshimura ,Laboratory of Animal Functional Genomics, and Avian Bioscience Research Center, Graduate School of Bioagricultural Sciences, Nagoya University, Japan

Animals living outside the tropics use changes in photoperiod to adapt to seasonal changes in environment, but the molecular mechanisms underlying photoperiodic time measurement are not fully understood. The Japanese quail is a robust model for the study of these mechanisms because of its rapid and dramatic response to changes in photoperiod. Rapid induction of thyroid hormone-activating enzyme (*DIO2*) gene expression in the ependymal cells (EC) lining ventrolateral walls of third ventricle of the mediobasal hypothalamus (MBH) is the earliest event yet recorded in the photoperiodic signal transduction pathway. The crucial question now is the identity of the photoperiodic transduction pathway regulating *DIO2* expression. To address this question we have dissected the molecular dynamics of gene expression regulating photoinduced thyroid hormone metabolism using a chicken high-density oligonucleotide microarray. Using one way ANOVA, two waves of gene expression were identified. The first was initiated ~14 h after dawn of the first long day and included increased thyrotropin (TSH) subunit expression in the pars tuberalis of the pituitary gland; the second occurred ~4 h later and included increased *DIO2* expression. When expression of TSH receptor and TSH binding assay were examined, TSH receptor was found in the EC of the MBH. Intracerebroventricular administration of TSH to short day quail stimulated gonadal growth, and expression of *DIO2* in the EC. This TSH induced expression of *DIO2* was shown to be mediated through a thyrotrophin receptor-cAMP signalling pathway. Increased pars tuberalis TSH therefore appears to trigger long day photoinduced seasonal breeding.

Wednesday, May 21

8:30-10:30 AM

Symposium 13: Interplay between Circadian and Social Behavior

Identification of a Regulator and Characterization of the Cellular Network Necessary for Male Sex Drive Rhythm in Drosophila

Shinsuke Fujii and Hubert Amrein, Department of Molecular Genetics and Microbiology Duke University Medical Center

It has been well established that the social context can profoundly affect the circadian rhythm of an animal. To investigate the effect of various social contexts on circadian behavior of *Drosophila*, we developed an assay that allows two flies to move and interact freely and enables investigators to track them simultaneously and continuously for up to 96 hours. Comparison of activity profiles of single flies and pairs of flies from the same or opposite sex revealed remarkable differences in locomotor pattern, indicating that the sociosexual context significantly modifies circadian behavior. Most surprisingly, diurnal activity of a single males or females shifts towards nocturnal activity when a male and female cohabitate the same arena. This shift is driven by nocturnal male courtship, which we refer to as Male Sex Drive Rhythm (MSDR). We used cell-specific genetic manipulation to disable the molecular clock and show that a subset of pacemaker neurons in the male (LNds and DN1s) is necessary to maintain MSDR. LNds and DN1s express FRU^M, a master regulator for male courtship behavior; in fact, we show that FRU^M expression is required in these neurons to generate a robust MSDR. Moreover, we find that several *fru* alleles affect MSDR without affecting overall courtship activity. However, FRU^M is not necessary for oscillation of PERIOD, suggesting that *fru* and the clock genes function in concert to regulate expression of downstream effectors of the molecular clock. Finally, FRU^M expression in females fails to generate a robust MSDR even though these females exhibit male courtship behavior, suggesting that additional male-specific components are necessary for MSDR.

The Social Clock of The Honeybee—From Social Organization to Plasticity in Clock Gene Expression

Guy Bloch , Yair Shemesh, and Mira Cohen, Department of Evolution, Systematics, and Ecology, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Israel

In honeybees (Apis mellifera) natural plasticity in circadian rhythms is associated with the division of labor that organizes their colonies. "Nurse" bees (typically < 2 weeks old) care for brood around-the-clock whereas bees older than 3 weeks of age typically forage for flowers with strong circadian rhythms. We found that nurses care for brood around-the-clock even under a light/dark illumination regime. Brain oscillations in the abundance of the putative clock genes Period and Cryptochrom-m were attenuated or totally suppressed in nurses as compared to foragers, irrespective of the illumination regime. However, nurses showed circadian rhythms in locomotor activity and molecular oscillations in brain clock gene expression shortly after transfer from the hive to constant laboratory conditions. The onset of their activity occurred at the subjective morning, suggesting that some clock components were entrained even while in the hive and active around-the-clock. These results suggest that the hive environment induces reorganization of the molecular clockwork. To test this hypothesis, we studied activity and brain clock gene expression in young bees that were confined to a broodless area on the honeycomb in a light/ dark illuminated observation hive. These bees experienced the hive environment and could interact with other bees, but not with the brood. By contrast to same-age nurses from these colonies, the confined bees showed molecular oscillations in clock gene expression and were more active during the day. These findings are consistent with the hypothesis that interactions with the brood modulate plasticity in the molecular clockwork of the honeybee.

Fight or Flight (to a New Phase?): On The Timing of Social Repulsion

William J. Schwartz and Matthew J. Paul, Department of Neurology, University Massachusetts Medical School

Behavioral ecologists refer to the concept of r*esource partitioning*, by which co-existing species may lessen competition within an ecological niche through their separation in space (e.g., utilizing terrestrial vs. arboreal habitats) and/or time of day. Relatively little is known about the mechanisms for such inter-individual temporal adaptations, or how the circadian system might be involved. The literature suggests that actual prolonged cohabitation might lead to robust effects on the rhythmicity of co-housed individuals, but that these effects are not easily reproduced by indirect or pulsatile social contacts. We have begun to study the conditions under which such cohabitation effects might be revealed and further analyzed in the laboratory. We will present data to show that co-housing pairs of Syrian hamsters can result in large, persistent changes in the free-running circadian period of one of the two hamsters of the pair, leading in some cases to a temporal separation of the hamsters' wheel running in constant darkness. Elucidating the mechanism(s) for this social interaction will require the measurement of individual as well as group activity data, which is now feasible using sensors small enough to implant subcutaneously in individual rodents. Social regulation of the rhythmicity of individuals may be a critical factor for a group's adaptation to the natural habitat, and a better understanding of this level of biological organization will likely generate a more complex, but ultimately more comprehensive, view of clocks and rhythms.

Circadian Food Anticipatory Activity, Social Enhancement, and Leadership in Shoals of Golden Shiners

Stéphan Reebs, Département de biologie, Université de Moncton, Moncton, Canada

Animals can use their circadian clocks to learn the time of day at which food is available and to anticipate its arrival. This anticipatory behavior is a cue that can prompt naïve conspecifics to join the informed individuals and to start searching for food, a phenomenon called social enhancement. If the conspecifics were already part of a group that included the informed individuals, and if these informed individuals know not only when but also where the food is available (a case of circadian-based time-place learning), then the naive may follow the informed at the right time of day in the hope of pilfering food. In such cases, the informed individuals seem to act as leaders for the group. Moreover, if a group contains individuals that have different knowledge, such as food availability at different circadian phases, then different leaders may emerge at different times of day, resulting in an adaptive temporal complementarity of leadership. My talk will illustrate these possibilities in captive shoals of golden shiners, a gregarious minnow that is known to roam widely within lakes.

Symposium 14: Clocks, Cell Cycle, and Cancer

Circadian Clocks and Cell Cycle Rhythms in Zebrafish

Nicholas S. Foulkes*, Daniela Vallone, and Thomas Dickmeis, Institute of Toxicology and Genetics, Forschungszentrum Karlsruhe

The zebrafish offers a unique set of properties (small size, low maintenance costs, high fecundity, optically transparent embryos, rapid development "in vitro" and amenability to genetic and chemical screens) that has made it a powerful model for studying various aspects of vertebrate biology. Although initially adopted by developmental biologists, the zebrafish is now being used to study much broader aspects of biology including physiology, behaviour and pathology. This animal model has proven particularly interesting for studying circadian clock biology. Unlike the situation in mammals, peripheral clocks in the zebrafish are entrained by direct exposure to light cycles. Likewise, cell lines derived from adult and embryonic stages also possess directly light entrainable clocks. Using this model we are exploring the links between the circadian clock and the timing of cell cycle progression during early growth and development. Using wild type larvae and adults, panels of mutants and also zebrafish cell lines, we are exploring the contribution of cell autonomous and endocrine factors in the establishment of circadian cell cycle rhythms.

Circadian Release of Hematopoietic Stem Cells

Paul S. Frenette[1,2,3], Simón Méndez-Ferrer[1], Daniel Lucas[1], and Michela Battista[1]; Mount Sinai School of Medicine, [1]Departments of Medicine, and Gene and Cell Medicine, [2]Black Family Stem Cell Institute, [3]Immunology Institute, New York

Haematopoietic stem cells (HSCs) are continuously released in the bloodstream under steady state conditions following robust circadian fluctuations, peaking 5 h after the initiation of light and reaching a nadir 5 h after darkness. These circadian oscillations are altered after disruption of the standard light cycle by exposing mice to continuous light or to a jet lag. We have recently evaluated the mechanisms (*Nature*, 2008), and shown that circulating HSCs/progenitors fluctuate in antiphase with the expression of the chemokine CXCL12 in the bone marrow (BM) microenvironment. Cyclical HSC release and *Cxcl12* expression are regulated by core genes of the molecular clock through noradrenergic signals from the sympathetic nervous system. These adrenergic signals are locally delivered by nerves in the BM, transmitted

to stromal cells by the b3-adrenergic receptor, leading to reduced nuclear content of Sp1 transcription factor, and rapid downregulation of *Cxcl12*. Neurally driven HSC release during the animal's resting period may promote regeneration of the stem cell niche, and possibly of other tissues.

Cell Cycle, DNA Damage and Neurospora Clocks

Josh Gamsby[1], Antonio Pregueiro[1], Han Cho[1], Jay Dunlap[1], and Jennifer Loros[1,2]. Departments of [1]Genetics and [3]Biochemistry, Dartmouth Medical School, Hanover.

The Neurospora clock gene *prd-4* encodes an allele of *chk-2*. *chk-2*$^{prd-4}$ is semidominant with a short period length (18 hr), and defective temperature compensation. Normally the CHK2 kinase is activated by DNA damage and phosphorylates its substrates leading to cell cycle arrest (CDC25) and repair or apoptosis (P53), and in Neurospora, one of CHK2's substrates is FRQ. CHK2^{PRD4} interacts more strongly with FRQ and has partially bypassed the requirement for DNA damage-activation. Therefore FRQ is precociously phosphorylated leading to a short period length, explaining the semidominance, the fact that Δ*prd-4* has a normal period length, and why a *chk-2*^{D414A} (kinase dead) point mutation abrogates the period shortening of CHK2^{PRD4}. Expression of *prd-4* peaks in the morning, and DNA damage resets the clock both in Neurospora and in mammalian (NIH3T3 and MEFs) cells in culture. Thus, CHK2^{PRD4} identifies an output from the clock that feeds back, conditionally, to affect input as well as the cell cycle. The circadian clock gates the cell cycle via regulation of WEE1 (work from the Okamura lab), and we now know that CHK2, which acts on CDC25C to affect the same cell cycle point as WEE1, also does: In mammalian cells synchronized by hydroxyurea, the time of entry into S-phase is determined by the serum shock and not by release from HU, and is dependent on Per2. DNA damage, either from MMS treatment or IR (the van der Horst lab), results in an all-advance PRC in mammalian cell in culture, just as seen in Neurospora.

Symposium 15: Clocks, Brain Function, and Dysfunction

Circadian Modulation of Associative Learning

Lisa C. Lyons, Florida State University

Learning represents a change in an animal's behavior in response to particular events or environmental stimuli. To fully understand learning, it is necessary to understand the mechanisms responsible for the induction and consolidation of memory as well as the factors responsible for modulation of these processes. Using the classic invertebrate models, Aplysia and Drosophila, we are investigating circadian modulation of short and long-term memory. In Aplysia, we found that the circadian clock modulates long-term, but not short-term memory, such that the greatest amount of memory is formed in phase with the animal's activity period. Circadian modulation of long-term memory formation appears to occur through suppression of learning-induced gene expression. The circadian clock regulates short-term memory in Drosophila with peak performance in memory occurring during the early to mid-subjective night. The rhythm in memory is eliminated in timeless and period mutants. Moreover, the rhythm in memory for olfactory conditioning appears dependent upon circadian modulation of learning, rather than modulation of the flies' responsiveness to sensory stimuli. Importantly, we found that the antennal circadian oscillator does not appear to modulate the circadian rhythm in short memory as cryptochrome mutants demonstrate robust circadian rhythms in memory. Thus, we hypothesize that central, rather than peripheral, circadian oscillators modulate the formation of short-term associative memory. These studies in Aplysia and Drosophila have significantly increased our understanding of circadian modulation of associative learning and set the stage for future studies investigating the molecular and cellular mechanisms through which the circadian clock modulates memory formation.

A Role for the Circadian Clock Gene Period2 in Synaptic Plasticity and Learned Behavior

Louisa Mei-Chen Wang[1], Joanna Molly Dragich[1], David K Welsh[3], Thomas J O'Dell[2] and Christopher Scott Colwell[1], [1]Department of Psychiatry and Biobehavioral Sciences; [2]Department of Physiology University of California–Los Angeles; [3]Departments of Psychiatry and Cell & Developmental Biology, University of California–San Diego

Genes responsible for generating circadian oscillations are expressed in a variety of brain regions not typically associated with circadian timing. The functions of this clock gene expression are largely unknown, and we sought to explore the role of the *mPer2* gene in hippocampal physiology and learned behavior. We found that mPER2 protein is highly expressed in hippocampal pyramidal cells and that this expression varies with a circadian rhythm. The rhythms in *mPer2* expression are autonomous and present in isolated hippocampal slices maintained in culture. Physiologically, *mPer2*-mutant mice exhibit abnormal long-term potentiation. The underlying mechanism is suggested by the finding that levels of p-CREB, but not p-ERK, are reduced in hippocampal tissue from mutant mice. Finally, *mPer2*-mutant mice exhibit deficits in the recall or consolidation of learned behaviors. Together, these results provide evidence that the clock gene *mPer2* plays a critical role in synaptic plasticity and in the recall of some forms of learned behavior.

Regulation of the Murine Monoamine Oxidase a Gene by Circadian Clock Components Implies Clock Influence on Mood

Gabriele Hampp, Jürgen A. Ripperger, Thijs Houben, Isabelle Schmutz, Christian Blex, Stéphanie Perreau-Lenz, Irene Brunk, Rainer Spanagel, Gudrun Ahnert-Hilger, Johanna Meijer, and Urs Albrecht*, University of Fribourg

The circadian clock has been implicated in addiction and several forms of depression. However, the molecular mechanisms involving the circadian clock in these processes are not known. Because dopamine may play an important role, we analyzed the murine promoters of genes encoding key enzymes important in dopamine metabolism. We find that transcription of the monoamine oxidase A (Maoa) promoter is regulated by the clock components Bmal1, Npas2 and Per2. A mutation in the clock gene Per2 in mice leads to reduced expression and activity of MAOA in the mesolimbic dopaminergic system, a reward circuit involved in addiction and depression. Furthermore, we observe increased levels of dopamine and altered neuronal activity in the striatum, leading to behavioral alterations in Per2 mutant mice in despair-based tests. These findings indicate a role of circadian clock components in dopamine metabolism highlighting a role of the clock in regulating mood related behaviors.

A Cell-Based Approach to The Study of Daily Behavior

Elzbieta Kowalska, Anke Mueller, Amelie Dumas, Pascal Bruegger, Paul Westermark, Dieter Kunz, HansPeter Herzel, Achim Kramer, and Steven A. Brown*, University of Zurich

Though daily behavior is governed by a central clock in the suprachiasmatic nucleus of the brain hypothalamus, "slave" oscillators of similar or identical molecular mechanism exist in most cells of the body. Our laboratory has exploited this duplication to gain insight into the way molecular clocks govern mammalian behavior. For example, using dermal fibroblast cells from skin biopsies of human subjects of early and late chronotype, we have studied the circadian period lengths of these two groups, as well as

their ability to phase-shift and entrain to environmental and chemical signals. We find not only period length differences between the two classes, but also significant changes in the amplitude and phase-shifting properties of the circadian oscillator among individuals with identical "normal" period lengths. We conclude that human chronotype may be influenced not only by the period length of the circadian oscillator, but also by cellular components that affect its amplitude and phase. We have also used peripheral oscillators as a screening tool to identify loci that influence the circadian oscillator in embryonic stem (ES) cell lines. Although ES cells divide rapidly and possess no detectable circadian transcriptional oscillations, wildtype cells display normal circadian rhythmicity once differentiated into neuronal or keratinocyte lineages. Using ES cells containing an integrated "genetrap" to abrogate production of candidate genes, we have looked for loci implicated in daily behavior by monitoring circadian clock function in these cells at a molecular level with an adenoviral bioluminescent reporter vector. With our screening method, we could observe the complete loss of circadian reporter gene expression after neuronal differentiation in cells lacking the NONO gene, which we have implicated previously in the circadian clock. By standard blastocyst injection, we have also generated NONO-genetrap mouse lines. Initial experiments indicate a shortening in the period length of the circadian clock in these animals, confirming the importance of NONO to the circadian oscillator.

Pittendrigh/Aschoff Lecture

The Mammalian Circadian Timing System: From Gene Expression to Metabolism

Gad Asher, Gwendal Le Martelot, Hans Reinke, David Gatfield, Camille Saini, Frederic Gachon, Thierry Claudel, Charna Dibner, Markus Stratmann, Alan Gerber, and Ueli Schibler*, University of Geneva

The mammalian timing system has a hierarchical structure, in that a master pacemaker located in the suprachiasmatic nucleus synchronizes self-sustained and cell-autonomous oscillators in most body cells. Daily feeding cycles are dominant Zeitgebers for the phase entrainment of many peripheral tissues, but rhythmic hormones, body temperature oscillations, and the peripheral nervous system also play important roles as timing cues. The temporal coordination of metabolism is a major purpose of peripheral circadian oscillators. In this lecture, I shall present several novel approaches aimed at deciphering inputs into and output from peripheral clocks operative in hepatocytes and fibroblasts. Genome-wide circadian transcriptome profiling studies in conjunction with a novel proteomic technique, dubbed DDDP (differential display of DNA-binding proteins) have unveiled candidate players of systemic signaling pathways that may participate in the synchronization of peripheral oscillators. These include HSF1, members of the fibroblast growth factor family, protein kinases, cytoskeleton proteins, and RNA-binding proteins. Recently, we found Sirtuin 1 (SIRT1) to be required for high amplitude and magnitude circadian clock gene expression, and this NAD-dependent protein deacetylase may thus connect metabolic with circadian cycles. Our studies with various mutant mouse models have revealed important output functions of REV-ERBalpha and the three PAR bZip proteins DBP, TEF, and HLF. While REV-ERBalpha participates in cholesterol and bile acid metabolism through the temporal modulation of SREBP activity and Cyp7a1 expression, PAR bZip proteins regulate the circadian import and oxidation of fatty acid via the activation of the nuclear receptor PPARalpha.

Sunday, May 18

11:00–12:30 PM

Slide Session A: Molecular Clocks I

11:00

1 • Genetic and Temporal Requirements for the Ectopic Induction of the Circadian Clock Program Reveal a Unique Role for the Clock Gene

Valerie L. Kilman*, Hilary Purdy, and Ravi Allada, Northwestern University

Circadian clocks rely on multiple cell-autonomous transcriptional feedback loops centered on the transcriptional activator Clock (Clk). Previously we found ectopic expression of Clk in naïve cells induces cycling per, tim, and cry. These ectopic clocks are used as a system to explore genetic requirements for clock induction. Ectopic clock induction fails in flies that lack key components of natural clocks such as cyc, CLK's heterodimeric partner. Ectopic clocks oscillate robustly in LD and gradually damp in DD. In common with natural peripheral clocks they require the cell-autonomous blue light photoreceptor cry for overt cycling. None of the other major components of the transcriptional feedback loops induce ectopic rhythms indicating that Clk is unique among clock genes in its ability to induce clock oscillations. In addition though the eyes are the major source of oscillating PER in the brain, several master regulator eye development genes fail to induce ectopic PER. This positions Clk as the critical and only known gatekeeper of clock cell oscillations. Clk expression restricted to adulthood can induce transgene-dependent clocks with some GAL4 drivers, suggesting Clk's role is acute and not developmental. Continual ectopic activation of Clk is required to sustain adult clocks. In other cell types, glia for example, ectopic PER was induced but oscillations were not detected. We are testing the hypothesis that these represent desynchronized clocks by using alternative methods of entrainment. Taken together, these studies indicate Clk holds a special place among clock genes in its ability to induce and sustain the circadian program.

11:15

2 • Linking CLK/CYC Activity to Phosphorylation and In Vivo Phenotypes

Jerome S. Menet and Michael Rosbash, Brandeis University

Eukaryotic circadian rhythms encompass an almost ubiquitous principle of molecular feedback loops. In Drosophila, CLOCK (CLK) and CYCLE (CYC) function as a heterodimer and activate transcription. PERIOD (PER) inhibits CLK/CYC-mediated transcription cyclically. In the present study, we found new CLK mutations that alter its sensitivity to PER transcriptional repression in tissue culture. These mutations target two evolutionary conserved serines (S15 and S19) in the CLK bHLH domain. Alanine substitutions (S15/19A) decrease CLK sensitivity to PER repression, whereas aspartic acid substitutions (S15/19D) increase CLK sensitivity. We assayed the physiological relevance of these mutations by using a new dClk transgene rescue strategy in flies. A wild-type dClk transgene rescued well the arrhythmic loss of function ClkAR mutation with a circadian period close to 24h. dClk transgenes with the above mutations also rescued ClkAR but with a strong effect on circadian period. Interestingly, rescue with the better repressed CLK mutant (S15/19D) had a period about 1.5hrs longer than rescue with wild-type CLK, whereas the less sensitive CLK protein (S15/19A) rescued with a period about 1.5 hrs shorter than

wild-type clock. Although it is still unclear whether PER mediates S15/19 phosphorylation in the course of inhibiting CLK/CYC-mediated transcription, molecular analysis of these transgenic flies indicates that the potency of transcriptional repression is linked to circadian period. In addition, very recent results using a new S15/19 phospho-specific antibody reveal a light-mediated change in CLK phosphorylation status. These new results suggest a novel and unexpected role of light in regulating CLK-mediated transcription in Drosophila.

11:30

3 • Rhythmic SAF-A Binding Underlies Circadian Transcription of the Bmal1 Gene

Yoshiaki Onishi[1]*, Syuji Hanai[1], Tomoya Ohno[1,2], Yasuhiro Hara[1,2], Norio Ishida[1,2], [1]Institute for Biological Resources and Functions, AIST and [2]Graduate School of Life and Environmental Sciences, University of Tsukuba

lthough Bmal1 is a key component of the mammalian clock system, little is understood about the actual mechanism of circadian Bmal1 gene transcription, particularly at the chromatin level. Here we discovered a unique chromatin structure within the Bmal1 promoter. The RORE region, which is a critical cis-element for circadian regulation of the Bmal1 gene, is comprised of GC-rich open chromatin. The 3'-flanking region of the promoter inhibited rhythmic transcription in the reporter gene assay in vitro even in the presence of RORalpha and REV-ERBalpha. We also found that the nuclear matrix protein, SAF-A binds to the 3'-flanking region with circadian timing, which was correlated with Bmal1 expression by footprinting in vivo. These results suggest that the unique chromatin structure containing SAF-A is required for circadian transcriptional regulation of the Bmal1 gene in cells.

11:45

4 • Regulation of Per1 by Aryl Hydrocarbon Receptor

Shelley A. Tischkau*, Bethany A. Karman, Stacey L. Krager, and Canxin Xu

The Aryl Hydrocarbon Receptor (AhR) mediates the toxicological effects of dioxins and certain other polycyclic aromatic hydrocarbons in biological tissues, including the ovary. Ovarian function is compromised after exposure to the prototypic AhR agonist, 2,3,7,8—tetrachlorodibenzo-p-dioxin (TCDD). A physiological role for AhR in regulation of ovarian follicle development has also been demonstrated using AhR null mice (AhRKO). The mechanism by which AhR functions in the ovary remain poorly understood. Because the AhR is a member of the PAS domain family of proteins, similar to many circadian clock genes, we hypothesized that AhR may interact with the ovarian circadian clock. Specifically, we examined 1) co-localization of AhR with the circadian clock genes Period1 (Per1) and Brain muscle ARNT-like1 (Bmal1) in the adult mouse ovary, 2) interactions between AhR and Bmal1 by co-immunoprecipitation, 3) changes in Per1 and Bmal1 24-h expression patterns in AhRKO mice and in mice treated with 1 μg/kg TCDD by gavage, and 4) effects of AhR activation on expression of Per1 in spontaneously immortalized granulosa cells. AhR co-localized with both Per1 and Bmal1 in the ovary. Bmal1 immunoprecipitated with AhR in ovarian extracts. Circadian expression patterns of both Bmal1 and Per1 were altered in AhRKO mice and in mice treated with TCDD. AhRKO mice had increased levels of Per1 and a circadian rhythm that was faster than in controls. Per1 levels were reduced after TCDD treatment and circadian expression patterns showed a dampening of rhythmicity. In culture, activation of AhR using either napthoflavone or 6-formylindolo[3,2-b]carbazole (FICZ), attenuated forskolin-stimulated Per1 transcript levels. Collectively, these data suggest that AhR may directly interact with Bmal1 to influence expression of Per1. Altered AhR signaling, as occurs after TCDD exposure, or in AhRKO mice, may disrupt the ovarian circadian clock, leading to a desynchronization of events required for proper follicular development. Supported by NIH ES012948 (SAT) and ACS/Institutional Cancer research grant 560

12:00

5 • Bmal1 Is a Direct Transcriptional Target of the Orphan Nuclear Receptor, NR2F1

Celina Montemayor, Fernanda R. Ruiz, Martin E. Young, and Fred A. Pereira*, Baylor College of Medicine

Orphan nuclear receptor NR2F1 (also known as COUP-TFI, Chicken Ovalbumin Upstream Promoter Transcription Factor I) is a highly conserved member of the nuclear receptor superfamily. NR2F1 plays a critical role during embryonic development, particularly in the central and peripheral nervous systems and in the inner ear. In the mouse cochlea, NR2F1 controls Notch signaling to regulate precursor cell differentiation into hair cells and supporting cells. In order to further identify pathways downstream of NR2F1, we obtained microarray gene expression profiles from wild type and Nr2f1-/- inner ear tissues. One of the most significant expression changes, as validated by real-time RT-PCR, is the core circadian gene Period1. Indeed, over-expressing NR2F1 in cells in culture resulted in complete deregulation of the circadian clock mechanism. We next performed chromatin immunoprecipitation (CHiP) and promoter-luciferase reporter assays and determined that NR2FI directly occupies the Bmal1 promoter and activates its transcription rate. Furthermore, nr2f1 transcript levels oscillate in a circadian manner in mouse embryonic fibroblasts (MEFs) and in NIH3T3 cells synchronized by serum-shock. Importantly, the Bmal1 transcript response to serum shock is altered in NR2F1-/- MEFs when compared to wild type controls. These results implicate NR2F1 in regulating both the circadian and Notch cyclic networks during inner ear development. Research supported by NIDCD 04585.

12:15

6 • An Sv-40 Poly Adenylation Signal Sequence in the 3'utr of Per2::Luciferase(Sv) Mice Lengthens Free-Running Period and Increases Per2

Seung-Hee Yoo[1], Shin Yamazaki[2], Ethan Buhr[1], Junghea Park[1], Shihoko Kojima[3], Carla B. Green[3], Joseph S. Takahashi[1*], [1]Howard Hughes Medical Institute, Northwestern University; [2]Department of Biological Sciences, Vanderbilt University; [3]Department of Biology, University of Virginia

Over the past couple years, the PER2::LUCIFERASE fusion knock in mouse model has been used as a reliable tool to monitor real-time circadian dynamics in many paradigms of research. Here we report an additional version of PER2::Luciferase fusion knock in mouse: mPER2::LuciferaseSV. To generate the mPER2::LuciferaseSV allele, we used an identical targeting strategy as with the original PER2::Luciferase, except in their 3'UTR regions. Instead of using the genomic mPer2 3'UTR, we used a poly adenylation signal sequence from simian virus 40. As a result, PER2::Luciferase and mPER2::LuciferaseSV mRNA have different 3'UTR sequences, but the same translated mPER2::Luciferase proteins. Previously we reported that the circadian period of PER2::Luciferase mice (Luc/Luc, 23.69± 0.07 hr) was indistinguishable from that of wild type mice. However, the free running period of mPER2::LuciferaseSV mice was 0.5 hr longer than PER2::Luciferase mice . Furthermore, bioluminescence signal from mPER2::LuciferaseSV was brighter than that of PER2::Luciferase. To investigate the basis underlying phenotypic differences between the two knock in alleles, we performed a series of experiments to survey the stability of mPer2 and its effect on expression of other clock components.

11:00

7 • Restricting Food Intake to the Light Phase Results in Weight Gain in C57BL/6J Mice

Arble, Deanna M.*, Laposky, Aaron D., and Turek, Fred W., Sleep & Circadian Biology, Northwestern University

Mice exhibit diurnal locomotor and feeding behavior with the majority of activity and caloric intake occurring in the dark phase. While the suprachiasmatic nucleus (SCN) controls most behavioral and physiological rhythms, an SCN-independent food-entrainable oscillator (FEO) has been identified. The independence between the SCN and FEO could serve adaptive functions; but it also presents the opportunity for developing internal desynchronization between multiple oscillators. In this study, we tested the hypothesis that the metabolic effects of a high-fat (HF) diet would depend upon the time of feeding. Nine-week old male C57BL/6J mice were entrained to a 12L:12D cycle and fed a 60% HF diet during only the light phase (HFL, N=6) or during only the dark phase (HFD, N=6) for 6 weeks. Activity was recorded continuously with IR beam breaks, body weight measured biweekly, and food intake calculated weekly. After 6 weeks on the HF diet, HFL mice (32.1 ± 1.3g post-feeding) weighed significantly more (+21%) than HFD mice (26.6 ± 1.6g post-feeding; $p<0.05$). Weighing at ZT0 and ZT12 revealed a substantial daily fluctuation in body weight, with HFL varying 2.1 ± 0.09g and HFD varying only 0.6 ± 0.06g ($p<0.001$). Importantly, HFL and HFD did not differ in the average amount of calories consumed per day, total activity counts, or relative amount of activity expended in the dark phase. These data indicate that the timing of food exerts a marked effect on energy homeostasis and maintenance, independent of total caloric intake or total activity level.

11:15

8 • Role of Cardiomyocyte Circadian Clock in Myocardial Metabolic Adaptation

Ju-Yun Tsai*, Michael W.S. Moore, Martin E. Young, Baylor College of Medicine

Marked circadian rhythmicities in cardiovascular physiology and pathophysiology exist. The cardiomyocyte circadian clock has recently been linked to circadian rhythms in myocardial gene expression, metabolism, and contractile function. For instance, the cardiomyocyte circadian clock is essential for the acute transcriptional response of the heart to elevated circulating fatty acids. When fatty acid availability exceeds the oxidative capacity of the myocardium, excess fatty acids spill-over into 'lipotoxic' pathways, potentially causing contractile dysfunction. These observations led us to hypothesize that disruption of the cardiomyocyte circadian clock impairs metabolic adaptation of the heart to chronic elevation of fatty acids. Wild type (WT) and cardiomyocyte-specific circadian clock mutant (CCM) mice were fed either a control or Western (high fat) diet for 16 weeks. Hearts were perfused ex vivo in the working mode for assessment of myocardial metabolism and contractile function, while a second set of hearts were used for transcriptional analysis. Western diet feeding induced anticipated alterations in myocardial metabolism (e.g. reduced carbohydrate metabolism; $p<0.01$) and impaired contractile function ($p<0.01$) for WT, but not CCM, mice. During assessment of myocardial metabolism with elevated fatty acids ex vivo (1.2mM), CCM hearts do not decrease reliance on endogenous substrate utilization (unlike WT hearts), suggesting abnormal triglyceride metabolism. Consistent with this, diurnal variations in expression of adiponutrin (adpn) and diacylglycerol acyltransferase 2 (dgat2), regulators of triglyceride metabolism, were abolished in CCM hearts ($p<0.05$). These data demonstrate that the cardiomyocyte circadian clock is essential for the metabolic adaptation of the heart to chronic elevation in fatty acids.

11:30

9 • Clock Gene Is Required for the Circadian Regulation of Plasma Lipids, Lipoproteins and Microsomal Triglyceride Transfer Protein (MTP)

Xiaoyue Pan and M. Mahmood Hussain, SUNY Downstate Medical Center

We have previously shown that plasma lipids and apoB-lipoproteins as well as tissue MTP exhibit diurnal variations (Pan and Hussain, JBC (2007) 282:24707). Here, we evaluated the role of food entrainment and Clock gene, a critical transcription factor regulating circadian rhythms, in the regulation of these changes. To examine the role of food availability, mice were fed between 0930 and 1130 h for 10 days and used to study temporal changes in plasma lipids and tissue MTP levels. These mice exhibited a major peak in plasma lipids and MTP levels coincident with the availability of food. To determine if food entrainment is affected by changes in light/dark schedules, food entrained mice were exposed to total dark or light for 5 days. Surprisingly, these animals did not show a significant change in plasma lipids and MTP at the time of food availability underscoring the importance of light entrained genes in their regulation. To evaluate the role of circadian genes, we used Clock mutant mice that express a dominant negative form of clock gene and display hyperlipidemia. The clock mutant mice did not show daily variations in plasma lipids and tissue MTP levels. The plasma levels were similar to those present in the nighttime in control mice and the nadirs observed in the daytime in mice exposed to night/day light cycle were absent in the mutant mice. Thus, Clock mutant mice lack the ability to reduce plasma lipids at dawn. These data demonstrate that light entrainment and Clock gene are required for the circadian regulation of plasma lipids and MTP. We suggest that hyperlipidemia observed in Clock mutant mice is due to their inability to reduce plasma lipids during the day and speculate that inadequate light entrainment and mutations in circadian genes may cause hyperlipidemias.

11:45

10 • Orexin: The Molecular Link between Plasma Glucose and Sleep/Wake Rhythms?

C.X. Yi[1]*, R.M. Buijs[2], M.T. Ackermans[3], H.P. Sauerwein[3], and A. Kalsbeek[1]

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The biological clock, located in the hypothalamic suprachiasmatic nuclei (SCN), uses its projections to the neuroendocrine and pre-autonomic hypothalamic neurons to control daily hormone rhythms, e.g. adrenal corticosterone and pineal melatonin release. Previously we have shown that a similar control mechanism also may exist for the daily rhythm in plasma glucose concentrations. During the transition zone from sleep to wake, glucose uptake as well as glucose production is increased. The present study aimed to understand how clock outputs can control separately, and in opposite directions, insulin sensitivity in glucose producing and glucose utilizing tissues. The GABAa receptor antagonist bicuculline (BIC) was chosen to activate two well-known suprachiasmatic nucleus target areas, i.e. the paraventricular nucleus (PVN) and the dorsomedial hypothalamus (DMH). BIC mediated activation of the neurons in the ventro-lateral DMH (vlDMH) resulted in a pronounced hyperglycemia and a strong increase in endogenous glucose production (EGP), neurons in the dorsal-medial DMH (dmDMH) and PVN responded gradually less. Immunocytochemical analysis of the BIC stimulated brains revealed a prominent activation of orexin, but not MCH, neurons in animals with vlDMH probe placements. ICV pretreatment with the orexin-1 receptor antagonist removed a major part of the increased EGP induced by BIC in the vlDMH. Together these data suggest that a withdrawal of the GABAergic inhibition of the orexin neurons at dusk not only is an important daily wake up signal but also an important mechanism to initiate the daily rise in hepatic glucose production.

12:00

11 • Linking the Development of Adiposity to Altered Circadian Rhythms

Molly Bray, Baylor College of Medicine

Research has supported a link between obesity and altered circadian rhythms (e.g., shift work, disrupted sleep) but the mechanism for this association is not known. We hypothesize that disruption of the adipocyte-specific circadian clock may contribute to the development of adiposity via altered adipocyte metabolism and/or alterations in energy balance. We, therefore, generated an adipose tissue-specific, circadian clock mutant mouse model (ACM) to address this hypothesis. The specificity of the ACM model was confirmed by quantitative RT-PCR in different tissues. In whole genome gene expression microarray experiments in visceral adipose, ACM animals showed altered expression of clock component genes, including increased bmal1 (6.5x, P<0.0002), and decreased rev-erba (3.7x, P<0.05), per3 (2.1x, P<0.05) and dbp (1.5x, P<0.01). A total of 2973 genes were differentially expressed in ACM animals compared to WT. Genes related to lipogenesis (e.g., acly, alox12, elovl4, ptgs1, prkag1) and lipolysis (e.g. adipor2, adrb2, fabp5, lipe, lpl, lypla1) were induced and repressed, respectively, in ACM mice. Pathway analysis revealed the insulin, adipocytokine, Wnt, MAPK, mTOR and JAK-STAT signaling cascades as being regulated by the adipocyte circadian clock. Both male and female ACM mice fed normal chow are significantly heavier (p<0.002) compared to wildtype animals, with weights beginning to deviate by six weeks of age, consistent with transcriptional changes. Furthermore, when challenged with high fat feeding (45% fat) for six weeks, ACM mice had significantly greater percent fat compared to wildtype animals (p=0.02). Disruption of the adipocyte-specific circadian clock appears to be associated with altered adipocyte metabolism and function.

12:15

12 • Polymorphisms in the Clock Gene Are Associated with the Metabolic Syndrome and Dyslipidaemia in Man

E.M. Scott*, A.M. Carter, K.F. Standeven, J. Surr, and P.J. Grant, Division of Cardiovascular and Diabetes Research, The Leeds Institute of Genetics Health and Therapeutics, University of Leeds, Leeds, United Kingdom

We have recently demonstrated a novel association between Clock polymorphisms and the metabolic syndrome in healthy families. The aim of the present study was to determine the association of Clock polymorphisms with the metabolic syndrome in subjects with cardiovascular disease. We performed genotype and haplotype analysis of 3 polymorphisms in the Clock gene (rs4864548, a C>T polymorphism in the Clock promoter; rs3736544, 2121G>A; rs1801260, 3111T>C) in 342 male subjects with myocardial infarction and 193 healthy male, age-matched controls. Metabolic syndrome was defined according to IDF criteria. There were no significant differences in genotype or haplotype distributions between the cases and controls. Analyses of the metabolic syndrome (MetS) and its subcomponents revealed that possession of the C allele of rs1801260 was associated with decreased prevalence of MetS (p=0.018), decreased prevalence of triglycerides above the MetS cut-point (p=0.001) and decreased prevalence of HDL below the MetS cut-point (p=0.028). Possession of the T allele of rs4864548 was associated with increased prevalence of HDL (p=0.016). Haplotype analysis indicated 3 common haplotypes: CAT, TGT and CGC (rs4864548-rs3736544-rs1801260) with frequencies of 35%, 35% and 25% respectively. The TGT haplotype was more prevalent in those with MetS (p=0.034), raised triglycerides (p=0.035) and decreased HDL (p=0.009). Conversely the CGC haplotype was less prevalent in those with MetS (p=0.023), raised triglyceride (p=0.001) and decreased HDL (p=0.008). These findings confirm and extend our previous results lending further support for our hypothesis that genetic variation in the Clock gene plays a role in the development of the metabolic syndrome in man.

11:00

13 • Circadian Changes in the Sleep Electroencephalogram (EEG) under Constant Sleep Pressure in the Rat

Roman Yasenkov and Tom Deboer*, Laboratory for Neurophysiology, Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands

Slow-wave activity (SWA) in the non-rapid eye movement NREM sleep (NREMS) EEG reflects sleep homeostasis and is a function of the previous amount of sleep and waking. In the rat, high frequency activity (HFA, EEG power density 10-25 Hz) seems to be under influence of circadian factors. We investigated whether circadian changes in HFA are independent of changes in sleep homeostasis. Rats (n=4), implanted with EEG and electromyogram electrodes, adapted to constant dark conditions for at least 1 week. A baseline day was recorded, followed by a continuous protocol of 2h sleep deprivation followed by 2 h rest (2h-2h) for 48 h. Vigilance states were scored and EEG spectral analysis was performed. The baseline day and the second day in 2h-2h were analyzed. The amount of waking over 24 h was higher in 2h-2h (56.7±1.7% SE) compared to baseline (48.5 ±1.2%, $p<0.005$, t-test). Circadian changes in vigilance states were still present but the amplitude was reduced to 25% of baseline ($p<0.05$, t-test). NREMS SWA (1.1-4.0 Hz) did not show a significant modulation over 24 h ($p>0.5$, ANOVA). The power density in the EEG spindle range (11.1-15.0 Hz) and between 15.1-25.0 Hz showed strong circadian modulation ($p<0.05$, ANOVA) virtually identical to baseline.

NREMS SWA is constant and circadian modulation in vigilance states is markedly reduced under the 2h-2h protocol. In contrast, HFA displays significant circadian modulation, indistinguishable from baseline. We conclude that circadian changes in HFA are endogenous and independent of sleep homeostatic mechanisms. Supported by EU Grant LSHM-CT-2005-518189.

11:15

14 • Light-Dark Differences in Heritability of Sleep-Wake Behaviors in the Inbred Mouse

D. Joseph Owens-Ream*, Aaron D. Laposky, Ketema N. Paul, Deanna Williams, and Fred W. Turek

The contribution of genetic factors to various traits related to the sleep-wake cycle is only beginning to be addressed using modern genetic approaches in humans and mice. To answer this question, mouse sleep-wake EEG/EMG recordings were used. To begin such approaches, the heritability of 19 traits in the light and dark periods of 115 mice from 15 separate inbred strains, with an average of 7.7 mice per strain were obtained. The sleep-wake state (i.e. wake, NREM, & REM) was determined by visual analysis for each 10-second epoch over a 48 hr time period. We used a factor analysis classification technique to place the 19 traits in four separate categories: wake/NREM duration, REM related traits, sleep/wake fragmentation, and power spectra. By calculating the trait intraclass correlation (ICC) in the light and dark periods, we determined that wake and NREM duration are heritable primarily in the dark period (ICCDark=0.59 vs. ICCLight=0.10). This is in stark contrast with the REM related traits which are primarily heritable in the light period (ICCLight=0.68 vs. ICCDark=0.32). We show here that many important sleep-wake behaviors are heavily light period dependent, and this polarity is consistent within our classification of the numerous sleep-wake indices. These highly heritable traits make for fertile ground for high resolution haplotype-based association mapping. The large phenotypic variance found between inbred strains, given their relative genetic homogeneity, indicates the presence of strongly acting sleep-wake genes.

11:30

15 • Long-term Effects of Artificial Dawn on Sleep Inertia: Does Melatonin Play a Role?

M.C. Gimenez[1]*, M. Hessels[2], M. van de Werken[1], B. de Vries[1], D.G.M. Beersma[1], M.C.M. Gordijn[1], [1]Department of Chronobiology, Center for Life Sciences, University of Groningen, The Netherlands, [2]Medical Design Engineering, Amsterdam, The Netherlands

Late chronotypes suffer from sleep inertia if they have to wake up early, e.g. on working days.

Objectives: to reduce sleep inertia complaints in people with difficulties in waking up early by means of artificial dawn and test whether this improvement is related to a shift in the melatonin rhythm.

Methods: An artificial dawn waking up alarm (Philips DAP B.V., Drachten, The Netherlands) was used by 51 subjects for 42 days (study 1) and by 41 subjects for 28 days (study 2) in their home environment. Study 1 consisted of 3 conditions of 2 weeks each; 0 lux (control), 50 lux and 250 lux. Study 2 consisted of 2 conditions; 0 lux (control) and self-selected maximal intensity. At the end of each condition, subjects collected saliva samples for melatonin assessment (study 1). Subjective ratings of sleep inertia were collected and sleep diaries completed. Results: No significant differences were found in dim light melatonin onset (DLMO) with increasing artificial dawn intensity (21:16 ± 1:04 h control, 21:25 h ± 51 min. 50lux, 21:17 h ± 54 min, 250lux). A significant reduction in sleep inertia duration (study 1, 16 min between 250 lux and control condition; study 2, 26 min) was found as well as significant improvements in several ratings of well being and sleep quality. Conclusion: The use of artificial dawn wake-up-light® has beneficial effects according to subjective ratings on sleep inertia, well being and sleep quality. However a shift of the biological clock, measured by melatonin onset, cannot explain these improvements.

11:45

16 • Sleep, Performance, and Error Rates in Anaesthetists Working Day and Night Shifts

J.F. Cheeseman*, A.F. Merry, C.S. Webster, M. Milovanovic, G.R. Warman, University of Auckland

Circadian disruption and fatigue are recognised as important safety concerns in the workplace and have been implicated as causes of drug error in anaesthesia. Our aims in this study were twofold: 1) to measure the quantity of sleep obtained by anaesthetic registrars (n=18) while working day and night shifts and 2) to measure performance of these registrars across these shifts. Sleep was measured using wrist actigraphy and sleep diaries. Performance was assessed using the Walter Reed Army Institute of Research five minute psychomotor vigilance task (PVT) and a novel task relevant drug recognition test (DRPT) to test the speed and accuracy of drug recognition. The quantity of daily sleep obtained was significantly less ($p < 0.01$) while working night shifts (mean 4:48h (SE 27 min)) than day shifts (mean 6:55 h (SE 13 min)). Less sleep while working nights corresponded with poorer performance on the DRPT. DRPT reaction time was significantly worse ($p < 0.05$, paired t-test) during night shifts (median reaction time 1345ms (SE 39ms)) than during days (median reaction time 1284ms (SE 22ms)). Many more lapses were made during the night (9.47 (SE 1.83)) than during the day (6.29 (SE 1.10)) ($p < 0.02$). The largest number of drug errors occurred at the conclusion of night shifts. Our results indicate that anaesthetists do not get as much sleep while working nights as they may need and that during these night shifts their ability to recognise drugs quickly and accurately may be compromised.

12:00

17 • Habitual Sleep Length and Subjective Perception of Seasonality Are Associated with Melatonin Deficit

Dieter Kunz, Nina Kaempfe, Georg Bohner, Randolf Klingebiel, and Richard Mahlberg, Universitätsmedizin Berlin

Although the responsiveness to photoperiod is well conserved in humans (Wehr 1993), only some 25 % of the normal human population experience seasonal changes in behavior. The aim of the study was to prove that the individual melatonin deficit marker DOC (degree of pineal calcification) is related to the lack of seasonal phenomena in humans. Out of 3000 patients in which cranial computer tomography (cCT) was performed for diagnostic reasons, the DOC score was determined (Kunz 1999) in 99 consecutive "healthy" subjects (44female, 55male; age 18-68yrs, mean35.3/SD13.4). Exclusion criteria were: pathological finding in cCT, acute/chronic illness, shift-work, alcohol/drug abuse, medication that influence melatonin excretion. The seasonal pattern questionnaire (SPAQ) was performed in a telephone interview. Twentysix subjects fulfilled criteria for seasonal affective disorder (SAD) or sub-SAD. Age was negatively and significantly correlated with seasonality only in females (r=-.41; p=.006). Overall seasonality score was negatively and significantly associated with DOC (r=-.224; p=.026). Controlling for age mean sleep length over the year was negatively and significantly correlated with DOC (r=-.372; p=.011) and even more pronounced in females (r=-.51; p=.031). Data match to an earlier study in which the length of nighttime melatonin excretion was associated with individual sleep length (Aeschbach 2003). Moreover, data prove for the first time in humans, that the lack of seasonality is associated with a reduced individual capacity to produce melatonin. Thus, because among all livings studied today humans show the most pronounced calcified pineal gland, pineal calcification may represent a human adaptation to modern life.

12:15

18 • Effects of Ramelteon on Insomnia Symptoms Induced by Rapid, Eastward Travel

Phyllis Zee, M.D., Ph.D.*; Gary Richardson, M.D.; and Sherry Wang-Weigand, M.D., Ph.D., Northwestern University Medical School

Introduction: Ramelteon, an MT1/MT2 melatonin receptor agonist, was evaluated for its ability to reduce insomnia symptoms associated with rapid, eastward travel. Methods: This randomized, double-blind, placebo-controlled study enrolled 110 healthy adults (mean age, 30.1 years) with a history of jetlag sleep disturbances. Subjects were flown eastward across 5 time zones, subjecting them to a 5-hour phase advance (Day 1). Subjects were randomized to receive ramelteon 1mg, 4mg, 8mg, or placebo 30 minutes before bedtime (local time) on the evening of Days 1, 2, 3, and 4. Sleep parameters were captured using polysomnography (PSG). The primary efficacy variable was the mean latency to persistent sleep (LPS) on Days 2, 3, and 4. Next-day residual effects were assessed using psychomotor and memory function tests. Adverse events were monitored throughout the study. Results: Compared with placebo, there was a statistically significant decrease in mean LPS with ramelteon 1mg (-10.64 min, P=0.030). Mean LPS with ramelteon 4mg and 8mg was also reduced vs placebo, although these differences did not reach statistical significance (-7.86 min, P=0.106 and -8.94 min, P=0.067, respectively). No consistent statistically significant differences were observed with ramelteon vs placebo on measures of next-day residual effects. The incidences of adverse events were similar with ramelteon and placebo. Conclusion: After a 5-hour phase advance due to eastward jet travel, ramelteon 1mg taken before bed for 4 nights significantly shortened mean LPS relative to placebo in healthy adults. All doses of ramelteon were similar to placebo on next-day effects and tolerability.

Monday, May 19

11:00 AM–12:30 PM

Slide Session D: Light Signaling

11:00

19 • Light Resetting and Entrainment in CLOCK-Drficient Mice

Robert Dallmann*, Jason P. DeBruyne, and David R. Weaver, Department of Neurobiology, University of Massachusetts Medical School

Mice lacking CLOCK protein have a relatively subtle circadian phenotype, including a slightly shorter period in DD, an advanced phase angle of entrainment in a light-dark cycle (LD) and differences in phase resetting after 4-hr light pulses in the early and late night (DeBruyne et al., 2006). Here, we more fully characterize the circadian phenotype of this line by studying Clock-/- mice under various lighting conditions. We examined the phase angle of entrainment in full LD (12L:12D), and skeleton (1L:10D:1L:12D, and 1L:23D) lighting cycles. In addition, we investigated the free-running period in constant light of various intensities, and generated a full phase-response curve (PRC) to 4-hour light pulses in free-running mice. Clock-/- mice showed an advanced phase angle of entrainment of 3–4 hours in full LD and 1L:10D:1L:12D skeleton lighting cycles, but entrained like wild-type mice to the 1L:23D cycle. Comparing entrainment under the two types of skeleton photoperiods revealed that exposure to 1 hr light in the morning leads to a phase advance of activity onset the following afternoon. In constant light, CLOCK-deficient mice did not obey Aschoff's rule, e.g., they did not lengthen period with increasing light intensity, while wild-type mice did. Clock-/- mice exhibited very large phase advances after 4-hr light pulses in the late subjective night, but had relatively normal responses to light at other phases (type 0 PRC). This abnormal response to light, restricted to a portion of the PRC, likely explains the other aspects of abnormal light responses seen in CLOCK-deficient mice.

11:15

20 • Mice Lacking the PACAP Type I Receptor Have Impaired Photic Entrainment and Negative Masking

Jens Hannibal[1,2]*, Phillip Brabet[3] and Jan Fahrenkrug[1], [1]Department of Clinical Biochemistry, Bispebjerg Hospital, Copenhagen, Denmark; [2]Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark; [3]Unité Propre de Recherche (UPR 9023) Centre National de la Recherche Scientifique, Montpellier, France

The retinohypothalamic tract (RHT) is a retinofugal neuronal pathway which in mammals mediates non-images forming light perception (NIF) to various areas in the brain involved in circadian timing, masking behavior and regulation of the pupillary light reflex. The RHT co-store the two neurotransmitters glutamate and PACAP which in a rather complex interplay are mediators of photic adjustment of the circadian system. To further characterize the role of PACAP/PAC1 receptor signalling in light entrainment of the clock and in negative masking behaviour we extended previous studies in mice lacking the PAC1 receptor by examining their phase response to single light pulses using Aschoff type II regime, their ability to entrain to non-24 h LD cycles and large phase shifts of the LD cycle (jetlag) as well as their negative masking response during different light intensities. The most prominent finding was a markedly decreased sensitivity (~80%) to light stimulation at early night as evidenced by a significantly decreased phase delay of the endogenous rhythm in PAC1 KO mice compared to wild type mice. In accordance a reduced ability to entrain to T-cycles longer than 26 h, a prolonged time to re-entrain to large phase delays and an impaired masking behaviour at lower light intensities were observed in PAC1 KO mice. The obtained data at late

night were less consistent. Our findings substantiate a role for PACAP/PAC1 receptor signalling in NIF and indicate that the system is particularly important at lower light intensities.

11:30

21 • CDK5—A Modulator of Glutamate Signaling—Determines Amplitude of Phase Shifts

J. M. Ding[1*], V. Chintalgattu[1], S. Vijayakumar[1], M. Smith1, J. Lesauter[2], A. K. Fu[3], L.C. Katwa[1], R. Silver[2], and N. Y. Ip[3] [1]Department of Physiology, East Carolina University Medical School, Greenville, NC, [2]Department of Psychology, Barnard College, New York, NY, [3]Departments of Biochemistry and Molecular Neuroscience, Hong Kong University of Science and Technology, Hong Kong, China

The SCN is synchronized to the environment through light-induced phase resetting. Light exposure during the early or late night produces phase delays or advances respectively, but induces no change in phase during the day. Cyclin-dependent kinase 5 (CDK5) is a cell signaling molecule that regulates diverse functions, such as modulating Glu neurotransmission, through the phosphorylation of a plethora of pre- and postsynaptic proteins. The CDK5 activity is regulated by two activators, p35 and p39. Accumulation of p25, the cleavage product of p35, produces hyperactivation of CDK5. Since Glu is the neurotransmitter of light entrainment, we investigated the role of CDK5 in the mouse SCN. A light pulse at ZT16 decreased the protein levels of both p25, p35, and the kinase activity of CDK5 in the SCN, while a light pulse at ZT 22 increased CDK5 activity and that of its two activators. A light pulse at ZT6 did not change CDK5 levels. When p25 was overexpressed in tetracycline-dependent transgenic mice, the magnitude of phase delays and phase advances were increased and decreased, respectively. Importantly, the magnitude of phase resetting returned to basal level when the p25 transgene was turned off by switching to the doxycycline diet. Anatomical studies showed CDK5 immunoreacticity in vasopressin-, pERK-, and GRP- containing cells and fibers and in unidentified compartments of the shell. Ultrastructural analysis indicates that the synaptic density in the SCN increases when P25 is overexpressed. These results support a role of CDK5 in the regulation of photic entrainment in the SCN.

11:45

22 • A Role For PRMT5 in The Regulation of Light Signaling and Clock Function in Arabidopsis

Sabrina Sanchez, Esteban Hernando, Matías Rugnone, Pablo Cerdán, and Marcelo Yanovsky*, Universidad de Buenos Aires y CONICET

Interlocked transcriptional feed-back loops are the basis of circadian oscillations in many organisms. In Arabidopsis these loops involve the Myb transcription factors CCA1 and LHY, and the pseudo-response regulators (PRRs) TOC1, PRR3, PRR5, PRR7 and PRR9. Using a forward genetic approach and positional cloning, we identified protein arginine methyltransferase 5 (PRMT5) as a key regulator of clock function and light signaling. prmt5 mutants show increased period length of circadian rhythms and reduced photomorphogenic responses to red, far-red and blue light suggesting that PRMT5 mediates light regulation of the circadian clock. prmt5 mutants flower very late under long and short day conditions due to an enhanced expression of the MADS box transcription factor FLC. Although FLC has been shown to regulate clock function, the flc mutation suppresses the late flowering phenotype of prmt5 but has no effect on its circadian phenotype. PRMT5 methylates histone and non-histone proteins, and regulates transcription, RNA processing, and signal transduction in many eukaryotic organisms. To further understand the mechanisms of PRTM5 action in plants we compared the prmt5 transcriptome with that of wild type plants using Affymetrix microarrays. Many more genes showed increased than decreased

expression, suggesting that PRMT5 acts as a transcriptional repressor. The gene showing the highest enhancement in expression was PRR9, a key component of the Arabidopsis circadian oscillator, whose expression is also acutely regulated by light. Thus, our results uncover a role for arginine methylation in clock regulation in Arabidopsis, presumably through the direct or indirect regulation of the PRR9 clock gene.

12:00

23 • Photoperiodic Flowering Occurs under the External and Internal Coincidence Mechanisms in Arabidopsis

Takato Imaizumi*, Mariko Sawa, Dmitri A. Nusinow, and Steve A. Kay, University of California–San Diego

To explain the mechanisms underlying photoperiodic flowering, the "external coincidence model" is currently the most consistent with the genetic evidence. In the long-day plant Arabidopsis, at least two circadian-clock regulated processes, both of which could be explained by that model, play important roles in day-length measurement. The first process is FLAVIN BINDING, KELCH REPEAT, F-BOX 1 (FKF1)-dependent daytime CONSTANS (CO) gene expression. FKF1 is expressed in the late afternoon and it seems that only when FKF1 expression coincides with light is the daytime CO expressed. The second process is CO-dependent FLOWERING LOCUS T (FT) gene induction. Because of the first mechanism, daytime CO expression occurs in long days and CO protein is activated by light to induce FT gene expression. Because FT protein induces floral identity gene expression, it is likely that with these two mechanisms plants ensure that FT expression occurs under appropriate conditions. We demonstrated that FKF1 protein absorbs blue-light and forms a complex with GIGANTEA (GI) protein, which is another activator of CO transcription. Our results suggest that the timing of FKF1 and GI complex formation determines the timing of daytime CO expression. Both FKF1 and GI are circadian clock-regulated genes. The peak expression of both proteins coincides in long days, but not in short days. Therefore, our results indicate that differentially-regulated internal timing of both FKF1 and GI expression coincide under long day conditions to maximize the induction of daytime CO expression, which is crucial for day-length sensing mechanisms.

12:15

24 • XAP5 Circadian Timekeeper Coordinates Light Signals to Properly Time the Circadian Clock and Photomorphogenesis in Arabidopsis

Ellen L. Martin-Tryon and Stacey L. Harmer*, University of California–Davis

Numerous, varied, and widespread taxa have an internal circadian clock which allows anticipation of rhythmic changes in the environment. We have identified XAP5 CIRCADIAN TIMEKEEPER (XCT), an Arabidopsis gene important for light regulation of the circadian clock and photomorphogenesis. XCT is essential for proper clock function: xct mutants display a shortened circadian period in all conditions tested. Interestingly, XCT plays opposite roles in plant responses to light depending both on trait and wavelength. The clock in xct plants is hypersensitive to red but shows normal responses to blue light. In contrast, inhibition of hypocotyl elongation in xct is hyposensitive to red light but hypersensitive to blue light. Finally, XCT is important for Rubisco production and plant greening in response to light. This novel combination of phenotypes suggests XCT may have a global role in coordinating growth in response to the light environment. XCT contains a XAP5 domain and is well-conserved across diverse taxa, suggesting it has a common function in higher eukaryotes. Knockdown of expression in *C. elegans* causes lethality, suggesting that studies in Arabidopsis may be instrumental to understanding the biochemical activity of XCT.

11:00

25 • The Functional Neuroanatomy of the PDF Receptor Reveals Output Circuits in the Circadian Pacemaker Network

Luoying Zhang*, Bridget C. Lear, and Ravi Allada, Department of Neurobiology and Physiology, Northwestern University

Previous work has characterized two groups of pacemaker neurons, morning cells (M-cells) and evening cells (E-cells) in the Drosophila brain, which are required for anticipation of lights-on and lights-off, respectively. M-cells release the neuropeptide pigment dispersing factor (PDF), which is required for synchronizing and maintaining molecular rhythms in the pacemaker neurons, as well as driving morning anticipatory activities and maintaining robust locomotor rhythms in constant darkness (DD). Functional clocks in M-cells have been demonstrated to be necessary and sufficient for robust locomotor rhythms in DD. In the present study, we performed tissue-specific rescue of PDF receptor (PDFR) mutant flies and surprisingly, found that PDFR expression in E-cells is necessary and sufficient for rescuing morning anticipation. Immunostaining analysis of the molecular rhythms as well as previous work suggest that PDF signaling is probably not exerting effects on the molecular clock in E-cells to regulate morning anticipation under light-dark conditions (LD). We also demonstrate that PDFR expression in M-cells is necessary but not sufficient for maintaining robust rhythms in DD. We propose that PDFR signaling among PDF+ M cells synchronizes circadian clocks, while action in the E cells acts as an output circuit for morning anticipation. These studies functionally define the direct output circuits for pacemaker neurons in Drosophila circadian behavior.

11:15

26 • Entrainment and Phasing of the Morning and Evening Oscillators in Drosophila : A New Function for the PDF Neuropeptide

P. Cusumano, A. Klarsfeld, E. Chélot, M. Picot, B. Richier, and F. Rouyer*, Institut de Neurobiologie Alfred Fessard, CNRS UPR2216, Gif-sur-Yvette, France

Rest-activity rhythms in Drosophila are controlled by a multioscillators brain clock that perceives light through the rhodopsins of the visual system and the blue light photoreceptor cryptochrome. The behavioral brain clock relies on about 150 PER-expressing neurons, including dorsal and lateral neurons (LNs). The bimodal activity of the flies in Light-Dark (LD) conditions is controlled by at least two oscillators: the pigment-dispersing factor (PDF) -expressing LNs control the morning activity, whereas the PDF-negative LNs control the evening activity. Interestingly, light activates the output of the evening oscillator and inhibits the output of the morning one. We show here that both morning and evening LN-oscillators are autonomous and can use cryptochrome or the visual system to synchronize with the LD cycles. However, the evening oscillator is more sensitive to light, suggesting that it plays a major role in the entrainment of the brain clock. Finally, we show that pdf0 mutants display an antiphasic evening oscillator in the absence of cryptochrome and a normal phase in the absence of the visual system. This indicates that the PDF neuropeptide controls the phase of the evening oscillator, but not the morning one, through the visual system entrainment pathway. The mechanisms of this new PDF function are under investigation.

11:30

27 • Lateral Ventral Neurons Determine Relative Diurnal/Nocturnal Behavior in Drosophila

Sheeba Vasu, Keri Fogle, Maki Kaneko, Yu-Ting Chou, Saima Rashid, Vijay K. Sharma, and Todd C. Holmes*, University of California–Irvine

We have shown previously that acute light response and circadian regulation promotes higher spontaneous action potential firing rate of the large subset of lateral ventral neurons (LNv) during day versus night in Drosophila. To determine whether neuronal activity in the LNv regulates nocturnal versus diurnal behavioral activity levels, we screened the effects of electrical hyperexcitation in the LNv versus other neuronal subsets of the circuit by targeted expression of the low-threshold voltage-gated sodium channel (NaChBac). NaChBac expression selectively enhances nocturnal locomotor behavior over a wide range of alternating light-dark photoperiods only when expressed in neuronal subsets that include the LNv. Correspondingly, nocturnal sleep is less robust in NaChBac flies. Enhanced nocturnal behavior is seen with multiple NaChBac lines hyperexciting the LNv and the large LNv are sufficient for this PDF-dependent effect. Furthermore, the evening activity peak of the NaChBac flies tracks lights-OFF when the flies are subjected to progressively falling day lengths, a characteristic feature of nocturnal animals. NaChBac-expression in the LNv tends to accelerate PER degradation in the other clock neurons. To determine whether the small LNv are necessary for hyperexcitation-driven nocturnal behavior, we concurrently electrically hyperexcited the large LNv while specifically ablating the small LNv. These flies exhibit robust increases in nocturnal behavior and hyperexcitation of large LNv action potential firing, thus the small LNv are not an essential circuit component for large LNv-determined nocturnal behavior. In summary, the electrical activity of LNv subset of the circadian pacemaker circuit strongly determines diurnal and nocturnal behavior in Drosophila.

11:45

28 • A Function for the Large PDF Cells in Drosophila Arousal

Yuhua Shang and Michael Rosbash, Howard Hughes Medical Institute and National Center for Behavioral Genomics, Department of Biology, Brandeis University

Light stimulates Drosophila activity as well as that of other diurnal animals, but the relevant arousal pathways within the adult fly brain remain largely unknown. Consistent with the notion that peptidergic neurons may be relevant to arousal [(1)], constant firing of a broad set of peptidergic neurons with the traditional Gal4-UAS/Gal80 system caused high levels of nighttime activity. The expression pattern of this Gal4 driver includes the 5 PDF-containing large ventral lateral neurons (l-LNvs) of the circadian system. These cells have recently been shown to be light-sensitive [(2)], but they have never been assigned a circadian or a behavioral function despite being among the first circadian neurons identified in flies. We first show that hyperexciting the large LNvs is necessary for much of the early nighttime activity caused by hyperexcitation of the broader peptidergic cell population. We then combined three expression systems: Gal4-UAS/Gal80, LexA-LexAop, and the FLP/FRT technique (3-5) to develop a novel mosaic technique that achieves single or few neuron resolution, in this case specifically within the l-LNv population. The approach allowed us to alter the activity of a small subset of the l-LNvs and to simultaneously label only these cells. It showed that hyperexcited l-LNvs are sufficient to promote night-time activity without light-stimulation. The data indicate that the l-LNvs directly promote locomotor activity in response to light stimulation and also suggest a central brain pathway that involves other circadian cells.

12:00

29 • VIP Synchronizes Circadian Rhythms in Astrocytes

Luciano "flaco" Marpegan*, T.J. "plugin" Krall, and Erik D. Herzog, Washington University in St. Louis

Single cell circadian oscillators have been described for many cell types. Astrocyte primary cultures were previously shown to display damped circadian oscillations that can be sustained when co-cultured with adult SCN explants. It is not known if individual astrocytes are sustained circadian oscillators or if they communicate timing information to each other. Using low light imaging and photomultiplier tubes, we further characterized circadian oscillations of bioluminescent astrocytes both at the population and single cell levels. We found that individual astrocytes expressed low amplitude, unstable, and damped oscillations in Period2-luciferase activity and were unable to synchronize to each other. We next studied vasoactive intestinal peptide (VIP), a potent coupling factor for SCN neurons, as a candidate neuron-to-astrocyte circadian effector. We found that VIP (200nM) restored rhythmicity in damped cultures. In non-damped cultures, VIP induced an amplitude increase and a type 0 phase response curve with little or no shifts around circadian time 5 and a switch from large delays to large advances (-12 to 12 hrs) around CT 14. We confirmed expression of the VPAC2 VIP receptor by real time rtPCR in both astrocytes and SCN. VPAC2 mRNA abundance was rhythmic in the SCN and arrhythmic in astrocytes, suggesting that changes in VPAC2 expression does not mediate the variable effects of VIP at different time points. Taken together our results indicate that astrocytes are damped oscillators that can be entrained by neuronal derived signals and that VIP is a good candidate as a neuron-to-astrocyte entrainment factor. Supported by NSF and NIH grants.

12:15

30 • An Ensemble of Novel Circadian Oscillators in the Mediobasal Hypothalamus

Clare Guilding*, Alun T. Hughes, Timothy M. Brown, Sara Namvar, and Hugh D. Piggins, The University of Manchester

The mediobasal hypothalamus (MBH) is pivotal for optimal internal homeostatic regulation, and is reciprocally connected with the suprachiasmatic nuclei (SCN), site of the master circadian pacemaker. Core circadian clock genes/proteins such as per2/PER2 are expressed in the MBH, but it is unclear if circadian timekeepers are present here. In this study, we use longitudinal electrophysiological and PER2::LUC bioluminescence recordings as well as visualisation of PER2 bioluminescence activity in vitro in acute adult brain slice cultures, to investigate circadian oscillators in the mouse MBH. Circadian rhythms in spontaneous discharge activity persist at both the population and single cell level in the arcuate (Arc) and dorsomedial (DMH) but to a much lesser extent in the ventromedial (VMH) nuclei; while circadian rhythms in PER2::LUC are visualized in the median eminence/pars tuberalis (ME/PT), Arc, DMH, and ependymal cell layer of the third ventricle, but not in the VMH. PER2 rhythms are resolved in individual cells of the Arc and DMH. Continued rhythms of PER2::LUC expression from microdissected Arc/ME/PT and DMH cultures indicate that rhythms in each nucleus are not dependent on interconnections preserved in the whole slice culture, nor are they dependent on action potential generation since they persist on blockage of TTX-sensitive sodium channels. This is the first demonstration of the intrinsic capacity of multiple extra-SCN hypothalamic regions to display autonomous circadian pacemaker activity in both clock gene expression and electrical activity. Collectively, these results establish for the first time that a hierarchical ensemble of semiautonomous and slave circadian oscillators are present in the MBH.

11:00

31 • Clock Control

Loning Fu, Baylor College of Medicine

The tumor suppressor p53 is the most commonly mutated or deregulated gene among all human cancers. It plays a key role in suppressing myc oncogenic activation. Loss of function in p53 in combination with myc over-expression is sufficient for lymphomagenesis in mice. We have shown previously that myc expression is controlled by peripheral clocks as well as the central clock through the sympathetic nervous system (SNS) in vivo. Loss of function in the peripheral clocks leads to uncontrolled myc expression, increased cell cycle clock activity and neoplastic growth in mice. We found recently that the expression of p53 is also controlled by the SNS signaling and the peripheral clocks, and follows a circadian rhythm that is coupled with the myc expression and the activity of the peripheral clock in all mouse tissues studied. Loss of function in both Per and p53 genes leads to myc oncogenic signaling and a further increase in tumor development in mice. Our studies indicate that the clock-controlled p53 expression could be one of the key mechanisms coupling tumor suppression with our daily behavioral and physiological rhythms by preventing myc oncogenic activation.

11:15

32 • Oscillator-Independent Control of Cell Cycle Progression by Circadian Clock Proteins

Eugin Destici*, Malgorzata Oklejewicz and Gijsbertus van der Horst, Department of Genetics, Erasmus University Medical Center CA Rotterdam, The Netherlands

The circadian clock controls a variety of cellular processes. Recently, particular attention has been given to the connection between the circadian and cell cycle systems. It is often suggested that the clock controls the cell cycle by a cell-autonomous gating mechanism. To test this hypothesis, we used primary wild type cell lines and cell lines lacking all or some mCry alleles. Cells completely lacking mCry genes display an increased proliferation rate, suggesting they indeed have a deregulated cell cycle. However, experiments with cells containing either one allele for mCry1 or one for mCry2 suggest that mCry genes may circadian clock independently contribute to cell cycle control. Instead, we show that proliferation in these cells correlates with mCLOCK/mBMAL1 transcriptional activity, suggesting that the observed cell cycle deregulation is more a consequence of altered expression of mCLOCK/mBMAL1 target genes (identified by microarrays) rather than due to a lack of gating. This challenges the above hypothesis.

To determine whether cell cycle deregulation in the absence of functional mCry genes also extends to conditions of stress, we exposed mCry1/mCry2 double knockout cells to different genotoxic agents. We did not observe differences between wild type and mutant cells using several assays, in contrast to results obtained in vivo by others. This suggests that in vivo the clock might contribute to cell cycle control through a non-cell-autonomous mechanism. We are currently investigating this possibility in more detail.

11:30

33 • Opposite Actions of Hypothalamic Vasopressin on Circadian Corticosterone Rhythm in Nocturnal versus Diurnal Species

A. Kalsbeek*, L.A.W. Verhagen, B. Bothorel, R.M. Buijs and P. Pévet, [1]Neth Inst Neurosci, Hypothalamic Integration Mechanisms, Amsterdam, The Netherlands; [2]Université Louis Pasteur, Dept.Neurobiol Rythmes,UMR CNRS-ULP 7518, IFR 37, 7168 Strasbourg, France; [3]Instituto Invest Biomedicas UNAM, Ciudad Universitaria, Mexico

Relatively little is known about the function of the biological clock and its efferent pathways in diurnal species. Several lines of evidence suggest that the activity cycle of the biological clock (SCN) itself is similar in nocturnal and diurnal mammals. Previously, we showed that, in the rat, vasopressin (VP) derived from the SCN has a strong inhibitory effect on the release of adrenal corticosterone and is an important component in the generation of a daily rhythm in plasma corticosterone concentrations. In the present study we investigated the role of VP in the control of the daily corticosterone rhythm in a diurnal rodent, i.e. Arvicanthis ansorgei. Contrary to our previous (rat) results, VP administered to the hypothalamic PVN in A. ansorgei had a stimulatory effect on the release of corticosterone. Moreover, both the morning and evening rise in corticosterone were blocked by the administration of a VP receptor antagonist. These results show that with regard to the daily corticosterone rhythm in diurnal and nocturnal rodents, temporal information is carried along the same pathway from the SCN to its target areas, but the response of the target area may be quite different. We propose that the reversed response to VP is due to a change in the phenotype of the target neurons that are contacted by the SCN efferents, i.e. glutamatergic instead of GABAergic.

11:45

34 • Robust Adverse Effects of Chronic Circadian Desynchronization in Animals under a Physiological "Challenge"

Fabian Preuss[1]*, Yueming Tang[2], AliKeshavarzian[2], Fred W. Turek[1], [1]Department of Neurobiology and Physiology, Northwestern University; [2]Rush University Medical Center, Department of Medicine, Division of Digestive Disease and Nutrition

A number of adverse mental and physiological effects have been associated with shift work in humans, leading to the general hypothesis, that chronic disruption of circadian rhythms has significant adverse effects on health. However, despite a few sporadic reports of decreased longevity in rodents exposed to long-term constant changes in the light dark (LD) cycle, and a recent report that chronic LD phase advances in aged mice increase mortality, there is very little evidence in animal models that continuous disruption of normal circadian temporal organization is in fact detrimental to the health of the organism. Here we demonstrate the effects of long term phase shifting on body weight and intestinal physiology. Mice exposed to a 12L:12D regime phase shifted by 12 hours every 5th day for 90 days showed no difference in body weight (25.6±.5g phase-shifted versus 26.0±.7g control). However, when additionally challenged with Dextran Sodium Sulfate (DSS) inducing colitis (7 day 2%DSS treatment plus 3 days of recovery), phase shifting led to a drastic increase in the progression of the induced disease state: more rapid ($p=0.006$) and increased weight loss (21%±3.6 versus 12%±2.7,$p=0.044$). Tissue analysis reveled increased Myeloperoxidase activity (178±17 vs 121±6, $p=0.011$) suggesting increased infiltration of neutrophils (inflammation) and increased histopathological damage (15.1±1 vs 10.0±1.2, $p=0.012$) in multiple regions of the colon of shifted animals. These results suggest that animals, and most likely humans, seem to tolerate phase shifts without direct health issues for extended periods of time, however it appears to leave the organism overall more vulnerable.

12:00

35 • Scheduled Exposures to a Novel Environment with a Running-Wheel Differentially Accelerate Re-entrainment of Mice Peripheral Clocks to New Light-Dark Cycles

Yujiro Yamanaka*, Sato Honma, Ken-ichi Honma, Hokkaido University Graduate School of Medicine

Effects of scheduled exposures to a novel environment with a running-wheel were examined on re-entrainment of mouse circadian rhythms to 8 hour phase-advance or -delay shifts of light-dark (LD) cycles. We measured spontaneous locomotor activity and Per1-luciferase (luc) activity in the cultured suprachiasmatic nucleus (SCN) and peripheral tissues. Wild type and Per1-luc transgenic mice were exposed to the novel environment by transferring them to a new cage with a running-wheel for 3 hours from the onset of shifted dark period. After 4 exposures, they were released into constant darkness (DD) to evaluate phase-shifts of the circadian rhythms. The control animals were treated with the same procedure except for cage exchange. In the phase-advance shift, behavioral rhythms fully re-entrained to new LD cycle in the exposed group, whereas in transients in the control. The Per1-luc rhythms in the SCN almost completely re-entrained in both control and exposed groups. In the skeletal muscle and lung, the rhythms fully re-entrained only in the exposed group, whereas neither group showed phase-shifts in the liver. In the phase-delay shift, the circadian rhythms in behavior and Per1-luc almost completely re-entrained in both groups. These findings indicate that the scheduled exposures to a novel environment with a running-wheel differentially accelerate re-entrainment of the mouse circadian rhythms to 8 hour phase-shifted LD cycles, which is depended on tissues and direction of phase-shifts.

12:15

36 • Chemosensory Rhythms in Drosophila

Abhishek Chatterjee*, Parthasarathy Krishnan*, Paul Hardin*, Texas A&M University

We previously showed that circadian oscillators in olfactory sensory neurons (OSNs) are necessary and sufficient to sustain rhythms in electroanntenogram (EAG) responses. To understand the cellular and molecular mechanisms that drive these rhythms, we measured single unit activity from different classes of antennal sensillae in wild-type, clock mutant, odorant receptor (OR) mutant, and G-protein coupled receptor 2 (gprk2) mutant flies. The amplitude of spontaneous spikes, but not the frequency of spontaneous or odor induced firing, is under clock control in food odor-tuned ab1 and ab3 basiconic sensillae and pheromone-tuned T2 trichoid sensillae. Mutants that fail to localize ORs to dendrites display constant low spike amplitudes, and reduced or increased levels of GPRK2 in OSNs result in constant low or high spontaneous spike amplitudes, respectively. The circadian control of spike amplitude persists in OSNs even in the absence of odorants, indicating that the clock modulates basic properties of the OSN membrane. To determine whether clock control extends to other sensory systems, we assayed electrophysiological responses within the gustatory system. Tastant-evoked spikes from labellar gustatory receptor neurons show a robust circadian rhythm in spike amplitude. This rhythm in gustatory responses parallels behavioral rhythms in proboscis extension reflex. These results reveal that the clock controls chemosensation, thus laying the groundwork for future studies of the regulation and function of chemosensory rhythms.

Slide Session G: Molecular Clocks II: Post-Translation

11:00

37 • Evolutionarily Conserved Features of Vertebrate Cki Delta and Drosophila Dbt in the Circadian Mechanism

Jin-Yuan Fan, Michael J. Muskus, Fabian Preuss, and Jeffrey L. Price*, Division of Molecular Biology and Biochemistry, School of Biological Sciences, University of Missouri

While vertebrate CKI epsilon/delta's and their Drosophila ortholog DBT are involved in the circadian mechanism, the extent of evolutionary conservation of their circadian function has not been clear. The N-terminal catalytic domains of these casein kinase I isoforms are highly conserved (~85% amino acid identity), but the C terminal domains diverge significantly. Moreover, their circadian targets may not be entirely the same. The evolutionary conservation of their circadian function was investigated by expressing various forms of vertebrate CKI delta and fly DBT in fly circadian cells with the GAL4>UAS binary expression approach. Overexpression of wild type CKI or of wild type Drosophila DBT produced only a small increase in circadian period. Mutations which produced short-period circadian rhythms in the organism of origin included the dbtS (isolated in Drosophila) and the tau (isolated in Syrian hamster) mutations, and overexpression of CKI or Drosophila DBT carrying either of these mutations produced comparable period-shortening in flies. Intriguingly, CKI or DBT carrying the tau mutation altered the rhythm of PER phosphorylation, as assessed by PER mobility shifts on SDS-PAGE, differently from CKI or DBT carrying the dbtS mutation, suggesting that the dbtS and tau mutations shorten period differently. CKI carrying the dbtL mutation did not lengthen period as did DBTL, but a CKI carrying another mutation reducing kinase activity did lengthen circadian period. The results imply a high degree of evolutionary conservation for the vertebrate CKI and Drosophila DBT, and for the mechanisms that are affected by the dbtS and tau mutations.

11:15

38 • DBT-Directed Phosphorylation of the Drosophila Period Protein: Mapping and Functional Analysis

Lino Saez*, Saul Kivimäe, and Michael W. Young, Laboratory of Genetics, The Rockefeller University

Protein phosphorylation plays an essential role in the generation of circadian rhythms, regulating the stability, activity and subcellular localization of certain proteins that constitute the biological clock. This study examines the role of the protein kinase Doubletime (DBT), a Drosophila ortholog of casein kinase I?/?. An enzymatically active DBT protein is shown to directly phosphorylate the Drosophila clock protein Period (PER). Several DBT-dependent phosphorylation sites are identified within PER, and their functional significance is assessed in a cell culture system and in vivo. The perS mutation, which is associated with short-period (19h) circadian rhythms, alters one of the identified phosphorylation sites. Inspection of this and neighboring sequence variants indicated that phosphorylation regulates PER activity in an integrated fashion that involves two sequence motifs in the PER protein that are preferentially phosphorylated by DBT.

11:30

39 • The Role of Casein Kinase 1 in Circadian Timing in Mammals

Qing-Jun Meng*, Liz Maywood, Wei-Qun Lu, Jian Li, Martin Sladek[1], Michael Hastings[1], and Andrew S. I. Loudon, Faculty of Life Sciences, University of Manchester, [1]Division of Neurobiology, MRC Laboratory of Molecular Biology, Cambridge

Post-translational modifications of key clock proteins are essential regulators of the biological clock. Phosphorylation by Casein kinase 1 is a key step in the degradation pathway of mammalian PER proteins. Our recent studies suggest that wild-type CK1ε selectively targets PERIOD proteins and not CRYPTOCHROMES for degradation. To further address the relative role of CK1ε and δ, we isolated primary fibroblasts and organotypic tissue slices from different CK1ε mutant mice on a PER2::LUC background. PMT photon counting revealed that both SCN and lung fibroblasts from the CK1ε -/- mice demonstrated robust rhythmicity, with a slightly longer period than in WT tissues. The CK1 inhibitor (PF670) is known to be highly selective for both CK1δ and CK1ε. PF670 dose-dependently lengthened circadian period up to 32h in SCN, lung and fibroblasts from WT mice, and also in transcriptional oscillations of Rat-1 cells stably transfected with clock gene reporters. CK1 inhibition also significantly lengthened period in cells and tissues derived from tau mutant and CK1ε -/- mice. A prediction for action of PF670 is that PERIOD protein degradation will be slowed by CK1 inhibition. We confirmed this in COS-7 cells that were transfected with PER2::YFP, and pre-treated with cycloheximide. Here, we observed a significant dose-dependent reduction in PER2 degradation, as revealed by real-time fluorescence video microscopy, demonstrating that CK1 plays a central role in targeting PER2 for degradation in mammals. Overall, our data demonstrate that inhibition of CK1 leads to lengthening of circadian period and reduced PER2 protein degradation, and that these effects occur in CK1ε knock-out cells and tissues. Our current studies aim to define relative contribution of CK1ε and CK1δ in circadian timing.

11:45

40 • Identification of Chemical Compounds Capable of Tuning the Mammalian Circadian Clock

Zheng Chen[1]*, Seung-Hee Yoo[2,3], David Ferster[2], Joseph S. Takahash[1,2,3], and Steven L. McKnight[1], [1]Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX [2]Department of Neurobiology and Physiology, Northwestern University [3]Howard Hughes Medical Institute and Center for Functional Genomics

Living organisms exhibit circadian rhythms of gene expression, metabolic processes and behavioral activities. Evidence has been accumulating in recent years supportive of the notion that the circadian cycle is fundamentally a metabolic cycle. We hypothesize that small chemical compounds could mimic endogenous metabolites to modulate the circadian clock. These compounds might serve as biological tools to probe the circadian clock or as therapeutic agents to correct dysfunctional circadian clocks associated with diseases. To identify such compounds, we have undertaken a high-throughput chemical compound screen using a cell-based assay. Fibroblast cells derived from Per2:luciferase knockin mice were treated with compounds in 384-well plates and luminescence was continuously monitored for several days. Alteration in period, phase and amplitude was determined and hit compounds were selected in the primary screen. Follow-up validation was conducted to determine dose response, tissue and animal efficacy. Results will be presented on representative compounds capable of modulating the circadian clock. We will discuss potential research and clinical applications of these clock-tuning compounds.

12:00

41 • Post-transcriptional and Post-translational Regulation of the Neurospora Circadian Clock

Yi Liu, Guocun Huang, Jinhu Guo, and Joonseok Cha, Department of Physiology, The University of Texas Southwestern Medical Center

FREQUENCY (FRQ), the FRQ-interacting RNA helicase (FRH), WHITE COLLAR-1 (WC-1) and WC-2 form the core circadian negative feedback loop in Neurospora. Both posttranscriptional and post-translational mechanisms play critical roles in clock functions. In the circadian negative feedback loop, we showed that the FRQ-FRH complex inhibits WHITE COLLAR (WC) complex activity by recruiting the casein kinases CKI and CKII to phosphorylate the WC proteins, resulting in the repression of frq transcription. In addition, CKI and CKII phosphorylate FRQ to promote FRQ degradation, a process that is a major determinant of circadian period length. Furthermore, we demonstrated that PKA is another important clock component by regulating several critical processes in the circadian negative feedback. First, PKA and the casein kinases mediate sequential phosphorylation of WC-1 with PKA as a priming kinase. Second, PKA phosphorylates and stabilizes FRQ, a role that opposes the function of CKI and CKII in regulating FRQ stability. In addition to FRH's role in the circadian negative feedback loop, it is also a co-factor of exosome, a multisubunit protein complex important for RNA processing and degradation. We show that the FRQ-FRH complex is associated with frq mRNA and the knock-down of FRH expression leads to high frq mRNA levels, the result of impaired frq mRNA degradation. Our results further suggest that FRH recruits frq mRNA to the exosome for its degradation and the impaired function of exosome causes aberrant circadian phenotypes. Together, these results suggest that the rhythmic FRQ-FRH complex plays an important role in the posttranscriptional regulation of the Neurospora circadian clock.

12:15

42 • Saturation Mutagenesis Screen of CRYPTOCHROME Proteins Reveals Novel PER Binding Domain Common to Both Crys and a CRY2-Specific Repression Domain

Ellena A. van der Schalie[1]*, Julie Baggs[2], Jeanne Geskes[2], John Hogenesch[2], and Carla B. Green[1], [1]Department of Biology, University of Virginia, [2]Department of Pharmacology, Institute for Translational Medicine and Therapeutics, University of Pennsylvania School of Medicine

Cryptochrome (CRY) plays an integral role in the negative feedback loop of the circadian clock by repressing the CLOCK:BMAL1 heterodimer, leading to down-regulation of Period and Cryptochrome genes. Although CRY1 and CRY2 are similar in sequence, Cry1-/- mice exhibit a short period locomotor phenotype, while Cry2-/- exhibit a long period (van der Horst et al 1999; Vitaterna et al 1999). In order to elucidate the molecular mechanisms underlying common and differential roles of the CRY proteins in the molecular circadian clock, saturation mutagenesis was used to generate mutant libraries of the mCry1 and mCry2 genes, which were then screened in a high-throughput cell-based functional assay to isolate mutants deficient in repression of CLOCK:BMAL1-mediated transcriptional activation. The mutants were then sequenced and the impact of these mutations on the primary amino acid sequence was determined. The resulting amino acid changes were located in regions that have not been previously described. The mutants were put through a battery of secondary screens testing protein expression levels, subcellular localization, and effects on a functional molecular clock. Further analysis of a cluster of residues that were hit in the CRY2 screen, but not in the CRY1 screen revealed two residues important for PER1 and PER2 binding to both CRYs, thereby elucidating a PER-binding domain in the CRY proteins. In addition, two other residues in this cluster were important for repression only in CRY2, suggesting that CRY1 and CRY2 may achieve repression through different mechanisms.

11:00

43 • Importance of the Transcription Factors CLOCK and BMAL1 in Central and Peripheral Circadian Oscillators

Caroline H. Ko[1,6*], Ethan D. Buhr[1], David K. Welsh[3,4], Richard Yamada[5], Martin R. Ralph[6], Daniel Forger[5], Steve A. Kay[3], Joseph S. Takahashi[1,2]; [1]Department of Neurobiology and Physiology; [2]Howard Hughes Medical Institute, Northwestern University, Evanston, IL; [3]Department of Cell and Developmental Biology, [4]Department of Psychiatry, University of California–San Diego, La Jolla, CA; [5]Department of Mathematics, University of Michigan, Ann Arbor, MI; [6]Department of Psychology and Zoology, Center for Biological Timing and Cognition, University of Toronto, ON, Canada

CLOCK and BMAL1 are transcription factors that are central to the maintenance of mammalian circadian rhythmicity. Genetic alterations of these transcription factors result in a defective circadian clock: Clock?19-mutant mice display longer (~4hrs) behavioral rhythms that become arrhythmic with prolonged exposure to constant darkness; Bmal1-/- mice display an immediate and complete loss of circadian behavioral rhythms in constant darkness. The peripheral oscillators of Clock-/- mice are arrhythmic (DeBruyne et al., Curr Biol, 2007); although, surprisingly, these mice exhibit normal, rhythmic behavior (DeBruyne et al., Neuron, 2006). Previously, we have shown that peripheral tissues express self-sustained circadian rhythms in the absence of the SCN (Yoo et al., PNAS, 2004); and that deletion of clock components may have different effects on central versus peripheral oscillators, owing to the intercellular coupling ability of SCN cell-autonomous oscillators (Liu et al., Cell, 2007). These studies highlight fundamental differences in the clock machinery and the relative contributions of clock genes in different tissues. To understand further the dynamics of positive clock components in central versus peripheral oscillators, we monitored real-time circadian expression of PERIOD2::LUCIFERASE bioluminescence in the SCN and various peripheral organ explants from wild-type, Clock?19-mutant, and Bmal1-/- mice. Preliminary results suggest that the central circadian pacemaker, the SCN, is comprised of a specialized tissue network to produce rhythmic signals; and that CLOCK and BMAL1 are indispensable in peripheral oscillators.

11:15

44 • Diurnal Variation in Myocardial Ischemia/Reperfusion Tolerance; Mediation by the Circadian Clock within the Cardiomyocyte

David J. Durgan*, Martin E. Young

Circadian rhythms in cardiovascular physiology (e.g. blood pressure and heart rate) and pathophysiology (e.g. myocardial infarction (MI)) exist. Humans exhibit a marked increase in MI frequency during the early hours of the morning. However, MIs occurring during the evening are more likely to result in subsequent heart failure (i.e. decreased tolerance). These phenomena have largely been attributed to rhythms in neurohumoral factors. Recent studies suggest a role for intracellular circadian clocks in modulating cardiovascular function. Therefore, we hypothesized that the cardiomyocyte circadian clock regulates tolerance of the heart to ischemia/reperfusion (I/R). Wild-type (WT) and cardiomyocyte-specific clock mutant (CCM) mice were subjected to 45 minutes of ischemia (through left anterior descending coronary artery occlusion) followed by reperfusion. The closed chest model was utilized to minimize acute influence of an immune response. I/R and sham interventions were performed either at ZT0, 6, 12 or 18. 24-hours following I/R or sham intervention, hearts were isolated and infarct size (i.e. region of nonviable tissue) measured. A marked diurnal variation in I/R-induced infarct size was observed in WT hearts (p=0.037),

with a 3.8-fold greater infarct size at ZT12 (peak) versus ZT0 (trough; p=0.024). This diurnal variation was absent in CCM hearts, which exhibit lower infarct sizes relative to WT hearts, independent of time (p=0.035). For example, at ZT12 infarct size in WT hearts was 5.3-fold greater than that of CCM hearts (p=0.034). These data reveal a diurnal variation in myocardial I/R tolerance that is mediated by the circadian clock within the cardiomyocyte.

11:30

45 • Circadian Activation of the Calcium-Activated Protein Phosphatase Calcineurin in the Heart and Beyond

Nita Sachan, Asim Dey, Joseph A. Hill, and Beverly A. Rothermel*, University of Texas Southwestern Medical Center

Calcineurin, a calcium-activated protein phosphatase, promotes cardiac hypertrophy and failure. In this study, we show for the first time that there are robust circadian oscillations in calcineurin-dependent activities in normal hearts. Calcineurin-dependent transcription and nuclear occupancy of the transcription factor NFAT regularly fluctuated as much as 20-fold over the course of a day. Phosphorylation of the protein phosphatase 1 inhibitor 1 (I-1), a direct calcineurin substrate, and phospholamban (PLB), an indirect target oscillated directly out of phase with calcineurin-dependent signaling. We propose that daily oscillations in calcineurin activity and phosphorylation of regulatory proteins form interdependent feedback loops helping coordinate cardiac function. At a time of day when cardiac demand is greatest, temporal regulation of calcineurin activity prevents calcineurin from antagonizing phosphorylation events that promote contractility. In failing mouse hearts, calcineurin oscillations persisted, however the peak and trough activities were elevated above peak activities seen in control hearts. Together, our results suggest that although sustained elevation of calcineurin activity may be pathological, normal cyclic activation helps the heart anticipate physiological demand. We find similar circadian oscillations in calcineurin activity in a broad range of tissues and propose a model for their integration with the transcriptional circadian clock.

11:45

46 • Differences in Liver Metabolism over Males and Females Depend on Circadian Timekeeping

Isabelle M. Bur, Anne M. Cohen-Solal, Norbert Chauvet, Gijsbertus T. J. van der Horst, Patrice Mollard, and Xavier Bonnefont*, Institut de Génomique Fonctionnelle, Département dEndocrinologie, Montpellier, France

An internal circadian clock paces the daily variations in rest and activity, feeding behavior, and metabolism. Although metabolic systems display marked differences between males and females, whether the clock itself contributes to this sexual dimorphism has remained unsuspected. We show that dimorphic liver metabolism is altered in absence of a functional circadian clock. Mutant male mice with a genetically inactivated clock express a number of sex-specific liver products, including several cytochrome P450 enzymes, at levels close to those measured in females. This feminized phenotype is likely due to the impairment of the dimorphic ultradian pulsatility of growth hormone (GH), as evidenced by 1) the alteration of body growth of clock mutant mice in a sex-biased manner, and 2) our ability to reverse it with hormonal injections that mimic male GH pulses. These results indicate that circadian timekeeping, sex dimorphism and liver metabolism are finely interconnected. This study also suggests that the circadian clock can tune ultradian pulsatility.

12:00

47 • Tissue-Specific Disruption of Mouse Casein Kinase 1 Delta Affects Circadian Function

Jean-Pierre Etchegaray[1]*, Kaz K. Machida[1], Elizabeth S. Noton[1], Cara M. Constance[2], Marianne N. Di Napoli[2], Jason P. DeBruyne[1], Haley M. Conde[3], Christopher M. Lambert[1], Steven M. Reppert[1], and David R. Weaver[1], [1]Department of Neurobiology, University of Massachusetts Medical School; [2]Department of Biology, College of the Holy Cross, [3]Department of Biotechnology, Worcester Polytechnic University

To assess the role of casein kinase I (CKI) delta in the circadian clock mechanism, we generated mice in which exon 2 of CKI-delta was flanked by loxP sites (floxed). In the presence of CRE recombinase, exon 2 is excised, leading to a deletion-mutant protein that fails to interact with PER proteins, does not promote PER degradation, lacks kinase activity, and does not interfere with the kinase activity of wild-type CKI-delta or CKI-epsilon in transfected cells. Germline deletion of exon 2 allows generation of animals with disruption of CKI-delta throughout the body. Mice heterozygous for the targeted allele tend to have longer circadian periods in constant darkness. Mice homozygous for the targeted allele do not survive, as reported for CKI-delta knockouts (Xu et al., 2005). To circumvent the lethality of homozygous mutants, we generated mice with liver-specific disruption of CKI-delta (expressing CRE recombinase driven by the albumin promoter and homozygous for the floxed allele of exon 2) and compared them with littermates homozygous for the floxed allele but lacking CRE. In mice with liver-specific disruption of CKI-delta, PER remained in the nucleus during daytime, reducing PER protein rhythm amplitude even though Per1 and Per2 transcript rhythms were relatively unaffected. In contrast, the amplitude of transcript rhythms for the output genes Dbp and Rev-erb-alpha was reduced. Liver tissue from mice with liver-specific disruption of CKI-delta had a longer period of PER2:luciferase bioluminescence rhythms. These data indicate that CKI-delta contributes to proper functioning of the circadian clock in mouse liver.

12:15

48 • Rhythmic Orchestration of the Unfolded Protein Response in the Liver Endoplasmic Reticulum

Gaspard Cretenet and Frédéric Gachon*, Inserm, Equipe Avenir, Montpellier, France CNRS, Institut de Génétique Humaine—UPR 1142, Montpellier, France

Many aspects of animal metabolism are regulated by a circadian clock. Recently, it has been shown that rhythmic expression of enzymes involved in liver detoxification (mainly cytochrome P450, Glutathion-S-transferase, or esterase) or their transcriptional regulator (CAR) is controlled by the clock directly or indirectly via the PARbZip transcription factors. Interestingly, all these enzymes are located in the endoplasmic reticulum (ER). To cope with this rapid protein increase inside the ER, organisms from yeast to human have developed an answer called "unfolded protein response" (UPR). UPR contributes to the maintenance of the integrity of the ER and is required for proper cytochrome P450 protein expression. It has been shown that the dilatation of the ER resulting in the activation of the UPR is rhythmic in mouse liver, and is being maximal during the dark period when the animals are eating and the expression of most detoxification enzymes is at its highest. Here we show that this UPR is rhythmic in mouse liver, with a rhythm that persists even under constant darkness, probably to permit circadian detoxification. This rhythmic UPR consists in the ultradian (12 hours period) activation of the transcription factors XBP1 and ATF4 that contributes to the ultradian expression of the UPR-activated mRNAs, including ER chaperone molecules. Interestingly, the UPR activation itself seems to be regulated by the resulting rhythmic expression of these chaperone molecules. Finally we describe a new clock that contributes to the proper organization of the ER, which could be required for an efficient detoxification of the mouse liver.

11:00

49 • Circadian Gene Polymorphisms in Delayed Sleep Phase Disorder

Daniel F. Kripke*, University of California–San Diego

Delayed sleep phase disorder is characterized by trouble falling asleep without a very late bedtime and trouble awakening in the morning. As bedtime is about 50% heritable, the condition is believed to be largely genetic, probably related to polymorphisms in genes of the circadian system. Our group has recruited 300 cases of DSPD and almost 300 matched controls. Cases and controls are perfectly distinguished by a combination of the Horne-Ostberg and BALM morningness-eveningness scales, bedtimes, and age. Current genotyping has identified suggestive association with a single nucleotide polymorphisms (SNP) in NR1D1 (Rev-erb-alpha) and a coding SNP in PER2. Two single nucleotide polymorphisms (SNPs)in ARNTL, two coding SNPs in PER2, and a SNP in RORC have suggestive correlations with the Horne-Ostberg scale in DSPS cases and controls. Among patients with bipolar disorder, suggestive association with the BALM scale was found in a PER3 coding SNP previously described by Ebisawa et al. and two linked intronic SNPs in CSNK1E. A promoter SNP in TEF possibly protective against unipolar depression has been identified. Although these associations are suggestive (i.e., nominal $P<0.05$), further studies and replication are needed before any of the relationships can be considered reliable. Analyses of the samples thus far suggest that bedtime is influenced by complex inheritance, in which a number of relatively uncommon polymorphisms in circadian system genes have individually-small effects on the delayed or advanced phenotypes.

11:15

50 • Age and Peripheral Human Molecular Circadian Rhythms

Lucia Pagani*, Lorenz Waldmeier, Fides Meier, Jan Izakovic, Christian Cajochen; Anna Wirz-Justice, Steven A. Brown, and Anne Eckert

Suprachiasmic nucleus (SCN) cells are not the only cells that possess the oscillatory machinery of the circadian rhythm. The same molecules are also present in peripheral tissues, such as the fibroblasts, which have a cell-autonomous and self-sustained circadian rhythm providing an excellent model to study the molecular and biochemical mechanisms of mammalian circadian systems in vitro. For human beings in general, circadian disturbances have been shown with age such as higher prevalence of early morning awakening and difficulties in maintaining sleep. Nevertheless, the origin of this phenomenon is unknown. In our study, we addressed the question of whether fibroblasts reflect age-related differences in the circadian period length in vitro, that parallel changes in subject circadian phase in vivo. For this purpose, we are currently investigating the period length of the skin fibroblasts from young and elderly subjects (young: ‘30 years old; elderly: ‘60 years old; sex-matched). In addition we collect data about the chronotype of the subjects by analysing the Munich Chronotype Questionnaire. Fibroblasts are isolated from skin biopsies collected from the buttock of the subjects and infected with an engineered lentiviral circadian reporter that permits the characterization of circadian rhythms in vitro. Measurements (3p. biopsy; n=6 total p. subject) are conducted over a time period of 5 days. Supported by EU Grant #LSHM-CT-2006-018741 and grant from Désireé&Niels Yde Found.

11:30

51 • Physiological Responses to Circadian Misalignment; Health Implications for Shift Workers

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There is considerable epidemiological evidence that shift work is associated with increased risk for obesity, diabetes, and cardiovascular disease, perhaps due to chronically sleeping and eating at abnormal endogenous circadian times. To begin to understand underlying mechanisms, we determined the effects of such 'misalignment' on metabolic, autonomic, and endocrine predictors of cardiovascular risk. 10 adults (5 female) underwent a 10 day laboratory protocol, wherein subjects ate and slept at all phases of the circadian cycle—achieved by scheduling a recurring 28-hour 'day'. Subjects ate three standardized meals and a snack each 28-hour 'day'. Plasma leptin, insulin, glucose, and cortisol were measured hourly, urinary catecholamines 2-hourly (totaling ~1,000 assays/subject), and blood pressure, heart rate, sympathovagal balance, oxygen consumption, and polysomnographic sleep daily. Core body temperature was recorded to assess circadian phase. Circadian misalignment, when subjects ate and slept ~12 hours out of phase from their habitual times, caused a systematic decrease of leptin (-18%, $P<0.001$), systematic increase in glucose (+6%, $P=0.01$), complete reversal of the daily cortisol rhythm ($P<0.001$), increase in mean arterial pressure (+3%, $P=0.01$), and reduction in sleep efficiency (-20%, $P<0.002$). Circadian misalignment, as occurs with shift work, leads to changes in certain metabolic, autonomic, and endocrine biomarkers in the direction of increased risk for obesity, diabetes, and cardiovascular disease. These findings, if borne out in longer term studies in an operational setting, could provide a physiological explanation for the increased cardiovascular risk in shift workers. Support: R01-HL64815, K24-HL76446, NCRR-GCRC-MO1-RR02635, R01-57875, and Pickwick Fellowship.

11:45

52 • New Insights and Techniques for Understanding the Health Impact of Circadian Disruption

Mark S. Rea*, Andrew Bierman, Mariana G. Figueiro, and John D. Bullough, Lighting Research Center, Rensselaer Polytechnic Institute, Troy, NY

Light and dark regulate the timing of the SCN so that, in the natural world, circadian rhythms are coordinated with the local 24-hour cycle of day and night. Several lines of research, from epidemiology to controlled studies with animal models, indicate that light-induced disruption of the natural and regular 24-hour light-dark cycle increases morbidity and mortality. Ecological studies of human exposures to light are virtually nonexistent, however, making it impossible to determine if, in fact, light-induced circadian disruption directly affects human health. We will present a novel method for characterizing and quantifying circadian disruption based on recordings of light and activity over an extended period of time. A newly developed, head-worn field measurement device, the Daysimeter, recorded circadian light exposures and activity from day-shift and rotating-shift nurses. Although total light exposures for dayshift and rotating shift nurses do not differ significantly, the two groups were distinctly different in terms of circadian disruption as quantified using phasor analyses of the circular cross-correlations between light exposure and activity. Circadian disruption also was quantified for rodents subjected to a regular light-dark pattern and ones subjected to a "jet lag" schedule. This paper provides a quantitative foundation for systematically

studying the impact of light-induced circadian disruption in humans and in animal models. This basic information provides an essential bridge between ecological studies of humans and parametric, biologically meaningful studies of circadian disruption with animal models. Project Sponsors: NIH# 1R01OH008171 and NIH # 1U01DA023822-01

12:00

53 • A Survey of Circadian-Related Sleep Disorders and Melatonin Use in the New Zealand Blind Population

G.R. Warman*, C. Bolton, C. Inglis, A. Fernando, J. Cheeseman, M.D.M. Pawley, and A. Wirz-Justice, University of Auckland

Information on the overall prevalence of sleep timing disorders in blind populations is scarce. Here we have conducted a nationwide survey in New Zealand's blind population. A telephone interview including the Pittsburgh Sleep Quality Index (PSQI), Horne-Östberg Questionnaire (HO), Munich Chronotype Questionnaire (MCTQ), and questions regarding sleep problems and medication use was conducted on 313 blind and visually impaired members of the Royal New Zealand Foundation of the Blind (157 subjects with no conscious light perception in at least one eye (NLP), and 156 subjects with light perception in both eyes (LP)), and 156 age, sex and deprivation matched sighted subjects (S). Response rates were 87-88%. Subjects in the NLP and LP groups reported suffering from more circadian-related sleep problems (Table), but only 6 individuals in the NLP group and 3 in the LP group have attempted to use melatonin.

	NLP	LP	S	p
PSQI scores	7.6	6.7	5.3	<0.05
Intermittent sleep problems	66%	64%	46%	<0.05
Sleep at unconventional times	55%	43%	32%	<0.05
Reg. pattern in sleep disturb.	12%	3%	4%	<0.05
Drifting Sleep	26%	21%	4%	<0.05
Sleep medication use	45%	37%	19%	<0.05
Melatonin use	4%	2%	0%	

Chronotype was assessed in 138 NLP, 149 LP and 156 S subjects. Age-related changes in chronotype (MCTQ) previously described in sighted populations were observed in our blind and visually impaired population, and in each of the different sub-groups (NLP and LP). The current study highlights the high proportion of sleep timing problems in visually impaired New Zealanders (estimated at up to 5,000). The observation of age-related changes in chronotype in a blind/visually impaired population may shed light on the endogeneity of these changes. It should be noted, however, that the population used displayed varying degrees of light perception and the number of participants with no light perception in both eyes is low (n=63). This study was funded by the Health Research Council of New Zealand (05/212)

12:15

54 • Practical Weekend "Catch-Up" Sleep Interventions to Stabilize Rhythms and Vigilance in Teens

Stephanie J. Crowley*, M.S.[1,3]; Mary A. Carskadon, Ph.D.[1,2,3], [1]Brown University, Department of Psychology; [2]Warren Alpert Medical School of Brown University; [3]E.P. Bradley Hospital Sleep and Chronobiology Research Laboratory

Introduction: Teenagers who sleep later and longer on weekends versus school-days may experience weekend phase delays and subsequent performance decrements. We examined two interventions to

minimize these changes. Method: Ten adolescents (15-16 years, 4 males) completed a 4-week study, keeping fixed sleep/wake times (time in bed (TIB)=7.5h) before and after two weekend "conditions" (TIB=9.0h; bedtime was delayed 1.5h). Weekend risetime was delayed 3h in a "usual" DELAY condition and 1h in a NAP intervention condition with a 2-h mid-afternoon nap. Six adolescents (15-16 years, 2 males) kept the DELAY schedule and experienced a LIGHT intervention of short wavelength light (454-484nm, GoLite®, Apollo Health, Inc.) for 1h upon weekend wakings. Phase shifts were measured from salivary DLMOs on Friday and Sunday evenings. Deviation scores for mean reaction time (RT), median RT, and lapses (RTs>500msec) were computed from 5-minute PDA-based reaction time tasks done four times each weekday. Post-weekend Monday and Tuesday morning scores were averaged and correlated with weekend phase shift (negative=delay) in each condition. Results: DLMO shifted significantly later, on average, for all conditions (mean minutes ±SD: DELAY=45±31 (range=-97 to +4); NAP=41±34 (range=-92 to -3); LIGHT=28±30 (-52 to +29 minutes)). DLMO delayed > 30 minutes in 70% of DELAY, 60% of NAP, and 50% of LIGHT. For LIGHT only, greater phase delays correlated with more lapses (r=.82,p=.05), but not mean RT or median RT (rs=.71,ps=.11). Conclusions: Neither the NAP nor LIGHT intervention stabilized rhythms, on average. The LIGHT phase shift range (advance and delay) may explain correlations with vigilance in this condition. Support: 1 F31 MH078662-01 awarded to S.J. Crowley; Apollo Health, Inc.

Wednesday, May 21

Slide Session J: Systems Biology and Modeling

11:00

55 • Quantification of Single-Cell Bioluminescence Data

Pal O. Westermark* and Hanspeter Herzel, Humboldt University Berlin

Nowadays, the circadian oscillators in the SCN and in peripheral tissues such as fibroblasts are thought to be self-sustained at the single-cell level. This notion is based on recent studies where the clock has been tracked in different tissues at the single-cell level using bioluminescence imaging. We apply established methods from engineering and physics to extract parameters such as period, damping and noise level from single cell data of wild-type and mutant cells (Liu et al., Cell 129, 2007). Corrleation functions are surprisingly well fitted by weakly damped oscillators driven by biochemical fluctuations. We illustrate that visual inspection has limited power to distinguish noisy damped oscillations from self-sustained oscillations. Thus the question of whether single cells are self-sustained oscillators or not is far from being settled. The understanding of the nature of the single-cell oscillator is crucial for understanding entrainment and synchronization properties. Since damped oscillators driven by random fluctuations and neurotransmitter coupling are easily synchronized further experimental studies with long time-series are required to judge whether the damped oscillator idea presented here stands up to scrutiny.

11:15

56 • Visualization of Frq Message and Protein Dynamics through Luciferase Reporters in The Neurospora Crassa Circadian System

Luis F. Larrondo*, Jennifer J. Loros, and Jay C. Dunlap. Department of Genetics, Dartmouth Medical School

The Neuropora crassa circadian oscillator is based on a negative feedback loop where the FREQUENCY protein (FRQ) acts on the white collar transcriptional complex (WCC) to inhibit its own transcription. In recent years our lab has developed a fully codon-optimized FIREFLY luciferase gene and adapted

for expression in Neurospora. A frq-luciferase transcriptional fusion clearly reveals the dynamics of frq expression under "close-to-wild-type" growth conditions for many days. We have now also engineered a FRQ-LUC translational fusion. When expressed from the endogenous frq locus, this gene fusion reports overt circadian rhythms of conidiation having a period of about 24 hrs, while FRQ-LUC rhythms and relative abundance can be easily analyzed by bioluminescence measurements with high temporal resolution Thus, now we can track in real time both frq message and FRQ protein dynamics, making these analyses an excellent substrate for testing and expanding existing Neurospora clock mathematical models.

11:30

57 • A Mathematical Model of the Decoupled Mouse Circadian Clock

Henry P Mirsky *, Andrew C Liu, David K Welsh, Steve A Kay, Francis J Doyle III, University of California–Santa Barbara

Existing models of the mammalian circadian clock produce oscillations with an ~24 period but were constructed from a meld of cellular, tissue-level, and organismal data. It has been argued, however, that the correct phenotypes driven by intracellular (i.e. core) clock networks can be observed only when individual neurons are decoupled from one another. Recently, for example, Liu et al. showed that dispersed neurons with Per1 and Cry1 knockouts exhibit arrhythmic behavior, in contrast to the previously-reported results, developed from experiments involving interacting cells, which indicated that rhythmicity was retained. Here we take the network structure and the phase data from the true, decoupled clock to develop a new mathematical model. Per1, Per2, Cry1, Cry2, Clk, Bmal1, Rev-ErbA, and RorC genes are included. These genes and their products are linked in a network of interactions with numerous positive- and negative-feedback loops. The model is fit to a 24-hour period, the phase relationships among the components (i.e. four-hour leads of Rev-ErbA over Per1 and Per2, four-hour leads of Per1 and Per2 over Cry1, Cry2, and RorC, four-hour leads of Cry1, Cry2, and RorC over Clk and Bmal1, and twelve-hour leads of Clk and Bmal1 over Rev-ErbA), and a lead of mRNAs over proteins of approximately four to six hours. The model then predicts de novo numerous phenotypes associated with gene knockouts, including changes in rhythmicity, expression level, amplitude, and period length.

11:45

58 • Regulation of Clock Controlled Genes in Mammals

Katarzyna Bozek, Christof Dame, Achim Kramer, and Hanspeter Herzel*, Institute for Theoretical Biology

The complexity of tissue and day-time specific regulation of thousands of clock controlled genes (CCGs) suggests that many regulatory mechanisms contribute to their transcriptional control. Our analysis is based on a meta-analysis of DNA-array data from rodent tissues. We search in the promoter regions of 2065 CCGs for high-scoring transcription factor binding sites. In order to compensate the relatively high GC-content of CCG promoters we employ a novel background model to avoid a bias towards GC-rich motifs. We find that most of the transcription factors with overrepresented binding sites exhibit themselves circadian rhythms. Among the predicted factors are known regulators such as BMAL1, VBP, HLF, E4BP4, CREB, RORa and the recently described regulators HSF1, STAT3 and HNF4a. As additional promising candidates of circadian transcriptional regulators we find PAX4, C/EBP, IRF, E2F, and NF-Y. Moreover, GC-rich motifs (SP1, EGR, ZF5, AP2) and AT-rich motifs (MEF2, HNF1, Oct1) are significantly overrepresented in promoter regions of CCGs. We discuss the connections of the predicted factors to the endocrine system and present putative tissue specific binding sites such as HTF and IPF1 for heart or myogenin for skeletal muscle. Finally, we study experimentally a gene with many predicted binding sites in its promoter and find that it is indeed regulated in a circadian fashion.

12:00

59 • Predicting the Electrical Activity of the SCN

Casey O. Diekman[1,3]* and Daniel B. Forger[2,3]. [1]Dept. of Industrial & Operations Eng., University of Michigan; [2]Dept. of Mathematics, University of Michigan; [3]Center for Computational Medicine & Biology, University of Michigan

Despite the wealth of experimental data on the molecular biology and electrophysiology of neurons in the suprachiasmatic nucleus (SCN), the neural code of the SCN remains largely unknown. Using a detailed model of the ionic currents within SCN neurons, we simulate a network of 20,000 inhibitory GABAergic SCN neurons. This model predicts that individual SCN neurons can either: 1) randomly fire out of phase with other SCN neurons 2) fire in synchrony with a subpopulation of other SCN neurons or 3) show random bursts of firing. Based on simulations and phase response curve analysis, we link these three behaviors to: 1) the circadian phase of an individual neuron 2) synaptic distributions within the SCN 3) heterogeneity of the circadian phase of the SCN neurons and 4) magnitude of post-synaptic currents. These results provide clues to how the SCN may encode information about circadian phase. Casey Diekman is a NSF Graduate Research Fellow. Daniel Forger is an AFOSR Young Investigator.

12:15

60 • The Endogenous Circadian Pacemaker Imparts a Scale-Invariant Pattern of Heart Rate Fluctuations across Time Scales Spanning Minutes to 24 Hours

Kun Hu[1]*, Frank A.J.L. Scheer[1,2], Ruud M. Buijs[2,3], and Steven A. Shea[1], [1]Division of Sleep Medicine, Brigham and Women's Hospital; 2Department of Hypothalamic Integration Mechanisms, Netherlands Institute for Brain Research, Amsterdam, The Netherlands; [3]Department Physiology, Instituto Biomedicas, UNAM Mexico

Heartbeat fluctuations in mammals display a robust temporal structure characterized by scale-invariant/ fractal patterns. These scale-invariant patterns likely confer physiological advantage because they change with cardiovascular disease and these changes are associated with reduced survival. Models of physical systems imply that to produce scale-invariant patterns, factors influencing the system at different time scales must be coupled via a network of feedback interactions. A similar cardiac control network is hypothesized to be responsible for the scale-invariant pattern in heartbeat dynamics, although the essential network components have not been determined. Here we show that scale-invariant cardiac control occurs across time scales from minutes to ~24h, and that lesioning the mammalian circadian pacemaker (suprachiasmatic nucleus; SCN) completely abolishes the scale-invariant patterns at time scales >~4 h. At time scales <~4h, the scale invariance persisted following SCN lesion but with a different pattern. These results indicate previously unrecognized multi-scale influences of the SCN on heart rate fluctuations that cannot be explained by a simple pacemaker of 24h rhythmicity. We conclude that the SCN serves as a major node in the cardiac control network and imparts scale-invariant cardiac control across a wide range of time scales with strongest effects between ~4-24h. These results demonstrate that experimental manipulations (e.g., SCN lesion) can be used to begin to model and understand the origin of scale-invariant behaviour in a neurophysiological system.

11:00

61 • Identification of Novel Genes Involved in Light Dependent CRY Degradation through a Genome-Wide Rnai Screen

Sriram Sathyanarayanan, Xiangzhong Zheng, Shailesh Kumar*, Dechun Chen, Bruce Hay and Amita Sehgal

In almost all organisms, circadian clocks regulate various physiological processes and synchronize them according to the environmental light: dark cycles. In Drosophila, the circadian clocks receive light through a dedicated photoreceptor CRYPTOCHROME (CRY). In response to light, CRY promotes the degradation of a clock protein TIMELESS (TIM) and then is itself degraded. Although CRY functions as a photoreceptor for the central clock that drives the activity: rest cycle, it also acts as an integral clock component in peripheral tissues. Thus, identification of molecules regulating CRY may provide better insight into the mechanisms that underlie circadian entrainment as well as those that maintain the free-running state of the clock. To identify novel genes involved in circadian entrainment, we performed an unbiased genome-wide screen in Drosophila cells using a sensitive and quantitative assay that measures light-induced CRY degradation. We systematically knocked down expression of about 21,000 genes and identified those that regulate CRY stability. These genes include ubiquitin ligases, signal transduction molecules and redox molecules. Most of the genes identified by the screen are specific for CRY degradation and do not affect the degradation of TIM in response to light, suggesting that these two pathways are distinct. We further validated the effect of three candidate genes on CRY stability in vivo by assaying flies mutant for each of these genes. This work identifies a novel regulatory network involved in light-dependent CRY degradation and demonstrates the power of a genome-wide RNAi approach for understanding circadian biology.

11:15

62 • Fundamental Differences between Light and Temperature Entrainment Mechanisms in Drosophila

Franz Glazer, Hana Sehadova, Carla Gentile*, Alekos Simoni, Astrid Giesecke, Ralf Stanewsky, Queen Mary University of London

The endogenous clock controlling circadian rhythms is able to synchronize with environmental time cues in order to keep the living organisms in tune with the natural time. Light is the only of these time cues whose role in the clock entrainment is somehow understood. Temperature cycles also work as zeitgeber for the clock, promoting both molecular and behavioural entrainment in different organisms. However, nothing was known until recently about the mechanisms and genes involved in the entrainment to temperature cycles. Two genes, nocte and norpA have been established to function in this process because the relevant mutations block temperature entrainment. nocte encodes a large glutamine rich protein, which is expressed in peripheral and central clock cells. Together with the phospholypase C encoded by norpA it may be involved in signalling temperature signals from the periphery to the clock neurons in the brain. Indeed, analysis of the molecular rhythms of different per-luc transgenic flies in whole fly and in independent body parts under temperature entrainment conditions suggest that peripheral tissues are required to send temperature signals to the brain clock. These results imply that the mechanisms driving the entrainment to temperature or light cycles are fundamentally different, although ultimately both pathways need to integrate in the central brain clock.

11:30

63 • A Circadian Clock in the Inner Retina Regulated by Dopamine

Guo-Xiang Ruan, Shin Yamazaki, and Douglas G. McMahon*, Department of Biological Sciences, Vanderbilt University

The mammalian retinal circadian clock exerts extensive control over retinal physiology and function. We have developed a protocol for long-term culture of intact mouse retinas, which allows retinal circadian rhythms to be monitored in real-time as luminescence rhythms from the PER2::LUC clock gene reporter. We studied the location within the retina of retinal molecular rhythms and the actions of two key neuromodulators, dopamine and melatonin. mPer2Luc knockin mouse retinas were isolated, cultured and assayed for luminescence rhythms. PER2::LUC retinal rhythms were routinely measured from whole-mount retinal explants for 10 days and for up to 30 days with media changes. Imaging of vertical retinal slices demonstrated that the rhythmic luminescence signal was concentrated in the inner nuclear layer. Persistence of rhythms in C57BL/6J mouse retinas that lack melatonin synthesis and during blockade of melatonin receptors with luzindole demonstrated that retinal PER2::LUC rhythms do not require communication via melatonin. Dopamine, acting through D1 receptors, reset the phase of retinal PER2::LUC rhythms producing both advances and delays. Our results reveal an inner retinal circadian oscillator that exhibits rhythms independent of melatonin. The phase of this oscillator is regulated by dopamine which likely mediates synchronization of inner retinal rhythms to the light/dark cycle. (Supported by NIH RO1 EY015815 to D.G.M).

11:45

64 • Melatonin Modulates Visual Processing in the Mouse Retina

G. Tosini[1]*, N. Pozdeyev[2], C. Liu[1], M. Kasamatsu[1], P.M. Iuvone[2,3], M.T. Martinez-Merlos, [1]Circadian Rhythms and Sleep Disorders Program, Neuroscience Institute. Morehouse School of Medicine; Departments of [2]Pharmacology and [3]Ophthalmology, Emory University School of Medicine

Several studies have reported that melatonin plays important roles in the circadian regulation of retinal rhythms. No previous study has investigated the functional roles of specific melatonin receptors in a mouse retina. In the present study, we have crossed mice with targeted deletion of the MT1 melatonin receptor gene (MT1-/- mice) onto the C3Hf+/+ background, a strain that makes melatonin but does not develop retinal degeneration. We have investigated the regulation of the circadian rhythms of dopamine and its metabolite, DOPAC, as well as the scotopic electroretinogram (ERG) responses of the C3H f+/+ MT1-/- mice and wild type controls. The lack of MT1 receptors did not affect photoreceptor viability. The circadian regulation of the dopamine and DOPAC levels was also unaffected by the lack of the MT1 receptor. In mice exposed to a light-dark cycle, a- and b-wave amplitudes at midnight were significantly greater in wild type mice than in C3H f+/+ MT1-/- mice. The ERG amplitudes were significantly greater at midnight than at midday in wild types, but not in C3H f+/+ MT1-/- mice. Surprisingly, we did not detect any circadian rhythms in the a-wave or b-wave amplitudes of the ERG in C3H f+/+ controls or C3H f+/+ MT1-/- mice exposed to constant darkness for two days, although a clear circadian rhythm in the implicit time of the a-wave was detected in both genotypes. These data suggest a complex relationship between melatonin and environmental lighting conditions in the control of retinal physiology.

12:00

65 • The Spectral Sensitivity of Human Circadian Photoreception Is Dynamic and Changes Depending on the Irradiance and Duration of Light

Joshua J. Gooley*, Shanthakumar M. W. Rajaratnam, George C. Brainard, Richard E. Kronauer, Charles A. Czeisler, and Steven W. Lockley, Brigham and Women's Hospital

In humans, a short-wavelength sensitive photoreceptor system, distinct from that used for vision, is thought to mediate circadian and neuroendocrine responses to light. To determine the relative contribution of cone photoreceptors and melanopsin, we examined circadian phase-resetting and melatonin suppression in subjects (18-30 yrs, n = 48) exposed to monochromatic green light (555 nm) or blue light (460 nm) for 6.5 hrs during the biological night. In a 9-day laboratory study, subjects were administered light across a 3-log-unit range of photon densities and circadian phase was assessed by a constant routine procedure before and after the light intervention. As predicted, melatonin suppression sensitivity during the entire 6.5-hour duration was about five times greater for 460 nm, as compared to 555 nm light exposure. The dose-response of melatonin suppression, however, showed a change in sensitivity across time such that 555 nm light was relatively more effective at the start of the 6.5-hour exposure as compared the end. Similarly, at low irradiances, light-induced phase delays showed a long-wavelength shift in sensitivity. These results suggest that the cone photoreceptors predominate circadian photoreception at low irradiances and at the beginning of a light exposure, whereas the melanopsin-containing retinal ganglion cells function as the major circadian photoreceptors at high irradiances and during long-duration exposures. Thus, circadian photoreception in humans represents a dynamic process in which the relative contribution of the classical visual photoreceptors and the melanopsin cells is determined by the irradiance, duration, and spectral composition of light. Support: NCCAM (AT002129, SWL); RO1NS36590 (GCB); NSBRI through NASA NCC 9-58 (CAC, GCB, SWL); NIMH (MH045130, CAC); NIH/NHLBI (T32 HL07901, CAC).

12:15

66 • The Spectral Quality of Light Affects Brain Responses to Emotional Stimuli in Humans

Vandewalle Gilles[1], Schwartz Sophie[2], Grandjean Didier[3], Wuillaume Catherine[1], Balteau Evelyne[1], Phillips Christophe[1], Luxen André[1], Dijk Derk-Jan[4], Maquet Pierre[1], [1]Cyclotron Research Centre, University of Liège, Belgium; [2]Laboratory for Neurology and Imaging of Cognition, University of Geneva, Switzerland; [3]Geneva Emotion Research Group, University of Geneva, Switzerland; [4]Surrey Sleep Research Centre, University of Surrey, United Kingdom

Light therapy is an effective treatment for seasonal affective disorder, but acute effects of light exposure on emotion-related brain activity have not been demonstrated. We recently provided evidence that melanopsin based photoreception, most sensitive to blue light, is involved in the modulation of brain activity by light exposure. The amygdala, which is involved in emotion regulation, was one of the brain areas activated following light exposure. However, that protocol did not include an emotional task. It therefore remains unknown whether exposure to blue light would also affect the processing of emotional stimuli within the amygdala. Seventeen subjects performed a gender decision task on emotional voices (word-like utterances with angry or neutral prosody) in fMRI while being alternatively exposed to 40s of blue (473nm) and green (527nm) monochromatic lights. Irradiance levels of half of the blue and green illuminations were set at 7x1012 ph/cm2/s, the other half at 3x1013 ph/cm2/s. Orders of irradiances and wavelengths were counter-balanced. The experiment took place 3h after habitual wake time in March and April. Behavioural data (reaction time and affective judgement) confirmed the emotional valence of the stimuli. Results

show that, as compared to green light, blue light increased brain activity related to negative stimuli in the bilateral amygdala and in the "voice area" in the bilateral superior temporal sulcus. These results constitute the first demonstration of an acute influence of light, and of its spectral quality, on the emotional system. Our findings support the involvement of non-classical photoreception in these effects. Supports: FNRS,FMRE,PAI/IAP,ULg,Wellcome Trust.

Slide Session L: Neural Clocks and Output

11:00

67 • Ca2+ Dependent Protein Kinase 9 (CDPK9) Regulates a Subset of Circadian Output Pathways

Ido Kron, Dror Hilman, Esther Yakir, Miri Hassidim, Shai Yerushami and Rachel Green*, Hebrew University

The circadian system of higher plants regulates a broad range of physiological and cellular output processes, including the expression of around 16% of the genome, with periods of ~24 hours and widely different phase and amplitude patterns. However, the mechanisms by which rhythms generated by the oscillator can be transformed into divergent outputs are poorly understood. In a bioinformatics-based screen for output regulators in the model plant, Arabidopsis thaliana, we have identified a circadian-regulated gene, Ca2+-DEPENDENT PROTEIN KINASE 9 (CDPK9). CDPK proteins have a characteristic structure with a highly variable N-terminal domain followed by a serine/threonine protein kinase domain fused to a carboxy-terminal calmodulin-like domain containing EF-hand calcium-binding sites that allows the protein to interact directly with Ca2+. Using plants that mis-express CDPK9, we demonstrate that CDPK9 controls the phase and amplitude, but not the period, of expression of certain circadian output genes. By contrast, the expression of oscillator genes and other circadian output pathways, including the expression of other circadian output genes, is unaffected. Our results show that CDPK9 has a role in regulating a subset of output pathways. Moreover, CDPK9 may represent a cross-talk point between Ca2+ and circadian pathways. We suggest that the regulation of circadian output genes by CDPK9 may serve a paradigm for starting to understand how multiple interacting pathways from the oscillator can control gene expression.

11:15

68 • Diurnal and Nocturnal Temporal Silencing of Period1::D2gfp Neurons in the Suprachiasmatic (SCN) Nucleus of Mice

Mino D. Belle*, Fiona Scott, and Hugh D. Piggins, Faculty of Life Sciences, University of Manchester

The suprachiasmatic nucleus (SCN) of the mammalian hypothalamus plays a key role in the circadian organisation of behaviour. In nocturnal rodents, SCN neurons in vivo respond to light during both subjective night and subjective day. However, a clock-controlled gating mechanism restricts photic-dependent phase-shifting in rhythms to the night. Unfortunately, the basis for this mechanism is unknown. Here, we provide the first electrophysiological evidence demonstrating unequivocally that diurnal and nocturnal rhythms in the resting membrane potential (RMP) of Per1::d2GFP SCN neurons may largely underpin photic-gating within the SCN. In the morning (ZT2-4.25) Per1::d2GFP neurons were at normal RMP, firing action potentials at about 5 Hz. During mid- to late-afternoon (ZT5.25-10.75) Per1::d2GFP cells rested from -33 to -25 mV. Those resting at -33 mV generated an L-type Ca2+-dependent membrane potential oscillation that was TTX insensitive, while at -25 mV the neurons were completely silenced. This extreme depolarization was not observed in non-Per1::d2GFP neurons which continued to generate

action potentials throughout the day. At night (ZT12.75-15) the RMP of Per1::d2GFP neurons was around -70 mV and these cells were also silent. Interestingly, at about ZT0 and ZT12, corresponding to projected lights-on and lights-off, respectively, Per1::d2GFP neurons were at normal RMP and generating action potentials. We conclude that a family of cation channels work in concert and in a temporal manner to orchestrate Per1::d2GFP neuronal silencing in the SCN of mice. Our results also suggest that Per1-containing neurons are an integral part of an elaborate network of cells within the SCN responsible for coding time of dawn and dusk.

11:30

69 • Blocking the Fast Delayed Rectifier Current at Late Night Phase Shifts or Abolishes the SCN Electrical and Molecular Rhythm

Anne Sinning[1], Johan Brask[2], Gabriella Lundkvist[2], Johanna Meijer[1], Stephan Michel[1]*, [1]Laboratory for Neurophysiology, Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands; [2]Department of Neuroscience. Karolinska Institutet, Stockholm, Sweden

The suprachiasmatic nucleus (SCN) is the central circadian pacemaker in mammals. Most SCN neurons exhibit a circadian rhythm in firing rate, membrane potential, intracellular calcium levels and clock gene expression. Among the membrane ionic current modulated by the circadian clock, the fast delayed rectifier (fDR) was previously shown to be necessary to maintain circadian rhythmicity in spike frequency. We now show that blocking the fDR for a short time (4 h) at the end of the night can also prevent or phase shift circadian rhythmicity. Application of the fDR blocker 4-aminopyridine (0.5 mM) during the late night phase advanced (2.3 ±0.3 hrs, n=5) or abolished the electrical activity rhythm (n=3). Measurements of rhythm in mPER2 expression in SCN organotypic slices using a luciferase reporter revealed similar phase advances (1.5 ±0.6 hrs n=3), and long-term blockade of fDR completely abolished the mPER2 rhythm (n=3). Levels of intracellular calcium were increased as measured using the calcium-sensitive dye Fura-2. Interestingly, this response depended on baseline activity of the SCN neuron indicated by the baseline calcium signal. Only cells in a quiescent state responded with calcium increase. Calcium signaling may provide a possible link between membrane and transcriptional gene regulation. Our data emphasize the importance of circadian controlled conductance to maintain the phase and rhythmicity in the mammalian pacemaker cells.

11:45

70 • A Cellular Analysis of Circadian Rhythms in Mesencephalic Trigeminal Nuclei

Hiler, D.J.[1]*, Davidson, A.J.[2], Yamazaki, S.[3], and Geusz, M.E.[1], [1]Dept. of Biological Sciences, Bowling Green State University, Bowling Green, OH; [2]Neuroscience Institute, Morehouse School of Medicine, Atlanta, GA; [3]Dept. of Biological Sciences, Vanderbilt University, Nashville, TN

The circadian timing system contains multiple circadian pacemakers in tissues throughout the body that are regulated by the suprachiasmatic nucleus (SCN). We have recently reported the discovery of a circadian pacemaker located within the mesencephalic trigeminal nuclei (Me5) of the brainstem. Using luminescence imaging of brain slice cultures made from transgenic mPer1::luc mice, we characterized the period and phase of circadian rhythms in gene expression in the Me5. The Me5 are a wide band of cells originating in the lateral periaqueductal gray of the midbrain and extending into the pons. It contains primary mechanosensory neurons that regulate jaw movement through the mesencephalic motor nuclei (Mo5) during chewing and biting, suggesting that Me5 cell depolarization from daily feeding behavior may entrain the pacemaker. Because of their large size (30 to 40-μm diameter), abundance, and morphologically identifiable sub-types,

the Me5 neurons could be a valuable alternative to the SCN for studying circadian properties of mammalian neurons. The membrane properties of Me5 neurons have been examined for comparison with homologous large neurons of dorsal root ganglia. As a model system for circadian research, it will be necessary to identify circadian rhythms in isolated Me5 neurons. Individual Me5 neurons were visible in luminescence images of rPer1::luc brain slice cultures. We also imaged transgene expression in individual dissociated Me5 neurons from mPer1::luc mice. We are continuing to characterize the pharmacological and cellular properties of Me5 neurons to identify which sub-types may contain a circadian pacemaker.

12:00

71 • Dissecting the Logic of a Circadian Neural Circuit

Ben Collins* and Justin Blau, New York University

We used the simple larval light avoidance assay to understand how M(orning) and E(vening) pacemaker neurons interact to control rhythmic outputs. M cells are required by larvae to avoid light in a circadian manner and E cells project to M cell axonal termini and inhibit light avoidance, presumably by inhibiting release of an excitatory M cell signal. By stopping the clock in M and E cells, we found that they likely fire at opposite times of day, thus providing an electrical explanation for their function in adult locomotor behavior. Molecular rhythms in M cells are altered in the absence of E cells, and vice-versa. Thus M and E cells are coupled oscillators and are not cell-autonomous. Furthermore, rhythmic light avoidance requires not only the presence of E cells, but also functional clocks in both M and E cells. Thus rhythmic larval behaviour requires interactions between excitatory and inhibitory signals released by M and E cells. We made similar observations for adult rhythms. Although M cells are believed to be sufficient for both dawn anticipation in LD cycles and DD rhythms, both are lost without an E cell signal, as in larvae. Conversely, hyperexcitable E cells prevent morning anticipation and lead to stable 27hr rhythms in DD; the period of the M cell clock is lengthened, confirming its non-autonomy. We propose that coupling between oscillators releasing excitatory and inhibitory signals is a general mechanism to generate rhythmic outputs, analogous to the negative feedback loops in molecular clocks.

12:15

72 • Functional Genomics on Drosophila Pacemaker Neurons Identifies a Rhythmically Expressed K+ Channel as a Major Contributor to Pacemaker Neuron Excitability

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While the mechanism by which molecular clocks tick is relatively well understood, how the central clock cells transmit time-of-day information (clock output) to the rest of the organism remains largely unknown. One possibility is that the clock drives rhythmic expression of output genes whose products generate oscillations in neuronal signaling. Indeed, circadian rhythms in electrical activity have been described in clock neurons, although the connection to their molecular clocks remain undefined. With this in mind, we performed microarray analyses on RNA amplified from purified LNvs (ventral Lateral Neurons)—the major pacemaker cells in the fly brain. By comparing transcript profiles at two times of day and in clock mutants, we have identified a set of rhythmically-expressed clock-controlled genes that underlie rhythmic LNv output. One of these genes is Ir (Inward rectifying potassium channel), which has a well-known role in determining resting membrane potential and cell excitability. Rhythmic Ir expression is important for normal circadian behavior since constitutive over-expression of Ir in LNvs severely weakens adult activity rhythms. Conversely, knockdown of Ir in LNvs by RNAi gives ~25 hr rhythms. These data are consistent with the idea that cycling Ir contributes to the rhythmic output of LNvs, and suggest an important direct link between the molecular clock and intrinsic neuronal excitability.

Molecular Clocks

P1

Post-transcriptional Regulation of the Neurospora Circadian Clock by the FRQ-FRH Complex

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FREQUENCY (FRQ), the FRQ-interacting RNA helicase (FRH), WHITE COLLAR-1 (WC-1) and WC-2 form the core circadian negative feedback loop in Neurospora crassa. FRQ forms a complex with FRH and acts as the suppressor of frq transcription by inhibiting WC activity. Besides its role in the circadian negative feedback loop, FRH is a co-factor of exosome, a multiple subunit protein complex important for RNA processing and degradation. We show that the FRQ-FRH complex is associated with frq mRNA and the knock-down of FRH expression leads to high frq mRNA level, the result of impaired frq mRNA degradation. Our results further suggest that FRH recruits frq mRNA to the exosome for its degradation. Consistent with this, the impaired function of exosome causes aberrant circadian phenotypes. More interestingly, we found that FRQ is also involved in the posttranscriptional regulation of frq mRNA decay. Taken together, these data suggest that the FRQ-FRH complex plays an important role in the posttranscriptional control of the circadian clock.

P2

The Role of the Negative Feedback Loop Genes, Cryptochrome and Period, In Circadian Rhythmicity

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The mammalian circadian system generates an approximate 24 hour rhythm through a complex autoregulatory feedback loop. This feedback loop consists of positive and negative loops that function together to generate circadian rhythms. The negative feedback loop is comprised of four genes: Cryptochrome 1, Cryptochrome 2, Period 1, and Period 2. These codependent proteins have distinct yet complimentary roles within the core circadian mechanism; however, their mutually dependent nature makes their function complex and therefore poorly understood. The tetracycline transactivator system (tTA) was used to gain temporal and spatial control over each of the Period and Cryptochrome genes in vivo. Using this system it was possible to overexpress in a tissue-specific manner the function of each of these genes independently. Effects of the overexpression were quantified by measuring the circadian period in constant darkness (DD) and constant light (LL). The use of tTA over conventional knockout or overexpressing lines provides the advantages of phenotype reversibility as well as tissue specificity. Tissue specificity minimizes secondary effects that may be present in system wide knockouts. Phenotype reversibility allows for the behavior of each gene to be studied in the overexpressed as well as WT state

in individual animals in both DD and LL. Using this system in the suprachiasmatic nucleus has provided a great level of control over the circadian system, which will allow for a fine dissection of behavior and physiology necessary for insight into the crucial, complex, and codependent roles of the genes in the negative feedback loop of the circadian system.

P3

A Novel REV-ERBα Ligand Resets the Peripheral Clock in a Phasic Manner—Insight into the Role of REV-ERBα in the Peripheral Clock

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The nuclear receptor REV-ERBα has been identified as a key negative feedback regulator of the biological clock. The ligand binding domain of REV-ERBα lacks the typical AF2 domain, which has been shown to be important for co-regulator binding. As a result, REV-ERBα interacts with co-regulators in a ligand-dependent manner. The multimeric co-repressor complex (containing REV-ERBα, ligand and nuclear receptor co-repressor-NCoR) binds to ROR elements of the Bmal1 promoter and represses Bmal1 transcription. This stabilizing negative loop is important for precise control of the circadian pacemaker. In this study, we identified a REV-ERBα ligand (GSK414112A) which can enhance the REV-ERBα/NCoR interaction by 70% within 1 hour. Efficacy was confirmed in Pai-1::Luc transient transfection assays in which incubation with GSK414112A elicited significant down-regulation of transcription of Pai-1 (a known REV-ERBα target gene). In order to explore REV-ERBα action on resetting responses of the molecular clock, we first established rhythmic transcription profile and expression level of Rev-erbα in Rat-1 fibroblasts. When the compound was applied to the cellular models stably transfected with Bmal1::Luc or Per2::Luc, at different phases of the circadian oscillation, GSK414112A induced phase-dependent bi-directional phase shifts. When the phase changes were plotted against time, a clear phase response curve (PRC) was revealed, with significant peak-to-trough amplitude of ca. 5 hours. The phase resetting effect was also observed when the drug was applied to primary lung fibroblasts and ectopic lung slices from transgenic mPER2::Luc mice. These data demonstrate a role for REV-ERBα in resetting the clock at both the cellular and tissue level and reveal pharmacological action of a novel REV-ERBα ligand. We have modelled our data to define phasic action of this compound on the retinoid receptor pathways controlling the circadian clock. This compound is the first known pharmacological agent which can reset the circadian clock in a phase-dependent manner and may offer novel approaches to pharmacological treatments of rhythm disorders.

P4

PAS-Domain Interactions of Drosophila and Mouse PERIOD Clock Proteins

Sven Hennig*, Holger Strauss, Julia Arens, Sabrina Schulze, and Eva Wolf,

Most organisms exhibit a day-night activity cycle of approximately 24 hours due to a circadian pacemaker which is operated by autoregulatory translational and transcriptional feedback loops. The function of PERIOD proteins, as central components of the circadian clock, is controlled by synthesis, cellular localization, phosphorylation, degradation as well as specific interactions with other clock components. Furthermore PERIOD is able to form homodimers via its tandemly organized PAS (PER-ARNT-SIM) domains. We have solved crystal structures of PAS (PER-ARNT-SIM) domain fragments of

Drosophila PERIOD (dPER) and mouse PERIOD2 (mPER2) to get insights into their regulatory role in the circadian clock at an atomic level. In the case of Drosophila PERIOD we were able to show the importance of the dimer interfaces between the PAS-A domain and Trp482 as well as the C-terminal alpha-F-helix. The mPER2 crystal structure represents the first three dimensional structure of a mammalian clock protein. The mouse homolog shows a homodimer stabilized by interactions of Trp419 (Trp482 in dPER) with the PAS-B-beta-sheet surface. No alpha-F homolog is found. Interestingly a mPER2 fragment without the alpha-F equivalent region has a 100-fold higher affinity for homodimer formation than a Drosophila PERIOD fragment without the alpha-F-helix.

P5

Kinases and Phosphatases Regulate Stability and Nuclear Entry of Drosophila CRYPTOCHROME

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Department of Genetics University of Leicester

Drosophila melanogaster CRYPTOCHROME (CRY) is generally considered a circadian photopigment involved in entrainment. However, several reports suggest a dark function for CRY, involving it in the negative feedback core of the clock. Here we show that light phosphorylates CRY targetting it for degradation via the proteasome pathway and that light-mediated degradation of CRY continues shortly also in darkness. Moreover, kinases and phosphatases affecting PERIOD and TIMELESS also modulate the stability of CRY in darkness and light and its accumulation into the nucleus. We conclude that CRY is part of a multiprotein complex that assembles in the cytoplasm and regulates the level and nuclear entry of clock proteins

P6

Possible Involvement of the Members of P160 Family of Coactivators in the Circadian Oscillation of Clock Genes

Masaaki Ikeda*, Cheng Piao, Fang Yang, and Megumi Kumagai, Yoshihiro Nakajima

The members of p160 family of coactivators, which include SRC-1, GRIP1 and pCIP, are known to be involved in the transactivation of nuclear hormone receptors by biding them directly and recruiting CBP/p300 cofactors. Transcription of Bmal1 gene have be shown to be activated by a orphan nuclear receptor ROR alpha and repressed by Rev-Erb alpha via ROR-responsive elements (ROR-RE) located in the promoter /enhancer region of the Bmal1 gene. In the previous study, we demonstrated that the basal and ROR-alpha mediated transcription of Bmal1 was enhanced by SRC-1, GRIP1 and pCIP via ROR-RE in the Bmal1 gene. For the purpose of search the factors that enhance the amplitude of clock gene oscillation, we utilized a real-time oscillation monitoring system and demonstrated that the amplitude of the Bmal1 oscillation was enhanced by the p160 family of coactivators without changing the phase and period. To better understanding of the functional involvement of the p160 coactivators in the enhancing amplitude of clock genes oscillation, we constructed a dominant negative pCIP expression construct (pCIP-DN). Co-transfection of the pCIP-DN reduced the amplitude of the Bmal1 and Cry1 oscillation in the NIH3T3 cells. These results showed that p160 coactivators exert important roles in the formation of circadian oscillation.

P7

Molecular and In Vivo Functional Studies on Sequential Phosphorylation of Mcry2

Nobuhiro Kurabayashi*, Arisa Hirano, Tsuyoshi Hirota, and Yoshitaka Fukada, Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Japan

Cryptochrome proteins are critical players for molecular oscillations in the circadian clocks of central and peripheral tissues in mammals. mCRY2 is phosphorylated at Ser557 in the mouse SCN and liver, in which the Ser557-phosphorylated form accumulates in parallel with mCRY2 protein (Kurabayashi et al., Chronobiol. Int., 2006; Harada et al., JBC, 2005). The priming phosphorylation of mCRY2 at Ser557 allows subsequent phosphorylation at Ser553 by glycogen synthase kinase-3 (GSK-3), resulting in proteasomal degradation of mCRY2. In this degradative process, the phosphorylation of mCRY2 at Ser557 is a potential key event, and hence it is important to characterize the protein kinase responsible for Ser557-phosphorylation. To this end, we prepared nuclear extract and cytosolic fraction from the mouse liver, and they were separately fractionated by anion-exchange column chromatography. Then we estimated Ser557-phosphorylating activity of each fraction by an in vitro kinase assay. We detected several activity peaks, among which we directed our attention to a single peak fraction devoid of active-MAPK, based on our previous observation that MAPK plays a minimal role in phosphorylating mCRY2 at Ser557 in cells. Investigations of the effects of various protein kinase inhibitors on the Ser557-kinase activity are underway in order to characterize the responsible kinase, In parallel, we examined physiological importance of Ser557-phosphorylation in vivo, and found that alanine-mutation at Ser557 caused significant effect on circadian period length. The sequential phosphorylation of mCRY2 appears to play a critical role in circadian time-keeping system.

P8

Feedback Loops Regulating Transcription of the Neurospora Clock Gene Wc-2

Andrea Neiss, Tobias Schafmeier*, Michael Brunner

FREQUENCY (FRQ) and the White Collar Complex (WCC), consisting of the subunits WC-1 and WC 2, are central components of positive and negative feedback loops of the circadian clock of Neurospora crassa. In the positive loop FRQ supports accumulation of WC-1 on a post-translational level and activates wc-2 transcription. We have analyzed the wc-2 transcriptional regulation. WCC indirectly inhibits wc-2 by controlling the expression of a putative repressor whereas FRQ activates wc 2 transcription by inhibiting the WCC. A putative transcriptional activator binds to the same region of the wc-2 promoter as the repressor. The proposed mechanism of wc-2 regulation is reminiscent to the transcriptional regulation of Bmal1 in mammals and Cycle in flies. Moreover, an internal promoter in the wc-2 coding region drives expression of an N-terminally truncated WC-2 isoform. Full size WC-2 and sWC-2 are expressed in an antagonistic fashion. Hence, sWC-2 expression appears to be a fail-save mechanism maintaining total WC-2 levels above a critical threshold.

P9

Towards the Beginning of Time: Rhythms in Embryonic Stem Cells

Jiffin K. Paulose*, Edmund B. Rucker, and Vincent M. Cassone

The presence of clock genes during early embryogenesis has led to much interest as to whether a functional clock exists during development and when such a clock would begin to function. Our previously reported clock gene expression in blastocyst-derived mouse embryonic stem cells (ESCs) corroborates in vivo

and in utero studies. Rhythmic glucose uptake and corresponding rhythms in cry1 and per1 mRNAs in differentiated stem cells (dESCs) suggest that the pathways involved in differentiation and clock function may act in parallel or converge upon one another. The upregulation of ROR, a clock-controlled gene that plays a major role in development, after differentiation further supports this hypothesis. We report here our further analysis of clock gene expression in dESCs as well as stem cells that have been directed towards neuronal and cardiac fates. Furthermore, we are currently utilizing luciferase-driven, real-time monitoring of gene expression to test the hypothesis that the switch to differentiation also switches on the clock. Supported by NIH P01 NS35846.

P10

MicroRNA Rhythms in the Chick Pineal Gland

Vikram R. Shende*, Philip D. Beremand, and Vincent M. Cassone, Texas A&M University

Transcriptional profiling of the chick pineal gland in vivo and in vitro have revealed global reductions in the number and amplitude of rhythmic transcripts, including those of clock genes, without appreciable decline in melatonin rhythmicity. This suggests post-transcriptional regulation of pineal melatonin rhythmicity. We analyzed the microRNA transcriptome of the chicken pineal gland to investigate the role played by these small RNA species in regulating the circadian output of various genes. We have identified four miRNA's that show circadian variation in their abundance. Their predicted targets include the chicken orthologues of mammalian clock genes, such as NPAS2 and Period3, among others involved in cell cycle regulation, metabolism and stress response. These studies will help us identify the minimal required components of the chicken core molecular clock, and the role played by post-transcriptional regulators in maintaining molecular and metabolic rhythmicity.

P11

Circadian Protein Interaction Networks

Thomas Wallach[1]*, Pablo Porras Millan[2], Astrid Grudziecki[1], Christian Haenig[2], Erich E. Wanker[2], and Achim Kramer[1], [1]Charité—Universitätsmedizin Berlin, Laboratory of Chronobiology, Germany; [2]Max-Delbrück-Center for Molecular Medicine, Neuroproteomics, Berlin, Germany

Cell autonomous circadian oscillation is generated by a transcription-translation negative-feedback loop with an essential delay primarily based on post-translational events including protein-protein interactions. Besides already described interactions within the circadian oscillator we hypothesize that so far unknown interactions as well as yet unknown protein components are involved in the molecular regulation of circadian rhythms. Therefore, we designed a study to identify those interactions using the Yeast-Two-Hybrid technique (Y2H). Briefly, one putative interaction partner is fused to a DNA-binding domain (bait plasmid) while the other to a transcription activation domain (prey plasmid). We have created an Y2H matrix composed of 48 known or assumed circadian components that were shuttled to bait and prey plasmids, respectively. We are currently performing an interaction screening where every single bait is mated with every prey using high-throughput robotic approaches. If an interaction occurs in yeast several reporter genes are activated (URA3, HIS3 and lacZ). This screening procedure allows us to map new interactions between already known clock components. Furthermore, we aim to identify new circadian components by screening a large prey library (> 12.000 genes) with our 48 candidates as baits. Both experimental approaches should result in protein-protein interaction maps, which should lead us to a better understanding of the molecular processes underlying circadian rhythms. First results will be presented at the meeting.

P12

Proteomic Analysis of Per1 and Per2 Multirotein Complexes In Vivo

Charo Robles*, Darko Knutti, Hao Anh Duong, and Charles J. Weitz, Harvard Medical School

The mammalian circadian clock is built on a molecular feedback loop, but the details and dynamics of the oscillatory mechanism are poorly understood. At the center of the negative feedback loop is the heterodimeric transcription factor BMAL1-CLOCK, which drives the expression of multiple target genes, including Per and Cry circadian clock genes. In turn, PER and CRY proteins assemble into one or more multiprotein complexes that enter the cell nucleus and repress BMAL1-CLOCK transcription by an unknown mechanism. To gain potential insight into the mechanism of the clock and factors controlling its dynamics, we performed a proteomic analysis of PER1 and PER2 multiprotein complexes in vivo. To this end, we generated transgenic mice expressing double epitope-tagged PER1 or PER2. The tagged PER1 and PER2 transgenes each robustly rescued circadian rhythms of behavior in Per1-/-; Per2-/- mice, indicating that the tagged PERs participate in all protein complexes essential for clock function. Next we immunoaffinity purified PER1 and PER2 multiprotein complexes from livers and lungs of transgenic mice, and we identified components of the complexes by mass spectrometry and confirmed their association with PER1 and/or PER2 by co-immunoprecipitation studies. This procedure identified most of the known or suspected components of the clock, along with a number of other proteins that we consider as candidates for clock components. Preliminary RNA interference experiments with one candidate suggest that it is important for circadian period length regulation.

P13

Analysis of Protein Interactions in the Neurospora Crassa Circadian Clock

Christopher L. Baker*, Arminja Kettenbach, Mi-Shi, Scott A. Gerber, Jennifer J. Loros, and Jay C. Dunlap, Department of Genetics, Dartmouth Medical School

The Neurospora circadian clock is a biochemical oscillator comprising interconnected positive and negative feedback loops. Many of the core clock components which make up these loops have been identified including frequency (frq), white collar-1 and 2 (wc-1, wc-2), frequency-interacting RNA helicase (frh). When these, and the other proteins known to interact with them, are considered together we can describe an emerging portrait of a dynamic multi-protein complex required to accurately maintain temporal control. With advances in technology such as direct HPLC nanospray ionization with hybrid ion-trap mass spectrometers, the ability to identify proteins in complex peptide mixtures is becoming a routine procedure. However, when considered at the level of a single purified protein, these methods generate large protein-protein interaction datasets that require laborious biological validation. Here we describe a subtractive proteomics strategy to identify new protein components of the Neurospora crassa circadian clock. Our hypothesis is that by separately purifying multiple known proteins with their interactors, and comparing the resulting lists, that new functional components will appear enriched on more than one list. Additionally, using a strain sensitized for homologous recombination we developed a fast and reliable method to create C-terminal tandem tagged proteins. We have applied this method to three well characterized clock proteins. These data confirm the direct physical interaction between FRQ, WCC, and FRH as well as potentially revealing novel protein-protein interactions within the clock.

P14

Proteomics Analysis of Bmal1 Multiprotein Complexes

Charo Robles, Darko Knutti, Cyril Boyault*, and Charles J. Weitz, Harvard Medical School

The heterodimeric transcription factor BMAL1-CLOCK lies at the center of the feedback loop that at least in part comprises the mammalian circadian clock mechanism. Little is known about the molecular context of BMAL1-CLOCK activity, and information about the negative limb of the circadian feedback loop that inhibits BMAL1-CLOCK is clearly incomplete. To gain insight into BMAL1-CLOCK transcriptional activity and its inhibition by the circadian feedback loop, we performed a proteomics analysis of BMAL1 multiprotein complexes in mammalian cells. To this end, we generated a mouse fibroblast line stably carrying a circadian luciferase reporter gene and an expression construct encoding a double epitope-tagged BMAL1, driven by its endogenous promoter. Next we immunoaffinity purified BMAL1 multiprotein complexes from the cells using monoclonal antibodies against the tags. By mass spectrometry we identified CLOCK and RACK1 (Receptor for Activated protein Kinase C-1), a multifunctional signaling protein, as the two most prominent constituents of BMAL1 complexes. Co-immunoprecipitation studies confirmed that endogenous native BMAL1 and endogenous RACK1 are present together in protein complexes in mammalian cells and in mouse tissues. We found that RACK1 binds directly to BMAL1 in vitro and that it potently and specifically inhibits CLOCK-BMAL1 transcriptional activity in mammalian cells. Preliminary RNA interference studies suggest that RACK1 contributes to regulation of circadian period length in mammalian cells.

P15

Functional Conservation of Exon 19 between CLOCK and NPAS2

Saurabh Sahar*, Kevin C. Pham, and Paolo Sassone-Corsi

The clock/clock mutant mice, which have a deletion in the exon 19 of CLOCK, are arrhythmic. However, even after a decade of analyzing arrhythmicity in this mutant, the mechanistic defects in CLOCK delta19 are not clearly understood. The CLOCK delta19 mutant protein dimerizes with BMAL1, binds to DNA, but is severely defective in transactivation of clock controlled genes (CCGs). NPAS2, a transcription factor primarily expressed in the mammalian forebrain, shares extensive sequence homology with CLOCK. However, so far it was believed that the homology is restricted to the N-terminal end. We have observed that a region corresponding to exon 19 in CLOCK is highly conserved in the C-terminal of NPAS2. Here we show that deletion of this region (henceforth addressed as NPAS2 delta19) has effects similar to CLOCK delta19. NPAS2 delta19 also dimerizes with BMAL1, binds to DNA and is defective in transactivation of CCGs. Moreover, we have found that unlike wild-type NPAS2, NPAS2 delta19 is not phosphorylated in the presence of BMAL1. CLOCK delta19 also shows a similar defect in phosphorylation. This defect is not due to the absence of any phosphorylatable residues, as confirmed by similar defect observed in a mutant with a smaller deletion in the exon 19 region devoid of any potential substrates for phosphorylation. These results suggest that exon 19 may either act as a docking site for kinases or help in the proper folding of CLOCK or NPAS2, consequently generating a more amenable substrate for phosphorylation. These events could then lead to enhanced transcriptional activation.

P16

Nocturnin Expression Is Regulated Post-Transcriptionally by miR-122

Shihoko Kojima[1*], David Gatfield[2], Carla Green[1], [1]University of Virginia; [2]University of Geneva

Nocturnin is a deadenlyase, which removes poly(A) tails from its target RNAs, and controls target RNA expression by either enhancing RNA degradation or silencing translation. Nocturnin shows rhythmic expression in many tissues in mice and this rhythmicity is particularly robust in liver. Nocturnin expression is also actely induced by simuli such as serum and TPA in cultured cells. Mice lacking Nocturnin are resistant to diet-induced obesity and hepatic steatosis, indicating Nocturnin may play a role in lipid metabolism. Here we report that Nocturnin expression is regulated by the microRNA miR-122. miR-122 is specifically expressed in liver, and Nocturnin 3'UTR harbors one putative recognition site for miR-122. Overexpression of miR-122 downregulated Nocturnin protein levels, however, the RNA levels remained same. On the other hand, both Nocturnin mRNA and protein expression were up-regulated by knocking-down miR-122, indicating that there may be a complex mechanism(s) involved in regulating Nocturnin expression via miRNA. miR-122 levels are not rhythmic in mouse liver and are not affected by acute stimulations that induce Nocturnin. These results show that Nocturnin expression in the liver is regulated by a post-transcriptional mecahnism. miR-122 is known to be a key regulator of lipid metabolism, and therefore the regulation of Nocturnin by miR-122 might be part of this important metabolic pathway.

P17

The Jumonji Protein Jarid1a/RBP2 Modulates Circadian Rhythms

Luciano De Haro, Sandhya Pulivarthy, and Satchidananda Panda, The Salk Institute

The promoter regions of core clock genes exhibit dynamic and extensive chromatin-remodeling events, the most extensively studied of which has been the rhythmic histone acetylation and deacetylation. Histone proteins are also modified by many other functional groups, including phosphate, ubiquitin and methyl moieties. However, their impact on circadian oscillator function has not been well understood. To address this, we have conducted a candidate-based approach to identify chromatin-remodeling enzymes that may play key roles in the generation and/or maintenance of circadian rhythms. Here, we show that the JmjC-domain H3K4 demethylase Jarid1a/RBP2 is a novel modulator of core clock function whose absence leads to abnormal circadian period phenotype due to altered expression of key oscillator genes.

P18

Biochemical Analysis of PER and BMAL1 Protein Complexes

Kiran Padmanabhan, Cyril Boyault, Charo Robles, Darko Knutti, and Charles J. Weitz, Department of Neurobiology, Harvard Medical School

The mammalian circadian clock is built on a molecular feedback loop in which the heterodmeric transcription factor CLOCK-BMAL1 drives expression of PERs and CRYs, which assemble into one or more multiprotein complexes, enter the cell nucleus, and inhibit the action of CLOCK-BMAL1. The details of this oscillatory mechanism and the factors governing its dynamics are poorly understood. To shed light on how the principal components of the clockwork function, we biochemically purified PER1- or PER2-multiprotein complexes from the livers of mice and BMAL1-multiprotein complexes from a fibroblast cell line. Whole cell extracts and nuclear extracts were subjected to isokinetic sucrose gradient analysis. We identified PER1 and PER2 complexes that ranged in size from around 300kDA to over 1 Mda, with PER proteins exhibiting distinct post-translational modifications in different

complexes. A similar analysis of BMAL1 indicated the presence of separable nuclear complexes that differed at antiphase circadian times. The components of the various sub-complexes are being isolated by co-immunoprecipitation and identified by MALDI-TOF mass spectrometry. With this analysis of PER1, PER2, and BMAL1 multiprotein complexes we hope to reveal essential cogs that operate within the circadian timing machinery.

P19

Mass-Spectrometric Mapping of PER2 Phosphorylation Sites and Analysis of Their Functional Relevance

Jens T. Vanselow*, Andreas Schlosser, and Achim Kramer, Charite, Universitaetsmedizin Berlin

Circadian rhythms in mammals and other organisms are based on cell-autonomous clocks. Molecular clockworks, comprising of transcriptional-translational feedback-loops, generate circadian oscillations. Most core clock proteins are phosphorylated in vivo and display a circadian rhythm in their phosphorylation pattern. The reversible protein phosphorylation regulates e.g. stability, subcellular localization, complex formation, and activity, respectively. A number of circadian phenotypes are attributable to an altered activity of a kinase or even to mutations of specific phosphorylation sites in core clock proteins. One example for the latter is the mutation of a single phosphorylation site in the human PER2 protein, which leads to the Familial Advanced Sleep Phase Syndrome, FASPS. An exact mapping of phosphorylation sites is a prerequisite for a detailed investigation of the role of the reversible protein-phosphorylation in the circadian clockwork. Phosphorylation sites of mPER2 were analyzed by nanoflow-liquid chromatography coupled to tandem mass-spectrometry (nanoLC-MS/MS). For a comprehensive phosphosite mapping, a novel method for the proteolytic protein digestion and the phosphopetide enrichment procedure was established. Furthermore, a software tool, Phosm, was developed for the efficient interpretation of mass spectra for the identification of distinct phosphorylation sites in MS-experiments. 21 phosphorylation sites of endogenous kinases in living cells on mPER2 have been identified by these methods, among them the FASPS-position. Phosphorylation is thought to be a signal for the proteasomal degradation of mPER2. Inhibition of the proteasome, thereby stabilizing protein species which are marked for degradation, led to the identification of further six phosphosites, which potentially induce the degradation of mPER2. The identified phosphorylation sites allow a differential functional analysis of protein-phosphorylation in the circadian clockwork. First results show an increase of the circadian period or the shortening of protein halflife, respectively, by certain phosphosite mutations in NIH3T3 cells. The established methods now allow a systematic mapping of protein phosphosites on single proteins. This permits a detailed research of regulatory functions of the protein-phosphorylation and therefore an understanding of many cellular processes.

P20

Investigating a Role for the Circadian De-Ubiquitinating Enzyme, USP2

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Transcription/translation loops function to sustain endogenous clocks. However, the ubiquitination and de-ubiquitination of proteins can also affect the clockwork and its output. USP2 (Ubiquitin Specific Protease 2) encodes two robustly circadian de-ubiquitinating enzymes called UBP69 and UBP45 whose peak expression closely resembles that of period and cryptochrome (Kita, et al., 2002; Oishi, et al., 2003). These enzymes stabilize known substrates by removing ubiquitins from proteins marked for proteasomal

degradation. To evaluate circadian functions of USP2 we produced mice with a targeted deletion of exons 3 and 4, which includes part of the catalytic domain of both UBP69 and UBP45. These mice exhibit normal circadian behavior but have alterations in total behavioral activity (see Yadav, et al., this meeting). Given the fact that USP2 targets and stabilizes FAS (Fatty Acid Synthase) protein (Graner, et al., 2004) through removal of ubiquitin, we looked at the expression of FAS in mice lacking USP2. FAS was expressed at lower levels in USP2-/- mice, and was arrhythmic in the retina, where FAS expression is rhythmic in WT animals. To assess potential new USP2 targets within the molecular clockwork we measured the transcript expression of several clock genes in USP2+/+ and USP2-/- mice and found Per1 mRNA to be arrhythmic in USP2-/- animals. PER1 protein is also decreased in brain tissue of USP2-/- mice. Our data suggest a potential role for a circadian de-ubiquitination event by USP2 both downstream of the clock (FAS) and within the clockwork (PER1).

P21

Subcellular Localization of the Circadian Deadenylase Nocturnin

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Nocturnin (Noc) is a rhythmic deadenylase (polyA-specific ribonuclease) that in mouse is expressed in multiple tissues at night. Deadenylation induces silencing or degradation in most transcripts. Our hypothesis is that NOC is a posttranscriptional regulator of the rhythmic expression of some circadian-related mRNAs. Here we asked whether mNOC localizes in cell subdomains where ribonucleoprotein complexes (RNPs) are processed. We expressed mNOC with two different tags: a C-term FLAG and an N-term GFP. Both constructions localized in the cytoplasm of NIH3T3 and HEK293 cells showing a diffuse signal, and in some cases, a few perinuclear dots. We then assessed by ICC whether mNOC co-localizes with markers of stress granules (SGs), sites where non-translating mRNAs are stored together with 40S ribosomal subunits, RNA-binding proteins (RBPs), some translational factors, and other proteins. mNOC did not colocalize with either of two SG markers. Next, we tested whether mNOC is in processing bodies (PBs), places where factors involved in 5' to 3' mRNA decay, RBPs, and other mRNA processing proteins are concentrated. mNOC-FLAG was not detectable in these structures. Finally, polysome analyses indicated that endogenous mNOC is not associated with ribosomes or polyribosomes. Our results show that mNOC localizes in the cytoplasm, outside of SGs, PBs, and polyribosomes. However we cannot rule out the possibility that mNOC translocates to some of those structures in other conditions or cell types. In addition, novel cytoplasmic subdomains where RNPs are remodeled have been described, and could represent the dots seen in the mNOC staining.

P22

Casein Kinase I E/D Are Essential Kinases For PERIOD Phosphorylation and the Circadian Clock

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Two kinases capable of phosphorylating PER in vitro and in cell culture are casein kinase Ie and Id (CKIe/d). Studies using cell culture systems suggested that phosphorylation of PER may affect its cellular location, stability and the entire clock mechanism. In Drosophila, genetic studies have demonstrated that DOUBLE-TIME (DBT), an ortholog of CKIe/d, is required for normal phosphorylation and turnover of dPER, and for behavioral circadian rhythms. Although these studies support an important role for CKIe/d in the clock mechanism, it has not been directly demonstrated that CKIe/d are essential for the phosphorylation of PER or other clock proteins in vivo. Moreover, it is unknown how phosphorylation of PER affects its function in the clock mechanism in vivo. To study possible roles of CKIe/d in vivo,

we disrupted activities of both kinases by overexpressing dominant negative mutants of CKIe/d in mPER2:Luciferase MEF and measured how phosphorylation of clock proteins and the clock mechanism are affected. In addition, we assessed how the clock is compromised when mPER:CKIe/d interaction is disrupted by overexpression of the CKIe/d binding domain of mPER2.

P23

Role of Dbt's Kinase Activity in the Circadian Mechanism of Drosophila

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A mutation (K38R) which specifically eliminates kinase activity was created in the Drosophila ckI gene (doubletime, or dbt). In vitro, the DBTK/R protein interacted with PER but lacked kinase activity. In cell culture and in flies, DBTK/R antagonized the phosphorylation and degradation of PER by wild type DBT, and it damped the oscillation of PER level and phosphorylation state in vivo. Nuclear PER was detected at all times of day in the fly eye and in larval neurons expressing the neuropeptide PDF. Overexpression of short-period, long-period or wild type DBT in circadian cells of adult flies produced the same circadian periods produced by the corresponding alleles of the endogenous gene. These mutations therefore dictate an altered "set point" for period length that is not altered by overexpression. Overexpression of the DBTK/R produced effects proportional to the titration of endogenous DBT, with long circadian periods at lower expression levels and arrhythmicity at higher levels. This first analysis of adult flies with a virtual lack of DBT activity in circadian cells demonstrates that DBT's kinase activity is necessary for normal circadian rhythms, and that a general reduction of DBT kinase activity does not produce short periods. The requirements for DBT's kinase activity on transcriptional feedback by PER, on nuclear localization of PER and in different groups of brain neurons are being examined, and a progress report will be presented.

P24

A Role for DOUBLETIME in Bridging CLK Phosphorylation by Other Kinases

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DBT dependent PER phosphorylation is essential for the generation of circadian rhythms in Drosophila. CLK phosphorylation is also regulated by DBT, but how DBT mediates CLK phosphorylation is not known. Flies containing the PERDELTA mutant, which is unable to bind DBT, display low levels of PER and CLK phosphorylation and exhibit no transcriptional repression. We expected that severely hypomorphic dbtar mutants would show a similar phenotype as perdelta, but found these mutants exhibited the opposite phenotype, high levels of PER and CLK phosphorylation and robust transcriptional repression. To reconcile these disparate results, we propose that DBT acts as a bridge to recruit other kinases into PER-CLK-DBT complexes and phosphorylate CLK and PER, thereby promoting transcriptional repression. Subsequent phosphorylation of PER and CLK by DBT promotes their degradation, thereby relieving transcriptional repression. To test this model, we demonstrated DBTAR is present in PER-CLK complexes. CLK is hypophosphorylated in dbtar and perdelta double mutants, confirming that DBTAR regulates CLK phosphorylation through PER binding. Expression of a dominant-negative dbt mutant (dbtK/R) in clock cells of wild-type files reduces PER phosphorylation, but has little affect on CLK phosphorylation. When DBT catalytic activity is virtually eliminated by expressing

dbtK/R in clock cells of dbtar flies, CLK remains hyperphosphorylated, but PER is not phosphorylated. Collectively, our data confirm that DBT bridges other kinases to phosphorylate CLK, and suggest that PER phosphorylation is dependent on DBT catalytic activity. Hyperphosphorylation of PER in dbtar mutants likely results from a defect in phosphorylation of residues responsible for PER degradation.

P25

Tissue-Specific Circadian Regulation of the Two Isoforms of the Orphan Nuclear Receptor RORgamma

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Circadian clocks control various aspects of physiology and behaviour. It was originally thought that the molecular clockwork was identical in all tissues. However, recent work has identified tissue-specificity and our previous work suggests that nuclear receptors of the ROR family may differentially regulate Bmal1 in a tissue-specific manner. This study aims to compare the expression of RORgamma isoforms and to define the mechanisms behind their tissue-specific expression. The tissue-specific expression of the two RORgamma isoforms in peripheral tissues was assessed in wild-type and Clock mutant mice at ZT2, 8, 14 and 20 using quantitative PCR. The regulation of RORgamma isoforms through elements present in their promoters was studied using luciferase assays in COS7 cells. In wild-type animals, the expression of RORgamma in the liver peaked at ZT20, while the expression was not rhythmic in muscle, testis and thymus. The expression of RORgammaT was detected at low levels in liver, muscle and testis and at high constant levels in thymus. In mice with a mutant CLOCK protein, the expression of RORgamma was no longer rhythmic in liver and was expressed at higher levels in muscle. The mutation had no effect on RORgammaT expression. Luciferase assay showed that CLOCK/BMAL1 induced the transcription of RORgamma via its E-boxes, whereas it did not activate transcription via RORgammaT E-box. Lastly, we observed that RORgamma could activate its own promoter. These results give insights into the molecular mechanisms that can lead to differential expression, among isoforms and between tissues, of a single gene.

P26

Rhythmic SAF-A Binding Underlies Circadian Transcription of the Bmal1 Gene

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Although Bmal1 is a key component of the mammalian clock system, little is understood about the actual mechanism of circadian Bmal1 gene transcription, particularly at the chromatin level. Here we discovered a unique chromatin structure within the Bmal1 promoter. The RORE region, which is a critical cis-element for circadian regulation of the Bmal1 gene, is comprised of GC-rich open chromatin. The 3'-flanking region of the promoter inhibited rhythmic transcription in the reporter gene assay in vitro even in the presence of RORalpha and REV-ERBalpha. We also found that the nuclear matrix protein, SAF-A binds to the 3'-flanking region with circadian timing, which was correlated with Bmal1 expression by footprinting in vivo. These results suggest that the unique chromatin structure containing SAF-A is required for circadian transcriptional regulation of the Bmal1 gene in cells.

P27

Temperature Compensation in the Neurospora Clock

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Temperature compensation is a defining, but poorly understood, property of circadian rhythms. We have cloned the genes responsible for the Neurospora clock mutants chrono (chr) and period-3 (prd-3) and find that the products of these genes, CASEIN KINASE 2 (CK2) regulatory (chr, encoding CKB-1) and catalytic (prd-3, encoding CKA) subunits, respectively, influence temperature compensation. In strains bearing a reduced genetic dose of ckb-1, as temperature increases, period length increases, i.e., such strains are distinctly overcompensated. We show that a direct target of CK2, FREQUENCY, the negative arm protein in the core clock, becomes more stable in ckb-1 hypomorphs as temperature increases. By contrast, strains with reduced casein kinase 1 (ck1-a) simply exhibit increased period length at all temperatures; thus, CK2's role is not shared by all common kinases. We have extended our analysis of temperature compensation by looking at other clock-affecting kinases (e.g., pka) and phosphatases (e.g., various pp2a iso-forms) under the control of an inducible promoter, and one of these appears to alter temperature compensation. Thus, surprisingly, over-expression of a particular phosphatase seems to affect temperature compensation in a manner complementary to that of under expressing CK2. We present a model to consolidate these results.

P28

Perturbations in the Nonsense-Mediated Decay Pathway Affect the Neurospora Clock

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RNA surveillance is effected by the nonsense mediated decay (NMD) pathway: incorrectly transcribed or processed messages bearing premature STOP codons can be identified and destroyed. NMD can also affect endogenous genes bearing upstream open reading frames (uORFs). In Neurospora, the core clock component frequency bears a number of uORFs in its 5 untranslated region. We wondered if NMD-mediated regulation of the uORFs in frq might be required for proper clock function. Thus, we knocked out a Neurospora homolog of one of the components of the yeast NMD machinery, upf-1, and had tentatively called this gene nmd-1. We see a significant reduction of period length as well as altered frq profiles; furthermore, we see changes in FRQ dynamics that are consistent with the observed period length reduction. Additionally, in an unbiased screen we have identified another upf whose deletion leads to a similar reduction of period length. Recently, annotation of the Neurospora genome, as well as work from the Feldman lab (unpublished), suggests that a long-standing clock mutant, period-6, is likely a mutation in nmd-1, consistent with our data. Efforts to suppress the effect of nmd-1 mutations by removing frq uORFs in nmd-1 strains have been unsuccessful, suggesting that the clock effect is not mediated by effects on frq specifically. However, we do see a reduction in the stability of FRQ in nmd-1 strains, which is consistent with reduced period length. This suggests that period alteration may be a pleiotropic consequence of removing nmd-1.

P29

The Neurospora Protein Phosphatase 4 Homologue Is an Important Circadian Clock Component by Regulating the Phosphorylation States of the Clock Proteins

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Circadian clocks regulate a wide variety of physiological and molecular activities in almost all eukaryotic organisms and in certain prokaryotic organisms. Post-translational modifications of clock proteins by phosphorylation plays an essential role in circadian negative feedback loops. We previously reported that the Ser/Thr protein phosphatases PP1 and PP2A have distinct functions in the Neurospora circadian clock. PP1 regulates the stability of FRQ and period length, and the loss of a regulatory subunit of PP2A results in low frq mRNA levels. Here we show that ppx-2, a homologue of protein phosphatase 4 is another important component of Neurospora circadian clock. Knock-out mutant of ppx-2 exhibits short period and low amplitude of conidiation rhythms in the constant darkness. In the ppx-2 mutant, FRQ is hyperphosphorylated, which results in faster FRQ degradation and low FRQ levels, suggesting that like PP1, PPX-2 dephosphorylates FRQ and inhibit FRQ degradation. Furthermore, the ppp-1 ppx-2 double mutant shows arrhythmic phenotype. Our results further suggest that PP4 and PP1 have overlapping and distinct roles in the post-translational regulation of the Neurospora circadian clock.

P30

Neurospora CHD-2 Remodels Chromatin at the Clock Gene Frequency and Is Needed for the Epigenetic Transfer of Time

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Transcriptional negative feedback loops are an underlying principle in circadian clock-regulated gene expression and a growing body of evidence indicates that epigenetics and chromatin-remodeling contribute to this process. We have previously determined that multiple ATP-dependent chromatin-remodeling enzymes in the SWI/SNF family function at frequency (frq); this includes the ETL1/SMARCAD homologue, CLOCKSWITCH. We now report that the Neurospora homologue of the chromo-helicase DNA-binding protein, CHD-2, also remodels chromatin at frq and is required for epigenetic transfer of time. Our studies on CHD-2 activity revealed that DNA sequences within the frq promoter are typically methylated and are hypermethylated in ?chd-2 strains. This normal DNA methylation requires both a functional circadian clock as well as the frq-antisense transcript, indicating that clock components contribute to the DNA methylation. Furthermore, the DNA methyltransferase, DIM-2, is required for frq methylation. Phenotypic characterization of ?dim-2 strains indicate a slight phase advance of approximately 2 hours suggesting that DNA methylation, like the antisense transcript, is necessary to establish proper clock timing. These data support a model in which the epigenetic state of time is transferred between dividing nuclei via DNA methylation which is then interpreted by CHD-2 to establish the chromatin state corresponding to the appropriate circadian time.

P31

Circadian Clock Genes in a Tidal Animal

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Many marine organisms coordinate their behaviour and physiology to the movement of the tides which, on most shores, occur twice each solar day with a period of ~12.4 hours. These rhythms persist under constant laboratory conditions, indicating the existence of circatidal clocks. The nature of such clocks, however, remains unclear. For example, are they true circatidal oscillators with an intrinsic period of 12.4 hours, are they circadian oscillators (24.8 hour period) coupled in anti-phase, or unitary circadian oscillators with a bimodal stimulation of behaviour and metabolism? Resolution of this issue depends upon identifying the genetic basis to circatidal timekeeping. To this end we are using the intertidal marine isopod crustacean Eurydice pulchra which displays robust circatidal swimming and metabolic rhythms. Initially we isolated full-length canonical circadian gene homologues including period, timeless, clock, cycle and doubletime/casein kinase1e. Using antisera against crustacean PDH and Eurydice PER we have localised putative circadian clock cells in the nervous system that share similarity with the neuroarchitecture of Drosophila. In heads of animals exhibiting robust circatidal behaviour, quantitative RT-PCR has revealed uniphasic circadian cycling in timeless, but no rhythm in period expression. Therefore, circatidal behaviour is not correlated with tidal patterns of these clock genes. If period and timeless constitute a circadian pacemaker in Eurydice, they may be involved in driving the circadian pattern of chromatophore dispersion we observe in Eurydice exhibiting tidal behaviour. This suggests that distinct circatidal (unidentified) and circadian (timeless dependent?) oscillators operate simultaneously in this animal.

P32

Characterizing a Novel Clock Gene in Neurospora

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Due to its relative simplicity and genetic tractability, the Neurospora crassa clock has become an important model for circadian rhythms. The molecular mechanism of circadian oscillation in Neurospora is well understood and revolves around a negative feedback loop involving the two main components, the FREQUENCY (FRQ)/FRQ-Associated RNA Helicase (FRH) complex (FRC) and the WHITE COLLAR-1/WHITE COLLAR-2 complex (WCC). Several additional factors affecting the core clock have been identified and their role in FRC / WCC dynamics and interaction characterized. While many factors might potentially influence the robustness or output of the clock, the period of the oscillator is generally thought to be tightly regulated by a limited number of tuning factors acting specifically on core clock components. Many of these components were initially identified through forward genetics, proceeding from the rare class of mutations that specifically affect period length. prd-2 is a single-locus, recessive mutation which significantly prolongs period of rhythmical conidiation while remaining robustly rhythmic and generally healthy. We report an initial molecular characterization of the prd-2 phenotype, consistent with an alteration of core clock dynamics. In addition, low-resolution SNP mapping suggested that the genetic locus does not correspond to any known clock components.

P33

Transgenic Drosophila with Conditional Circadian Clock Function

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The identification and characterization of clock gene mutations has been instrumental in uncovering the molecular basis of circadian time keeping. Studies of the molecular circadian phenotypes associated with classical mutations are confounded, however, by indirect and pleiotropic effects. For example, circadian gene expression rhythms are virtually absent from head extracts of adult timeless01, period0, or Clockjrk flies, yet only a small number of circadian transcripts may be controlled directly by CLOCK/CYCLE (CLK/CYC) and TIMELESS/PERIOD (TIM/PER). To help determine how directly different steps in the molecular circadian cycle are connected to one another as well as to the generation of output signals we have generated transgenic flies that conditionally express wild-type or dominant-negative versions of core clock genes using a temperature sensitive expression system. Circadian locomotor activity rhythms could readily be abrogated or restored in these flies. Both period length and rhythmic power could be reliably and reversibly manipulated with environmental temperature in flies with conditional expression of the per gene. Analysis of flies with conditional expression of the cyc gene uncovered a developmental requirement, as adult circadian locomotor behavior could only be restored if cyc had been expressed during previous development. We are currently determining the sequence of molecular responses that results from manipulation of clock gene function in these flies.

P34

Twenty-Four, a Novel Drosophila Circadian Clock Gene, Is Required for Robust Behavioral Rhythms and Wild-Type PERIOD Expression in Pacemaker Neurons

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To reveal novel circadian clock components in Drosophila, we performed a genome-wide overexpression screen, identifying several candidate genes. Here we report on the analysis of one that we designate twenty-four (twf) which results in a long period when overexpressed. Two strong hypomorphic twf alleles as well as RNAi-mediated twf knockdown in clock cells results in weak but long periods. The circadian phenotype of twf hypomorphs was not complemented by deletions that remove the twf locus. Twf expression in the pacemaker ventral lateral neurons is sufficient to rescue free-running locomotor rhythms. Poor rhythms are accompanied by dramatically reduced PERIOD protein levels in pacemaker neurons. PER oscillation is rescued by wild-type twf expression in pacemaker neurons. These effects on PER are specific as other clock proteins, CLOCK, CLOCKWORK ORANGE, PDP1, and PDF were not comparably affected. PER expression driven by heterologous promoter in S2 cells was increased or decreased by TWF overexpression or twf knockdown, respectively. Interestingly, TWF interacts with PER in vitro. Taken together, our data suggest that the TWF-PER interaction may facilitate PER accumulation, in turn, sustaining core clock rhythms.

P35

Regulation of Narrow Abdomen, a Unique Ion Channel That Functions in Circadian Neural Output in Drosophila

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In both Drosophila and mammals, the mechanisms and regulation of circadian neural output remain poorly understood. We have previously demonstrated that the unique ion channel narrow abdomen (na) functions within Drosophila circadian pacemaker neurons, downstream of the molecular clock, to promote locomotor activity rhythmicity (Lear et al., 2005). Recent evidence from mammals indicates that the NA subfamily of channels conduct neuronal sodium leak current, helping to determine resting membrane potential and neuronal excitability (Lu et al., 2007). We are now investigating NA regulation in order to determine whether the expression/activity of this channel may be circadianly regulated. In *C. elegans*, the novel genes unc-79 and unc-80 have been implicated as regulators of the worm NA orthologs (nca-1 and nca-2) (Humphrey et al., 2007; Jospin et al., 2007). unc-79 and unc-80 are highly conserved among animals, and the regulatory relationship appears conserved between *C. elegans* unc-79/ nca and Drosophila unc-79 (dunc79)/ na (Humphrey et al., 2007). We are characterizing the circadian function of both dunc79 and Drosophila unc80 (dunc80). Both dunc79 and dunc80 mutants express little or no NA protein, and both mutants exhibit circadian phenotypes similar to na mutants. We are currently examining the mechanisms by which these novel genes regulate NA, and we would like to determine whether these genes mediate circadian regulation of NA.

P36

Extreme Early-Running Behavior in Cast/Eij Inbred Mice

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Recently, we reported "early-runner" behavior in a subset of mice derived from two diverse strains—CAST/EiJ (CE) x C57BL/6J (B6). These "early-runners" typically initiate wheel-running 4-6 hours prior to dark onset. We identified a major QTL on chromosome 18 for this trait. Here, we tested similar mice under a wider range of conditions. Daily rhythms of activity or sleep were assessed in CE, B6, F1, F2, and backcross progeny, by wheel-running or in a piezoelectric system that scores sleep and wake. A 12h:12h Light:Dark (LD) cycle was followed by several weeks in DD. Instead of intense florescent light used previously, our current conditions utilized green lights at 100-lux. All B6 mice displayed normal activity onsets at dark, while CE mice displayed activity onsets several hours earlier. This was observed in both wheel-running activity and by percentage of sleep-wake using the piezoelectric technology. This was observed in three independent laboratories (UK, SRI, and NWU). In both backcrosses and intercrosses, progeny displayed onsets ranging from eight hours before dark onset to the time of dark onset. The median phase of activity/wake onset for CE mice and early runner progeny was about four hours before dark. Upon release to DD, all mice maintained the phase determined by the prior LD cycle. CE mice, and a high percentage of progeny from CE crosses, provide good models to study the phase relations between behavioral rhythms and their entraining stimuli, and may also provide a model system for human advanced sleep phase syndrome. Supported by NIH Grant MH067752.

P37

Functional Analysis of Mcry1: In Vitro Versus In Vivo

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Cryptochromes are the major regulators of the negative limb of the transcription-translation feedback loops that make up the mammalian circadian oscillator and are indispensable for circadian output such as rhythmic running-wheel behaviour under constant conditions. We have previously performed a detailed structure-function analysis of mouse cryptochrome 1 (mCRY1) in order to identify functional domains. mCRY1 is composed of a conserved core domain (photolyase homology region, PHR) and a distinguishing C-terminal extension (CT). The CT harbours a predicted coiled-coil domain (CC), involved in interaction with mPER1/2 and BMAL, and a bi-partite nuclear localization signal (NLS), which is determinant for nuclear import. Deletion of the CT leads to a complete loss of transcription inhibition capacity. Deletion of the CC, however, produces a protein which no longer can interact with mPERs and BMAL, but is sill nuclear and retains 70% of the capacity to inhibit CLOCK/BMAL driven transcription. The mCRY1-CC protein is partially functional, and therefore is an interesting mutant to analyse in vivo. We generated mCry1CC mice using a knock-in approach that allows cyclic expression of the mutant allele from the endogenous promoter. Homozygous mCry1CC mice are viable and express the knock-in allele at wild type efficiency. Analysis of behaviour and clock gene and protein expression in mCry1CC mice (particularly in a mCry2 deficient background) will allow us to understand how this partially active mCRY1 behaves in vivo and whether it can sustain rhythmicity in the SCN and periphery.

P38

Quantitative Genetic and QTL Analysis of Entrained Circadian Activity Rhythms in Drosophila

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Seventy two recombinant inbred lines of *Drosophila melanogaster* were assayed for entrained expression of circadian activity in a 12:12 LD cycle. Genetic variation, covariation and quantitative trait locus analysis was conducted for overall mean activity levels per ten-minute interval, for mean activity in the light, mean activity in the dark and for the ratio of activity in the light relative to the dark phase of the photoperiod. The same data were also analyzed for the number of ten-minute intervals showing any activity at all as a measure of sleep. Significant genetic variation for each variable, genetic covariation between variables, and respective quantitative trait loci are described.

P39

Genetic Components On Chromosome 13 Regulate Daily Physical Activity Pattern in Mice

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While there is considerable evidence from human twin studies and inbred strains of mice supporting a strong genetic basis for daily physical activity and exercise, little is known about the genetic components regulating the amount and pattern of physical activity in mammals. Substantial differences in daily

physical activity are observed between two commonly used strains C57BL/6J (B6) and A/J, and these two strains have been used to create chromosome substitution strains in which one chromosome from A/J has been substituted onto a B6 background by successive backcrossing. In the present study, we have made a surprising discovery that a single A/J chromosome 13 substitution onto the B6 genome can reproduce almost the entire wheel running phenotype of A/J strain. Chromosome 13 substitution mice C57BL/6J-Chr 13A/J/NaJ (CSS-13, n=13) were recorded and compared to wild type B6 (n=13) and A/J (n=8) controls on a LD 14:10 cycle. During the dark, three was a marked difference in activity between the three strains ($F(2,32)=36.39$, $p=7.4\times10\text{-}9$). The number of wheel revolutions was significantly greater in B6 mice (27315 ± 4095) compared to the A/J mice (17602 ± 1212, $p=2.1\times10\text{-}5$) and the CSS-13 mice (14012 ± 4386, $p=3.2\times10\text{-}8$), whereas no difference between CSS-13 and A/J mice ($p=0.16$). The diurnal profile of the activity rhythm, which also varies between B6 and A/J, is quite similar between CSS-13 and A/J mice. In conclusion, these data indicate that daily physical activity pattern in mice is greatly affected by genetic factors on chromosome 13.

P40

Behavioral Activity Phenotypes in Mice Deficient in the Circadian De-ubiquitinating Enzyme, USP2

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USP2 (Ubiquitin Specific Protease 2) is a deubiquitinating enzyme that stabilizes target proteins through removal of ubiquitin; known targets include fatty acid synthase, but additional targets are likely. USP2 exhibits a robust circadian rhythm at the RNA level, (Kita, et al., 2002, Pharmacogenetics. 12: 51), which depends on wild type CLOCK (Oishi, et al., 2003, J. Biol. Chem. 278: 41519). To evaluate its role in circadian function we have created mice (USP2-/-) carrying a deletion in a portion of the catalytic domain (Exons 3 and 4). USP2-/- mice exhibit normal clock-regulated activity rhythms, with the free running period of 23.59 ± 0.06h compared to 23.44 ± 0.008h in WT animals and are capable of light induced phase delays. Nonetheless, compared to control animals, USP2-/- mice are strongly hyperactive during subjective night under constant dark conditions. These mice also exhibit normal food anticipatory behavior in a restricted feeding paradigm, but when returned to ad libitum feeding subsequent to restricted feeding, they become extremely lethargic and hypoactive. These activity phenotypes suggest that USP2 regulates pathways outside of the clock that are associated with the generation of the activity itself. A known USP2 target, fatty acid synthase, is down-regulated in USP2-/- mice, and we have begun analysis of pathways associated with activity, in a search for additional targets of USP2.

P41

High Resolution Mapping of Frp-1 Using Multiple Strain Advanced Intercross Lines

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Frp-1 (Free running period 1) is a QTL (Quantitative Trait Locus) that affects circadian free running period of locomotor activity rhythm in mice from a (BALB/cJxC57BL/6J)F2 experimental population. The BALB/cJ allele of Frp-1 lengthens free running period ~0.2 hours. A major obstacle in cloning of QTLs in mice is the ability to achieve high resolution mapping for the detected loci. QTL analysis with F2

populations allows mapping to rather wide confidence intervals (~80MB) because of the limited number of recombination events in small chromosomal regions. To overcome this difficulty, we created 12th generation advanced intercross lines from multiple strain combinations (BALB/cJ x C57BL/6J, C3H/HeJ x C57BL/6J or BALB/cJ x C3H/HeJ x C57BL/6J). This design is based on further generations of random mating in order to accumulate recombination events across generations, which expands the map length, and which in turn allows reduction in the confidence interval. Theoretically, a mapping resolution of F12 is 6 times higher than that of F2. Since common inbred strains have been created from a relatively small set of ancestral haplotypes with a large number of recombinations, analyzing segregating populations from multiple strains is also a very powerful approach for high resolution QTL mapping. Combining analysis of the advanced intercross with haplotype analysis from the three strains enabled us to map Frp-1 to within 2MB of mouse chromosome 4. With this level of resolution, we were able to identify candidate genes for further quantitative complementation.

P42

Circadian Period in Chromosome Substitution Strain Mice

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The B.A chromosome substitution strains (CSS) are 21 strains each containing one entire chromosome (Chromosomes 1-19 and X or Y) from the A/J strain while the rest of the genome is from the C57BL/6J (B6) strain. The CSS permit examination of the effect of genome partitioning on complex genetic traits like circadian period. Prior studies show that A/J have shorter periods than B6, and genes on Chr 1 and 12 affect circadian period of locomotor activity. We determined the free-running circadian period of B6, A/J, B.A1 (Chr 1 from A/J) and B.A12 (Chr 12 from A/J) mice using infrared photobeam motion detectors. Period was calculated based on the last 8 days of 14 days in DD. A/J mice had shorter circadian period than B6 mice. B.A1 mice had the same period as A/J. However, B.A12 mice did not differ in period from B6 mice. The results suggest that the quantitative trait genes affecting difference in circadian period between B6 and A/J mice reside on Chr 1. Implications, caveats and future studies will be discussed.

P43

Super-Duper Short: A New Circadian Mutation in Syrian Hamsters

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The homozygous tau mutant hamster, whose free running period in constant darkness (tDD) averages 20h, has proven valuable in physiological and molecular studies of circadian rhythms. We initially observed tDD of 17.6h in 3 animals born to tau mutant parents. Because this is well below the range of tau mutants previously studied, we suspected a spontaneous mutation and undertook to systematically breed these hamsters. The precision of circadian activity rhythms of 29 such "super-duper short" animals (tDD =18.2+.07h, mean+SEM), as determined by the error of the fit of activity onsets in DD, was 0.66+0.12h, comparable to that of wild types (tDD=24.04+0.03h; error of fit 0.55+0.12h). Crossing of super-duper short animals to hamsters of the parental tss produces t=18 and t=20 in an approximately 1:1 ratio. F2 pups produced by crossing super-short F1 animals have thus far shown only the tDD=18h phenotype. The super-duper short animals entrain to 14L:10D, but activity onset precedes dusk by about 10h. A phase response curve constructed for 15min light pulses on day 10 of DD is clearly Type 0, with a crossover around CT15 and maximal shifts of as much as 12 circadian hours. Quantification of Per induction in SCN by such pulses and cloning and sequencing of CK1e are in progress. Supported by NIH RO1MH70019.

P44

Natural Allelic Variation in Circadian Clock Function in Brassica Rapa

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The geographic range of species can be broad, particularly among domesticated species that are widely cultivated. Crop species encounter widely differing environmental conditions, including variable daylength and temperature, across their latitudinal ranges. In many plant species, including Brassica rapa, daylength modulates the circadian clock and thereby affects flowering time. In addition, both clock function and flowering time are influenced by ambient temperature. There is considerable natural variation in the circadian clock, flowering responses to daylength, and temperature responsiveness, and this genetic variation undoubtedly contributes to the ability of these species to thrive in diverse environments. To date, efforts to elucidate the plant circadian clock mechanism have emphasized Arabidopsis thaliana. We have used the circadian rhythm in cotyledon movement to extend this study to the crop plant, Brassica rapa. We have analyzed a set of Recombinant Inbred Lines (RILs) derived from two diverse parents, R500 and IMB211 and have identified Quantitative Trait Loci (QTL) for period, amplitude and temperature compensation of the circadian rhythm in leaf movement, as well as for a number of morphometric parameters, including flowering time, size of floral organs, and hypocotyl length. The status of our efforts to identify the genes responsible for these QTL will be discussed. We have observed transgressive segregation of each of the traits studied, which suggests that there may be considerable potential for the modification of circadian clock function as well as floral traits in Brassica rapa crops grown under agroecologically relevant daylength and temperature settings.

P45

Length Variation in the Chinook Salmon (Oncorhynchus Tshawytscha) Clock Polyq: Does It Contribute to Seasonal Differences in Migration Timing?

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Circadian rhythms are the external expression of an endogenous clock that initiates temporal coordination of biological processes. The molecular machinery underlying these rhythms is conserved among taxa. We partially characterized duplicated Clock genes from Chinook salmon (Oncorhynchus tshawytscha) designated OtsClock1a and OtsClock1b. Comparative genomics revealed that these paralogs likely arose subsequent to the Salmonidae autotetraploidization event. We employed these genes as candidates for migration timing, an important adaptation of anadromous salmon. Based on length variation in the PolyQ domain of OtsClock1b, we found evidence for significant genetic differentiation between two temporally divergent migratory runs of Chinook salmon from two unique study systems: Feather River, California; and the South Island of New Zealand. Tests for selective neutrality indicate that OtsClock1b is likely under selection in both systems. Next, we examined Clock variation among 42 migratory runs in North America and found evidence of a latitudinal cline in OtsClock1b PolyQ allele frequency as well as average allele length. Compared to the frequency distribution of microsatellite alleles, the OtsClock1b alleles deviate from neutral expectations suggesting that the observed clinal variation is likely maintained by selection. Furthermore, a hierarchical gene diversity analysis of OtsClock1b PolyQ variation revealed that run timing explains 43.7% of the overall genetic variance among populations. In contrast, analysis of microsatellite data shows that run timing explains only 8.1%. Thus, evidence presented here suggests that OtsClock1b may influence the migration timing of Chinook salmon, supporting the notion that genes controlling daily rhythms may also regulate seasonal behavior.

P46

Evolution of Teleost Fish Circadian Clock Genes: Preservation of Different Ancient Duplicate Circadian Clock Genes in Different Teleost Fishes

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Interrogation of the five teleost fish genomes uncovered 19 per genes, 12 clock genes and 14 bmal genes. Phylogenetic and splice site analyses showed zebafish (Danio rerio) have per1a, per1b, per2a and per3; clock1a, clock1b, and clock2; and bmal1a, bmal1b, and bmal2a; fugu (Takifugu rubripes) have per2a, per2b, per1b and per3; clock1a, clock1b, and clock2; and bmal2a, bmal2b and bmal1a; tetraodon (Tetraodon nigroviridis) have per2a, per2b, per1b and per3; clock1a, clock1b but no clock2; and bmal2a, bmal2b and bmal1a; medaka (Oryzias latipes) have per2a, per2b, per1b and per3; clock1b and clock2; and bmal2a, bmal2b and bmal1a; stickleback (Gasterosteus aculeatus) have per2a, per2b, per1b but no per3; clock1b and clock2; and bmal1a, bmal2b. Further, syntenic analysis indicated per1a/per1b, bmal1a/bmal1b in zebrafish; clock1a/clock1b in zebrafish, fugu and tetraodon; per2a/per2b in fugu, tetraodon, medaka and stickleback; bmal2a/bmal2b in fugu, tetraodon, medaka are ancient duplicates. Relative rate tests showed that asymmetric evolutionary rates are observed in most of these ancient duplicate pairs, implicating one of the duplicates in these circadian duplicate pairs have been under positive selection or relaxed functional constraint since the duplication. Taken together, the extra copies of fish circadian clock genes were generated from the fish-specific genome duplication, divergent resolution including reciprocal gene loss after the duplication led to retaining different circadian clock duplicates in different fishes, most of which have diverged significantly since the duplication. It appears that like their diverse morphology, teleost fishes have evolved intricate and diverse timekeeping mechanisms that likely have contributed to their remarkable radiation.

Cellular and Developmental

P47

Increased Coherence of Cellular Rhythms in Mature NIH 3T3 Fibroblast Cultures

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Over the last decade NIH 3T3 fibroblasts have been a valuable platform for studying mammalian circadian rhythms in vitro. A significant problem in interpreting 3T3 experimental data, however, is introduced by the progressive phase desynchonisation between cells, leading to an overall loss of apparent amplitude over 3-4 cycles. To overcome this, base-line subtraction or single-cell assays are usually required. We observed, however, that under long-term (> 1 month) confluent culture conditions, 3T3 transcriptional rhythms (reported by bmal1::luc) continue to increase in coherence until barely distinguishable from those in SCN explants. This effect correlated with an increase in culture density, and could be mimicked by the application of cell cycle inhibitors. We conclude that mature fibroblast cultures will be of great utility for high-throughput pharmacological and mutational circadian screening.

P48

Actin's Role in Signaling Early Night Phase Shifts in Rodent SCN

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The cells of the circadian clock in the mammalian suprachiasmatic nucleus undergo a nearly 24-hour cycle of tightly controlled cellular events. Light signals from the eye are capable of changing the state of these cells in a time-dependent manner. In the early night, light signals are transmitted by the neurotransmitter glutamate (Glu), acting via an NMDA-R/nitric oxide (NO) pathway and causing a delay in the clock. This pathway also requires the release of stored Ca2+ through neuronal ryanodine receptors (RyR), and this Cai2+ has been shown to activate a set of proteases known as calpains. Calpains can act on cytoskeletal actin filaments (F-actin), dynamic structures that form intracellular networks involved in a variety of processes, including cell migration, neuronal plasticity, and even cell signaling. Our data support the hypothesis that activating the light signaling pathway leads to a reorganization of the actin cytoskeleton, and that this cytoskeletal rearrangement is a necessary component of the pathway leading to a phase delay. Additionally, light and Glu previously have been shown to induce transcription of the clock genes Per1 and Per2. Inhibiting the induction of these two genes using antisense ODN blocks the phase delay. We show here that blocking actin reorganization using Jasplakinolide inhibits Per2 induction and phase delay following Glu treatment. These findings provide evidence that changes in the cytoskeletal state are essential to SCN phase shifts in the early night in response to Glu exposure and that these cytoskeletal state changes are required to access the molecular clockworks.

P49

Atypical PKC Signaling in Circadian Plasticity in Rat Suprachiasmatic Nucleus

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Light pulses can reset the mammalian circadian clock at specific times: in early night, light delays clock phase, whereas in late night, light advances it. Glutamate (Glu) is the primary messenger of light from eye to the suprachiasmatic nucleus (SCN). In early night, Glu signaling acts through elevation of intracellular Ca2+, mediated by Ca2+ influx via NMDA receptor activation and intracellular Ca2+ release through neuronal ryanodine receptors (RyR). The circadian response to light is among the most basic forms of neural plasticity resulting from an exogenous stimulus. Studies of long-term potentiation (LTP), the best understood form of neuronal plasticity, provide clues to understanding circadian regulation. Atypical protein kinase C (aPKC), especially PKM, the independent catalytic domain of aPKC, has been reported to play important role in the maintenance of LTP. We hypothesized that aPKC contributes to circadian state changes stimulated by Glu signaling. We found that two forms of a PKC, PKC and PKM are expressed in rat SCN. Atypical PKC activity and protein level are increased significantly by Glu stimulation in early night. Additionally, specific aPKC inhibition, by either the pseudosubstrate peptide or antisense oligodeoxynucleotide, blocks Glu-induced phase delay, demonstrating a requirement of aPKC in Glu signaling to the clock. aPKC colocalizes with glycogen synthase kinase-3GSK-), a known substrate, in cells throughout the rat SCN. Importantly, Glu-stimluated phosphorylation of GSK-3 in the early night is abolished by the aPKC pseudosubstrate peptide. Together, the data suggest that aPKC mediates Glu-induced phase delays by regulating the activity of GSK-3 This research was supported by PHS grant HL086870.

P50

Calcium Signaling Induced by Glutamate in Rat SCN Neurons

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Mammals' daily rhythms of physiology and behavior are synchronized to day and night by the action of the signal of environmental light on suprachiasmatic nucleus (SCN). Glutamate (Glu) is the primary neurotransmitter that conveys photic information from the retina to SCN. The effect is specific to the time of day: in early night, this signal induces phase-delay of the clock, but phase-advances it in late night. Ca2+ plays a pivotal role in the Glu signaling. Using two-photon laser microscopy, we here studied neuronal Ca2+ signaling in the rat SCN brain slice. We found that Glu at CT 14 induces a strong intracellular Ca2+ (Ca2+i) increase, and this response can be abolished by tetracaine, a ryanodine receptor (RyR) inhibitor. On the other hand, Glu can induce only a small Ca2+i increase at CT 19, and this response is insensitive to tetracaine. Furthermore, high potassium (K+) treatment at either CT 14 or CT 19 induces a strong Ca2+i increase, and such response is sensitive to RyR inhibition. These findings indicate that the RyR-regulated Ca2+i store can be activated at both CT 14 and CT 19 by a strong depolarizing stimulus, high K+, but only at CT 14 can Glu activate this store. Thus, RyR-regulated Ca2+i release may be a bifurcation point for Glu signaling, but the Ca2+i store is activatable and potentially regulatory at both CT 14 and CT 19.

P51

Getting There on Time: NO/cGMP Signal Transduction and Circadian Entrainment

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The NO/GC/cGMP/PKG pathway is essential for light synchronization of the circadian clock. Phosphodiesterases (PDEs) are regulators of intracellular cyclic nucleotide concentrations. To study the regulation of cGMP levels in the hamster SCN, we have determined by RT-PCR the presence of PDEs in this model. In hamsters receiving specific PDE5 inhibitors (sildenafil, vardenafil or taladafil), reentrainment to a 6h phase-shift of the LD cycle took significantly shorter than controls. PDE5 inhibitors also elicited an increase in light-induced phase advances when injected 45 min before light stimulation at CT18. No differences were observed in either reentrainment rates after a delay in the LD cycle, or light-induced phase delays after a light pulse at CT14. We have studied the role of nitric oxide (NO) in the intercellular communication within the dorsal and ventral portions of the SCN. Administration of the NO scavenger 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO) blocked photic phase advances in a dose-dependent manner and inhibited light-induced cFos-ir, without affecting phase delays. In addition, preliminary results show an inhibition in the non-parametric entrainment to 23.5 h cycle (L:1 D:22.5) in hamsters receiving a single dose of PTIO before light stimulation. These results demonstrate that pharmacological inhibition of PDE5 or NO scavenging affects photic entrainment, indicating a potential benefit for circadian disorders which require an increase in light signaling to the clock. These findings could serve as a basis for pharmacological treatment for optimizing circadian adaptation to environmental changes, including transmeridian flight schedules (jet-lag). Also, a role for extracellular NO in the intra-SCN communication is suggested.

P52

Forskolin Triggers the Circadian Oscillation in the Mouse Immortal Hepatocytes

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Not only peripheral organs but also cells in culture can maintain the circadian oscillation of gene expression. Different agents are used for synchronisation of cells, including mediators of the cAMP response, such as forskolin. Majority of synchronisation studies have been performed on mouse and rat fibroblasts but little is known regarding the circadian behaviour of cell lines form other peripheral organs. Oscillating immortal hepatocytes in culture would be a useful model to study mechanisms responsible for the circadian behaviour of metabolic output genes in the liver. We performed the forskolin shock (10uM forskolin for 2 h) of the mouse hepatoma cell line Hepa1 and measured by real time PCR the expression of Dbp and Bmal1 in a 48-hour interval. After the immediate early increase of both genes that has been observed also in fibroblasts, the two measured circadian transcription factors start the cyclic expression with the opposite phase.The metabolic output gene Cyp51 from the cholesterol synthesis has a circadian phase similar to Bmal1. This is consistent with results from the circadially sampled mouse liver and is followed by the circadian expression of the CYP51 protein. As forskolin is a mediator of cAMP response, the circadian expression/binding of cAMP inducible transcription factors to CRE elements in promoters of cAMP responsive genes represents a possible cause for the cyclic expression of output genes in these synchronised cells.

P53

Synchronization Pathways in the Fibroblast Clocks

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Individual fibroblasts contain a molecular oscillator, which is composed of interlocked negative feedback loops. They generate circadian rhythmicity when they are stimulated by appropriate signals. However, the molecular mechanisms of synchronization of circadian clocks are not well understood. It has been shown that Dexamethasone (Dex) and Forskolin (Fsk) synchronize Rat-1 fibroblasts in a distinct manner from each other. We extended this analysis on immortalized MEF (Mouse Embryonic Fibroblasts) isolated from PERIOD2::LUCIFERASE mice (designated here as MEF/P2L). Although MEF/P2L exhibits synchronized rhythm by medium change, robust oscillations were induced by Dex and Fsk treatments in a concentration dependent manner, as compared to solvent controls (0.0005% EtOH and 0.1% DMSO, respectively). These oscillations, however, are affected by serum concentrations in the assay medium. As in the case with Rat-1 fibroblasts, Dex and Fsk show distinct patterns of rhythmicity that is different from each other, in MEF/P2L. Further characterizations of Dex- and Fsk-stimulated pathways may reveal important molecular details about the phase-resetting mechanisms in the peripheral tissues.

P54

Down Regulation of Circadian Clock Gene Period 2 Accelerates Breast Cancer Growth by Altering Its Daily Growth Rhythm

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Circadian organization is a fundamental property of life. Circadian clocks coordinate life processes within organisms and coordinate those organisms with their circadian environments. Mammalian circadian clocks consist of two interacting molecular transcription/translation feedback loops comprised of some nine core clock genes that beat about once every 24 hours. This molecular clockwork resides within each cell in the body, including cancerous cells. Per2, a core circadian clock gene, has tumor suppressor properties, and is mutated in spontaneous human breast cancers. We demonstrate that down regulation of functional Per2 gene expression increases Cyclin D and Cyclin E levels and doubles in vitro breast cancer cell proliferation. Down regulation of Per2 also accelerates in vivo tumor growth. This acceleration is accomplished by altering the circadian organization of tumor growth. Per2 down regulation triples the daily amplitude of the tumor growth rhythm and stably delays the daily peak times of this rhythm by 4 hours. Therefore, PER2, and perhaps other clock genes, represent a new class of potential therapeutic targets whose manipulation will modulate cancer growth and cancer cell proliferation.

P55

Expression of Clock Genes in Mouse Embryos

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Circadian molecular oscillations are present in nearly all animal tissues and organs. In mammals, "clock gene" rhythms in peripheral tissues are entrained to environmental cycles by the suprachiasmatic nucleus (SCN) through physiological systems and/or behavior. In the tissues examined, the expression of hundreds of genes also oscillate, influencing a wide range of cellular processes including cell proliferation. Some clock genes are known to be expressed in mammalian embryos (Johnson et al., 2002; Saxena et al., 2007), and preliminary data suggested time of day variation on Ell and E19 in whole embryos and fetal liver, respectively (Miller and Takahashi, personal communication). Like peripheral tissues of the mother, embryos are likely to be exposed to maternal rhythms in physiology and/or behavior. In the present study we surveyed the expression of four clock genes (per2, bmal1, cry1, clock) in mouse embryos from E8 to E18 (just before birth) using real-time polymerase chain reaction (RT-PCR). On E10, E14, and E18, embryos were collected and RNA extracted every 4 hours. On E18, RNA was also extracted from liver only. We found expression of clock genes at all ages but could not detect rhythms in either whole embryos or in livers. In contrast, robust circadian oscillations from E16 SCN and liver were measured in vitro using a luciferase reporter of PER2 protein levels (PERIOD2::LUCIFERASE mice; Yoo et al., 2002; mice from M. Harrington with permission from J. Takahashi), indicating that fetal tissues can generate oscillations. The apparent discrepancy between these approaches with respect to oscillations in the mammalian embryo are discussed.

P56

Circadian Genes Are Expressed during Early Development in Xenopus Laevis

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We have examined the developmental expression of genes known to regulate circadian rhythms in order to better understand the ontogeny of the circadian clock in a vertebrate (Xenopus laevis). In this study, core circadian oscillator genes (xPeriod1, xPeriod2, and xBmal1) as well as a rhythmic, clock-controlled gene (xNocturnin) were analyzed using in situ hybridization. No differences in expression pattern or level were observed in similarly staged embryos (from neurula to late tailbud stages) maintained in a 24 hour light-dark cycle (LD) at two circadian time-points (dawn and dusk). All genes examined were present in the developing nervous system (brain, eye, olfactory pit, otic vesicle and in decreasing levels in the spinal cord). These genes were also expressed in the developing somites and heart, with surprisingly unique expression patterns in various peripheral tissues, such as the pronephros, cement gland, and posterior mesoderm. Quantitation by real time RT-PCR of xBmal1 expression was performed in eyes isolated at dawn, midday, dusk, and midnight from embryos maintained in a 24 hour LD cycle. Rhythmic expression was not detected at stage 31 (ANOVA; p=0.176) but stage 41 eyes showed significantly increased levels of xBmal1 expression at midnight (RQ=1.98+/- 0.094) when compared to dawn (RQ=1+/-0.133; p=0.0004). We hypothesize that when circadian genes are not co-expressed in the same tissue during development that it may indicate pleiotropic function. Our preliminary results show that although clock genes are present during early brain and eye development that rhythmic gene expression occurs at later stages of development beyond stage 31.

P57

CLOCK Expression Is Restricted to Oscillator Neurons in Adult and Larval Brains and to Presumptive Oscillator Neurons in Developing Embryos

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The Drosophila circadian oscillator is composed of transcriptional feedback loops in which CLOCK-CYCLE (CLK-CYC) heterodimers activate their feedback regulators per and tim via E-box mediated transcription. These feedback loop oscillators are present in distinct clusters of dorsal and lateral neurons in the adult brain, but how this pattern of expression is established during development is not known. Since CLK is required to initiate feedback loop function, defining the pattern of CLK expression in embryos and larvae will shed light on oscillator neuron development. Using a novel CLK antiserum, we show that CLK is first detected in presumptive small ventral lateral neurons (s-LNvs), dorsal neurons 2s (DN2s), and dorsal neuron 1s (DN1s) during embryonic stage 16 (ES16), well before oscillator function is initiated in L1 larvae. PER then accumulates in all CLK-expressing cells except presumptive DN2s during late ES16 and ES17, consistent with the delayed accumulation of PER in adult oscillator neurons and antiphase cycling of PER in DN2s. PER is also expressed in non-CLK-expressing cells in the embryonic CNS, suggesting a role for PER outside the clock. Although PER expression in CLK negative cells continues in ClkJrk embryos, PER expression in cells that co-express PER and CLK is eliminated. The pattern of CLK expression established in embryos persists in larvae. These data demonstrate that brain oscillator neurons begin development during embryogenesis, that PER expression in non-oscillator cells is CLK-independent, and that CLK is required for PER expression in developing brain oscillator cells.

P58

Modeling the Circadian Clock : From Molecular Mechanism to Physiological Disorders

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Based on genetic and biochemical advances on the molecular mechanism of circadian rhythms a computational model for the regulatory mechanism of the circadian clock in mammals has been proposed. The model shows how the interlocked positive and negative regulatory feedback loops involving the Per, Cry, Bmal1, and Clock genes produce sustained circadian oscillations in continuous darkness. When incorporating the induction of Per expression in the light phase, this model also accounts for entrainment of the circadian clock by light-dark cycles. Besides the insights that it provides into the molecular mechanism of circadian oscillations, the model can be used to examine the dynamical bases of circadian clock-related physiological disorders in humans, such as the Familial advanced sleep phase syndrome (FASPS) or the Delayed sleep phase syndrome (DSPS). The lack of entrainment observed in numerical simulations can be associated with another sleep-wake cycle disorder known as the Non-24h sleep-wake syndrome. The computational approach suggests biological systemic strategies to restore normal periodic behavior of the circadian clock.

P59

Modeling Circadian Enhancers with Comparative Genomics

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Several experimental and computational studies show that partner elements around circadian E-boxes in insects and mammals influence such enhancers. Here we build a probabilistic sequence model for a 25bp sequence located in the center of the 69bp period enhancer reported in Hao et al., and also in the majority of known CLK/CYC targets in flies. The model is trained on multiple alignments of fly core clock genes and reveals a motif composed of a canonical E-box (E1) separated by 6 or 7 base pairs from a more degenerate version thereof (E2). We tested our model with functional genomics datasets and we found that CLOCK-induced genes were enriched in high scoring cis-elements. Surprisingly the fly model also predicts many known CLOCK/BMAL1 targets in mouse. This finding is supported by a phylogenetic analysis showing the evolutionary conservation of the E1-E2 structure in core circadian and output genes in vertebrates, fishes and insects. To study the importance of E2 in mediating protein-DNA interactions, we tested our consensus sequence by EMSA. We observe significantly different patterns of shifted proteins for the original and the mutated version of the consensus. Our data show that both E1 and E2 bind proteins, but with different affinities. Moreover a cooperative binding of the two complexes is likely, as indicated by the affinity for doubly occupied sequences compared to single ones. Supershift assays confirmed that shifted bands contained CLOCK/BMAL1. This suggests that E1-E2 can be bound by one or two CLOCK/BMAL1 heterodimers, but does not fully exclude the involvement of other binding proteins.

P60

A Mathematical Model of Neurospora Crassa Circadian Rhythms Provides Insights to Molecular Mechanisms

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The existence of biological clock is observed from cyanobacteria to humans. In all cases, a conserved transcription-translation time-delayed negative feedback loop is observed. Additional feedback loops are intertwined with this negative feedback loop. Due to the complexity of multiple feedback loops, a mathematical model provides a better understanding of the underlying system. In Neurospora crassa, the clock protein FREQUENCY (FRQ) oscillates with a period of about 22 h. FRQ inhibits its own synthesis by interacting with its transcription factor, WHITE COLLAR 1 (WC-1). Our model recapitulates phase differences observed between frq mRNA and FRQ protein, FRQ and WC-1 proteins, and distinctively different total vs. nuclear ratios of FRQ and WC-1. The nuclear FRQ is lower than the nuclear WC-1 even though the total FRQ is greater than the total WC-1. Due to the limiting amount of FRQ in the nucleus, it's possible to hypothesize that FRQ would act as a direct or indirect catalytic component inactivating WC-1, as oppose to non-catalytic physical binding and inactivation of WC-1. In our studies, however, we demonstrate that a non-catalytic, direct linear interaction of FRQ with WC-1 in the nucleus generates robust oscillations even though the nuclear FRQ is significantly lower than the nuclear WC-1.

P61

An Experimentally Integrated Approach towards Modelling the Arabidopsis Clock

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Mathematically defined models of cellular circadian rhythms have recently emerged for many experimental organisms. Because they are largely based on time series of transcriptional data, most models, by necessity, rely heavily on data fitting to find system parameter values, which have either not been derived experimentally or have been described qualitatively. In order to further refine our model for the Arabidopsis clock, we are systematically quantifying key mechanistic parameters. In the first instance, using isothermal titration calorimetry, we have determined the dissociation constant of the LHY/CCA1 dimer for its cognate DNA sequence. By integrating mathematical and experimental approaches to describe this system, we hope to obtain a more detailed understanding of the molecular mechanisms that sustain circadian rhythms in plants.

P62

Oscillator Stability and Phase Diffusion in Common Circadian Clock Models

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Circadian Clocks are cell-autonomous molecular oscillators with an approximate 24h period.

In the absence of entrainment signals or coupling to other cells, one expects that intrinsic and extrinsic noise at the cellular level perturbs the clock phase and hence will drive a cell population to desynchronization. To study the dephasing kinetics, it is necessary to understand how the noise perturbs a particular clock network. It is expected that the stability properties of the limit cycle orbit will affect its response to noise and ultimately influence the dephasing rate. Here we study how the dephasing

rate depends on model parameters, and whether the most important dependencies are shared across a library of models. We investigate whether the relation between the dephasing rate and oscillator stability predicted in simple phase models holds for more realistic descriptions of circadian gene networks. Our preliminary analysis shows that while the stability of the cycle does indeed contribute significantly, the complete dependency involves other geometric properties of the cyclic orbits. Through this in silico analysis we expect to identify principles that allow circadian timing systems to tick accurately in the presence of molecular and environmental fluctuations.

P63

Phase Response-Based Model Reduction Improves Analysis of Clock Models

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The primary function of a circadian clock is to regulate the timing of an organisms daily behaviors. A properly functioning clock coordinates with the environment, adjusting its phase to environmental cues, or zeitgebers. Thus, fundamental to the study of the molecular machinery of a clock is the study of each mechanisms contribution to the phase response behavior. Mathematical modeling is important to understand this complicated dynamical behavior at the gene regulatory network level. As more is learned about the biology of clocks, models are becoming increasingly complex, making it more difficult to discern the roles of the various components and feedback loops in terms of phase resetting. We present a novel model reduction technique that preserves the phase response behavior of a limit cycle model. We apply the technique to a model of the mouse clock, and illustrate the insights gained from studying the reduced models both of a single cell and of SCN tissue (i.e. a population of coupled cells). In particular, we show that one negative feedback loop is sufficient to maintain the phase response behavior of this multi-loop model. Additionally, we show that preserving the phase response behavior of a single cell preserves emergent characteristics of a coupled population, for example the populations period and ability to synchronize spontaneously.

P64

Understanding the Dynamics of Two-Oscillator Circadian Systems Forced by Two Independent Zeitgebers

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Experimentally observed phenomena such as forced desynchronization, or intriguing behavioural patterns under two simultaneously acting, contradictory Zeitgebers (e.g. light/dark and feeding cycles) have recently been the subject of quantitative modeling studies. In many cases, these phenomena were explained by hypothesizing a circadian system consisting of two coupled circadian oscillators, for instance a light-entrainable/food-entrainable (LEO/FEO) or an evening/morning system. In the present work, we feature a top-down modeling approach to study the complex dynamics exhibited by a two-oscillator system under the action of two independent, periodic drivers, complementing more detailed bottom-up modeling approaches. Simulations carried out using the Neurodynamix II software show that for very weak positive and symmetric inter-oscillator coupling, linear changes in the phase relationship between the Zeitgebers elicit characteristic non-linear responses in the phase relation between the two oscillators. With increasing coupling strength, complex phenomena like phase-jumps and bistability appear. The same effects caused

by increasing inter-oscillator coupling can be observed by decreasing environmental forcing strengths. We discuss the implications of our results on the interpretation of many specific chronobiological problems. Supported by FAPESP (06/61276-0).

P65

Using a Linear Fractional Analysis to Understand Rod/Cone and Melanopsin Contribution to Responses to Monochromatic Light Stimuli at Different Irradiance Levels

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We sought to determine analytically the contribution of rods/cones (mechanism A) or melanopsin (mechanism B) to light-induced pupillary constriction. Data were used from a study in which wild-type (mop+/+), melanopsin knockout (mop-/-)(mechanism A) and rodless-coneless(rd rd/cl)(mechanism B) mice were exposed to a 1-minute 480nm monochromatic light pulse over a large irradiance range (Lucas et al. 2003). We fit each dataset of normalized pupil radius as a function of log irradiance (logI) by a 4-parameter logistic function: the exponent values were for mechanism A 0.25 and for mechanism B 0.70. For any chosen pupillary radius, the logistic fits give different irradiances for the different genotypes, IA and IB. Let IC represent the stimulus required when both contribute to pupillary constriction (wild-type animals). The two mechanisms make fractional contributions IC/IA and IC/IB, with (IC/IA)+(IC/IB)=1. Consequently IC=(IA*IB)/(IA+IB). When IA<<IB, IC is approximately IA; when IB<<IA, IC is approximately IB. The Lucas et al. data demonstrate these properties: the response of wild-type animals to irradiances under 11 logI was well fit by mechanism A and to irradiances above 12.5 logI was well fit by mechanism B. Between 11 and 12.5 logI, the IC formulation is required for a good fit to data. Since very different exponents characterize the A and B mechanisms, no single 4-parameter logistic function can fit wild-type response accurately over the full irradiance range. This should be examined for other physiologic responses for which combinations of response components may be required, including other light wavelengths, melatonin suppression, and circadian phase-shifting.

P66

Wavelet-Based Analysis of Rhythmicity and Period Shift in Gene Expression from SCN Neurons

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Determining rhythmicity in patterns of gene expression presents challenges which have been addressed by a variety of mathematical approaches, each with its advantages and disadvantages. Fourier-based methods—the most commonly used—require a stationary process, while biological systems show cycle-to-cycle variability. The results from Fourier analysis of such systems can therefore be very volatile. Wavelet-based analysis provides a flexible and robust alternative. We provide three applications of wavelet techniques to problems in analysis of circadian signals:

1. Classification of signals as rhythmic or arrhythmic on a circadian scale via wavelet ANOVA; 2. Filtration of traces to identify features of interest; 3. Identification of period shifts by direct examination of wavelet coefficients. We chose to analyze PERIOD2-mediated bioluminescence recorded from SCN cells plated at low density. These data have 1-h resolution, tend to be relatively low amplitude, and show a wide

range of periods and cycle-to-cycle variability. We demonstrate that these methods are more robust than Fourier-based methods for the real signals recorded from SCN cells, which tend to show non-stationarity. Several cells classified as aperiodic by Fourier methods can be shown to have strong but transient periodic components in the circadian range, and conversely some cells classified as weakly periodic were found to have total power in the circadian range consistent with Gaussian noise. In addition, some cells clearly displayed a shifting of power from one frequency band to another over time, strongly suggesting that the period of oscillation of individual cells is not always constant. When applied to model data from a discrete stochastic simulation of SCN neurons, we show that wavelet-based methods allow comparison of fundamental properties of simulated data to real data, allowing an alternative method for assessing the model accuracy. We conclude that wavelet-based analyses can reveal novel circadian properties from real data, and serve as a starting point to develop more elaborate methods for the analysis of real and simulated circadian oscillators.

P67

Ultradian and Circadian Rhythms: Experiments and Models

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The importance of both ultradian and circadian rhythms in Nature is well known. In the literature it is possible to find many articles referring to the characteristics and properties of such rhythms and, in some cases, of their mathematical modeling. However, as far as we know, there is not enough information relating these types of rhythms either from a biological or mathematical point of view.

In this work we describe the main properties of the crayfish circadian rhythm structure and its mathematical simulation through different developmental stages and experimental situations. During the work we revealed the persistence of the ultradian rhythms even when they are masked by circadian rhythms. Moreover, when transients were analyzed in both models, biological and mathematical, we were led to consider the hypothesis that ultradian rhythms could represent a regression to a primordial vital dynamic state. Finally, we report on certain new experiments and their mathematical model, where we can observe the initial presence of a circadian rhythm and its subsequent regression to again an ultradian one by the influence of an external disturbance. Supported by IN200206 DGAPA grant.

P68

Synchronization in a Network of Noisy Circadian Neurons

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Besides the properties of the individual cells, the network topology of an ensemble of oscillators can have significant affects on the dynamics of the ensemble. In an investigation of the synchronization properties of mammalian circadian pacemaker cells in the suprachiasmatic nucleus (SCN) some of the network properties of interest are the size of the network, its connection topology, and the weighting of the connections. We present a stochastic model of an ensemble of circadian neurons consisting of the gene regulatory network in each neuron and vasoactive intestinal polypeptide (VIP) intercellular signaling which activates per mRNA transcription. A phase synchronization order parameter is measured for different connection topologies, weighting, and levels of stochastic noise. As stochastic noise is increased by lowering the volume, a cut-off is reached below which network topologies with low connectivity fail to synchronize.

P69

Biochemical and Network Modeling of the SCN

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In recent years, quantitative modeling has emerged as an additional tool complimenting experimental techniques in the study of circadian rhythms. Here we develop a detailed mathematical model for circadian timekeeping within the suprachiasmatic nucleus (SCN). Our proposed model consists of a large population of SCN neurons, with each neuron containing a network of biochemical reactions involving the core circadian components. Central to our work is the determination of the models unknown parameters, which were obtained from comparing the models output to experimental data. From these estimated parameters, additional experimental tests of the model are proposed. Our studies highlight the importance of low numbers of molecules of clock proteins and how this fact affects the accuracy of circadian timekeeping.

P70

Dynamics of Polymorphic PERIOD3, a Mathematical Model

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While the cyclic expression of PERIOD proteins PER1 and PER2 is fundamental to the mammalian circadian oscillator, the clock protein PER3 plays a more subtle role. A variable number tandem repeat polymorphism in the human PER3 gene affects markers of sleep homeostasis and cognitive performance without affecting circadian markers such as plasma cortisol or melatonin concentration. We present a mathematical model for intracellular PER dynamics incorporating this PER3 gene polymorphism. The model allows for variations in homeostatic sleep regulation while remaining consistent with the experimental results on the circadian phenotypes.

P71

An Integrated Model of Sleep-Wake Regulation with a Dynamic Circadian Oscillator: Jetlag, Morning-Types and Evening-Types

P.Y. Chen and A.J.K. Phillips*, and P.A. Robinson, University of Sydney

We construct a biomathematical model of the sleep-wake cycle, by coupling a physiologically-based model of the ascending arousal system with Jewett and Kronauers van der Pol oscillator model of the suprachiasmatic nucleus (SCN). This yields an integrated model which includes both circadian and homeostatic influences on the human sleep cycle, and is capable of quantitatively simulating jetlag, shift work, and morning/evening-types. Morningness and eveningness are simulated by varying model parameters such as circadian period, photic exposure, sleep duration, and circadian and homeostatic strengths within physiologically realistic ranges. The model predicts that a decrease in the strength of the homeostatic drive relative to the circadian drive results in morningness, consistent with empirical studies of age-related morningness. Additionally, the model predicts that increasing the endogenous period of the SCN results in eveningness. Jetlag is simulated by a rapid change in the phase of photic input, and the model realistically predicts reentrainment dynamics following transmeridian flights. Different photic schedules are compared, and under an ideal schedule in which photic input is temporally constrained to the most effective circadian phase for reentrainment, the model predicts no asymmetry between circadian phase advance and phase delay. Under nonideal photic schedules, the model reproduces the empirically observed

preference for phase delays. Hence, the model offers novel advice for travelers, suggesting circadian phase advance after eastwards flights beyond 8 timezones, if the treatment is close to ideal. Furthermore, by providing a physiologically-based theoretical framework, the model allows us to directly relate various entrainment phenomena to their underlying causes.

P72

On the Richness of Temporal mRNA Expression Patterns Following Light and Temperature Entrainment in Drosophila

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Environmental variables such as light and temperature induce gene expression responses in *Drosophila melanogaster*, and the 24 hours periodicity of such stimuli entrain the endogenous circadian clock with the environment. Recently, time series of mRNA abundances in Drosophila heads were measured with Affymetrix arrays in various conditions: 12h-light/12h-dark cycles (LD), 12h-ambient/12h-cold (AC) cycles, and constant conditions following each of the predicted cycles (AA and DD). A subset of about 50 genes robust circadian transcripts exhibit an advance of about 6 hours in ACAA compared to LDDD, which corresponds to the time lag between the minimum of the light level and the subsequent temperature minimum during the night, by clear sunny days. Here we extend the analysis of the relationship between profiles and phases elicited under either entrainment. We apply a cross-correlation analysis allowing to discuss patterns that are more general than those found using Fourier methods. We observe delays between light and temperature stimuli which are consistent with the original study, but also find new patterns, e.g. genes that have anti-phasic expression patterns in light and temperature, or genes that respond with richer waveforms, e.g. showing two peaks per day. Our analysis suggests the existence of multiple pathways relaying the temporal cures from environmental stimuli in interaction with the circadian clock.

P73

Small World Network Models of Intercellular Coupling in the Suprachiasmatic Nucleus

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The suprachiasmatic nucleus (SCN) of the hypothalamus is a multioscillator system that drives daily rhythms in mammalian behavior and physiology. Although intercellular communication within the SCN has been the focus of significant experimental effort, little is known about how SCN cells synchronize to each other to coordinate behavior. We previously developed a multicellular, molecular model of the mammalian circadian clock that incorporated recent data implicating the neurotransmitter vasoactive intestinal polypeptide (VIP) as the key synchronizing agent. This model assumed that cells were locally connected and that connection strengths were inversely proportional to the distance between cells due to the effects of VIP diffusion. Here, we consider an alternative connectivity scheme based on small world networks that contain both short and long range synaptic connections for VIP mediated coupling. Individual SCN cells exhibit experimentally observed heterogeneities in uncoupled oscillator phenotype (sustained and damped oscillators) and VIP release characteristics (VIP producing and non-producing cells). We show that the small world network models are able to robustly synchronize despite heterogeneities in individual cells and network connectivity as long as the number of long range connections is sufficiently large. The model is used to investigate the hypothesis that age related circadian

dysfunction is attributable to degeneration of the VIP coupling mechanism in the SCN rather than impairment of individual cell function. We find that partial removal of long range connections produces several experimentally observed behaviors including reduced VIP and CREB oscillation amplitudes in individual cells, a decrease in the mean period of the population, a higher percentage of non-oscillating cells, and reduced synchrony. The model thus predicts that loss of a few long range connections in the SCN can have large effects on circadian rhythmicity. Supported by NIH grant 78993.

P74

Quantitative Modeling of Sleep Deprivation: Advantages of Physiologically-Based Modeling

A.J.K. Phillips* and P.A. Robinson, University of Sydney

A physiologically-based quantitative model of the human ascending arousal system is used to make quantitative predictions about recovery from sleep deprivation in humans. The model includes circadian and homeostatic drives to the sleep-wake switch of mutual inhibition between monoaminergic nuclei and the ventrolateral preoptic area. It is calibrated using a small number of physiological and dynamical constraints, so as to reproduce normal human sleep-wake behavior and realistically respond to sleep deprivation. The resulting model is able to account for normal variability, explain pathologies including narcolepsy, and predict realistic repayment of sleep debt in both quantity and distribution. Recovery from deprivation is dependent on the circadian phase at which sleep occurs, and the model predicts that an optimal recovery routine should place sleep in phase with the circadian cycle, if amount of recovery sleep is to be minimized. The idea of wake playing a role in the recovery of sleep debt is also explored. We then make direct comparison between our model and the two-process model, identifying key similarities and differences. Hysteresis behavior is shown to be common to both models, but in our case it is understood as a direct consequence of the underlying physiology, rather than the product of arbitrary rules. Finally, we demonstrate the advantages conferred by physiologically-based modeling, by presenting the first accurate simulation of sleep latency times following sleep deprivation.

P75

Promoter Analysis of Mammalian Clock Controlled Genes

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Numerous studies reveal that about 2-10% of genes in mammalian tissues are clock controlled (CCGs) manifesting circadian oscillations in their mRNA levels. Mechanisms generating these oscillations are still debated. We apply bioinformatic tools to predict transcription factors (TFs) important in the regulation of CCGs. From selected published microarray studies we assemble a list of 2065 CCGs expressed in several mammalian tissues. We scan promoter regions of the genes in the search for overrepresentation of putative regulatory motifs as compared to a background set of promoters of genes not reported to be oscillating and having a matching promoter GC-content. The same overrepresentation search procedure is repeated on lists of genes expressed in specific tissues and sharing the same expression phase. We narrow our predictions to the clock-related TFs that either appear on our CCG list or have CCGs among their TRANSFAC annotated target genes. We estimate the false discovery rate by measuring the ratio of the overrepresented clock-related motifs to the overall number of clock-related motifs in TRANSFAC. The redundancy among the predictions is analyzed using motif similarity estimation methods. These procedures result in the identification of TFs whose binding sites are overrepresented in the promoters of all CCGs and of CCGs

specific for tissues or certain expression phases. Many of the known clock regulatory elements as E-boxes, D-boxes, and ROR elements are among our predictions. Our results indicate putative regulators of the circadian processes, participating in clock output pathways and synchronizing peripheral tissues.

P76

Phase and Amplitude Responses to VIP in the SCN Are Influenced by Cell-Cell Synchronization

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Vasoactive intestinal polypeptide (VIP) is thought to generate and sustain circadian rhythmicity and synchrony of the neurons in the mammalian suprachiasmatic nucleus (SCN). Exogenous VIP application phase shifts firing rate rhythms of SCN neurons. To begin to address the mechanisms that mediate VIP-induced shifts in the population rhythm, we treated organotypic slices of SCN from PER2::LUC mice with 10uM VIP at defined circadian times. The PER2::LUC waveform in these SCN slices exhibited large phase delays in the late subjective day to early night (CT5-CT18), and smaller phase advances in subjective early day and late night (CT0-5 and CT18-24). VIP application also induced changes in the amplitude of PER2::LUC rhythms with a transient increase when treated at CT6-7 or 11-12 and a transient reduction when treated at CT0-2, 9-10, 13-15 and 16-17.

We used a mathematical model of a population of neurons coupled via VIP to further explore possible underlying mechanisms. This model includes the canonical transcription/translation negative feedback loop in each cell. Then, 100 heterogeneous circadian oscillators were coupled via VIP binding which activates a signaling pathway that induces PER transcription. To match the experimental phase response curve, we introduced phase dependent sensitivity via modulating the expression of the VIP receptor by a circadian oscillator component. Results from this model suggest that VIP application at subject night desynchronizes the cells' rhythms leading to dampening of amplitudes, while VIP application at other circadian times increased synchronization and increased amplitudes. Supported by NIH grants 678993 and 63104.

P77

Arousal Responses to External Stimuli

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A recent neurophysiological model of sleep-wake dynamics is extended to incorporate external stimuli. The model includes the mutual inhibition of the sleep-active hypothalamic ventrolateral preoptic area (VLPO) and the wake-active monoaminergic (MA) brainstem populations as well as circadian and homeostatic drives. Our proposed framework describes an arbitrary stimulus in terms of its relative effect on the VLPO and MA nuclei. The drives span a two-dimensional drive space, a representation that yields detailed insights into the impact of perturbations from normal sleep-wake `flip-flop dynamics. The power of this system stems from its quantitative physiological formulation in which external influences are modeled according to their known neurological mechanisms. Resulting nonlinear neuronal dynamics can be calculated and used to infer time-dependent arousal state information. To demonstrate the approach, a mathematical model of caffeine is presented. Sleep delay and arousal enhancement following caffeine ingestion are explained in terms of its known adenosine antagonism. The impact of short, impulsive stimuli is also explored, with sleep fragmentation used as an illustrative example. Predictions of the recovery time

from a given acoustic stimulus as a function of its strength and the timing of its application are analyzed. Concepts developed here are expected to facilitate a more thorough understanding of how external influences modulate arousal. The ability to compare diverse pharmacological, sensory, and other stimuli on a unified scale of arousal impact, for example, has a potential clinical application in treating sleep-related disorders such as sleep apnea.

P78

A Systems Approach to Elucidate Network Structures of the Circadian Clock

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In biology, nonlinear behavior such as oscillations has been postulated to emerge from simple genetic structures. Pathways that employ these networks such as the circadian clock regulate cellular and organismal physiology and behavior. In this study, we have undertaken a systems approach to delineate the network structures of the mammalian circadian clock. Using a robust human oscillatory cellular model and RNA interference, we depleted known clock components in dose response and measured the consequences on oscillator function using kinetic imaging. Only a small portion of the perturbations resulted in severe disruption of oscillator function, even when multiple components were knocked down, highlighting the robustness of the circadian oscillator in maintaining rhythmicity over a wide range of disruptive conditions. Gene dosage networks were constructed using mathematical modeling and by applying known biochemical constraints. Several properties emerge from these networks, including proportional and disproportional genetic switches and paralog compensation of clock components. In addition to revealing many known and unknown aspects of oscillator function, these results highlight the power of systems biology in uncovering regulatory mechanisms of nonlinear pathways

P79

Gene Expression Analysis Grouping with Gener-Rhythm v.2.3

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The information obtained from gene expression analysis is fundamental in the exploration and understanding of natural biologic processes. It is relevant that genes that share a particular circadian, biologic, or temporal pattern may be involved in similar biologic processes and pathways. Gene expression can be regulated in order to inhibit or prohibit the functional part of any gene. Therefore, the analysis of this important information could render an understanding of how certain diseases proliferate, such as cancer, and will enable researchers and physicians alike an opportunity to battle diseases more affectively. We have created a tool that takes information from gene expression analyses, as raw or recoded data, and sort these data in such a way that genes are grouped together that share a common cyclic pattern. The Gener-Rhythm? v.2.3 software package allows this functionality and outputs extraordinary information, but has such a general use that any temporally based data can be analyzed and grouped together in this way.

P80

Synchronization and Singularity Behavior of a Cyanobacterial Circadian Clock In Vitro and In Silico

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Biological clock has been considered as limit cycle oscillator. In general, limit cycle oscillator has a phase singularity, the point at which phase is ambiguous and near which phase takes on all values. For example, it was reported that the specific stimulus at the specific time induces arrest of the circadian clock in mammalians cell (Ukai et al. 2007) and chlamydomonas (Johnson and Kondo 1992). Ghosh et al. (1971) mixed two yeast cells suspensions with different phases of metabolic oscillation and observed the phase of the mixture. They realized the mixture composed of the specific phase suspension had an unsettled phase. Recently, our group found that the cyanobacterial circadian clock could be reconstituted in vitro only by mixing the three clock proteins, KaiA, KaiB, KaiC, with ATP. Namely, the ratio of phosphorylated KaiC woscillates every 24hr in the mixture (Nakajima et al. 2005). To reveal this reconstituted system act as singularity behavior, we explored a singularity point in this assay. We prepared different phase circadian clocks (every 2 hr) and mixed them all combination, and then, we observed phase shift. As a result, we found the mixture with an ambiguous phase, in other words, a singularity point. Additionally, we attempted to make the toy model to illustrate the synchronization and singularity behavior of KaiC phosphorylation rhythm by using phase description. Furthermore, by using this model, we checked the robustness of phosphorylation rhythm of KaiC in vivo situation.

P81

Computing Cortisol Secretion Times with a Biophysical Model

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Cortisol regulates many vital biological processes, increases with stress, increases during sleep deprivation, and is associated with learning. Essential to the study of cortisol are methods that can accurately detect the timing and amplitude of each cortisol secretory pulse. We have developed a statistical approach using a biophysical model for identifying the time and amplitude of cortisol pulse secretion from human experimental data. Monte Carlo techniques are used to compute 25 model parameters; each parameter has a physiologic basis. We applied the model to plasma cortisol concentration data from two healthy women in whom plasma cortisol was assessed every ten minutes during a 24-hour constant routine. MATLAB routines were used to perform the Monte Carlo simulations and data analyses. The Monte Carlo simulations were run for 200,000 iterations and converged in approximately 100,000 iterations. After excluding the first 100,000 iterations, the marginal probability density was evaluated. The results for the two women, respectively, include: (1) the number of pulses : 20 and 16, (2) cortisol plasma infusion rate : 0.033 (1/min) and 0.017 (1/min), and (3) plasma clearance rate : 0.0317 (1/min) and 0.0108 (1/min). This work allows extraction of physiologic information from cortisol data in the form of model parameters. The

work will allow for the identification of potential mechanistic explanations for different observed cortisol rhythms, the effects of circadian phase or amplitude, and determination of parameter distributions for individuals in different experimental or pathological conditions. Support: US AFOSR F49620-95-1-0388, NIH NCRR-GCRC-M01-RR-02635, T32 HL07901-10 (DAD), NIH K02-HD045459 (EBK), NIH R01 AR43130 (GKA), AFOSR FA9550-06-0080, NSBRI HPF00405, R01 MH071847, and R01 EB006385 (ENB).

P82

The Implications of the Theory of Nonspecific Influences

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The "theory of non-specific influences", as a new approach to creation of means and methods of therapy, was proposed. This approach consists of a search for the most "nonspecific" influences of any nature, and implies that the point of application of these influences in an organism should be the highest level in its hierarchical system of biological rhythms. The higher the influence level in hierarchy of biological rhythms, the more nonspecific influence it will have, and the more the expected effect of harmonization of subordinated to the main oscillator functional systems it will be. Because neuroglia possibly stars in protection of mammals' circadian organization at the levels of the whole organism and retina being a universal carrier of nonspecific environmental influences, which transfers both external and internal oscillatory effects to the organism, the expediency of the future research directed to the study of the possibilities of nonspecific influences on glial nets of the retina and brain, and glio-neuronal interactions is substantiated. It is necessary also to pay especial attention to the theory of dynamical systems corollary, namely: whatever weak would be the influences applied on an organism, as an open nonlinear dynamical system; there always exists danger to bring a powerful response about from this system, threatening to become an unpredictable dangerous response to the application of the external factor. Elaboration new schemes of chronotherapy taking into account such positions of the theory of nonlinear dynamical systems, as the deterministic chaos and stochastic resonance, seems to be actually important.

P83

Understanding the In Vivo Behavior of the Kai Protein Oscillator through In Vitro Characterization

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In the in vitro Kai protein circadian oscillator, four phosphoforms of KaiC accumulate in a cyclic pattern arising from the KaiA-modulated autokinase and autophosphatase rates of KaiC, with one of the phosphoforms exerting negative feedback on KaiA via KaiB. How does this protein oscillator function in vivo, where it experiences conditions—for example, de novo protein synthesis and oscillations in protein concentrations—not experienced by the in vitro system? To gain insight into the Kai protein oscillator's operation in vivo, here we explore its behavior in vitro under a variety of conditions. We characterize the scaling of the period, amplitude, and phosphoform abundances with the concentrations of KaiA and KaiC. We find that the period of oscillation is remarkably stable under varying KaiA concentrations. We also examine oscillator behavior and the underlying kinetic rate constants and feedback properties obtained with various Kai protein mutants. We refine our previously-reported mathematical model of the oscillator to account for these observations. This enhanced model will provide a framework with which to understanding the robustness of the oscillator to the many perturbations it experiences in vivo.

P84

Discovery-Based Approaches to Identifying New Circadian Peptides in SCN

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The mammalian suprachiasmatic nucleus (SCN), site of the central circadian clock, generates a near 24-h timebase, synchronizes to environmental light-dark cycles, and coordinates rhythms of physiology and behavior throughout brain and body. Key to these functions is a complex suite of peptide-based cell-to-cell signaling molecules. To determine the chemical complexity of secreted intercellular messengers, peptides secreted at the SCN were collected using novel solid phase extraction strategies and evaluated using mass spectrometric-based peptidomic techniques. Stimulation paradigms reported to alter clock timing, including electrical stimulation of the retinohypothalamic tract (RHT), produce peptide-rich, stimulus-specific releasate profiles. The peptides include established circadian neuropeptides as well as peptides with no reported circadian function. One of these peptides, little SAAS, is released from the SCN endogenously as well as following a phase-delaying stimulus in early night and was selected for further characterization. Exogenous application induces a significant phase delay consistent with light-mediated cues regulating circadian timing. The proSAAS precursor is present in rat SCN across the circadian cycle. Little SAAS-positive cells exhibit a unique, regional-specific distribution pattern. Whereas little SAAS expression does not overlap with vasopressinergic neurons in the SCN shell, it is present in both the vasoactive intestinal peptide (VIP)-expressing ventrolateral core and the gastrin-releasing peptide (GRP)-expressing central regions. These findings demonstrate that discovery-based approaches to analyzing peptidergic signaling within the SCN are effective strategies for expanding our understanding of circadian physiology. Funded by P30 DA018310 to the UIUC Neuroproteomics Center on Cell to Cell Signaling (JVS).

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Proteomic Characterization of the SCN

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The timing of all biological processes in mammals is orchestrated by the core circadian clock situated in the suprachiasmatic nucleus (SCN) of the brain hypothalamus. A small group of essential genes constitute a molecular feedback loop which drives biochemical, metabolic and behavioral rhythms throughout the organism. These clock genes typically exhibit an oscillating pattern of protein expression over a 24 hour day. Beyond this handful of clock elements, it is estimated that between 2-10 % of all mammalian genes display circadian rhythmicity in transcriptional levels (Kondratov and Antoch [2007] TRENDS in Cell Biol.,17: 311-317). Clearly a vast number of unknown players may contribute to biological timekeeping. We have undertaken a proteomic survey of the whole rat SCN to identify novel proteins which show daily variation in expression. We compared SCN gene products at 3 different times of day by subjecting tissue lysates to two-dimensional difference in gel electrophoresis (DIGE) followed by protein identification by mass spectrometry. We identified approximately 30 proteins that show differential expression between day and night. These gene products mediate a variety of cellular functions, including metabolism, cytoskeletal structure and stress response. Furthermore, we are applying proteomic techniques to specific functional subcompartments of the SCN. Direct interaction of proteins with the cytoskeleton has proven to be an

unexpected means to regulate the circadian clock. We are using these proteomic methods to identify SCN gene products which directly interact with filamentous-actin (F-actin). These technologies offer us a new tool-set for circadian gene discovery and characterization. Supported by PHS grant P30 DA 018310.

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Co-localization of the Vesicular Transporters for Glutamate and GABA (VGLUT and VGAT) in Neurons from the Anterior Paraventricular Thalamic Nucleus Projecting into the SCN

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The anterior Paraventricular Thalamic Nucleus (aPVT) is reciprocally connected with the suprachiasmatic nucleus (SCN) of the hypothalamus, which is a circadian pacemaker in mammals. There is evidence that lesion of aPVT abolish the phase advances of circadian rhythmicity induced by light pulses. Electrophysiological studies indicate simultaneous release of GABA and Glutamate from aPVT into SCN neurons (see abstract by Alamilla-Gonzalez and Aguilar Roblero at this meeting). This work is aimed at determine whether GABA and Glutamate co-localize as transmitters in the PVT neurons that project to the SCN. We dissociated aPVT neurons by enzymatic digestion and gentle mechanical desegregation, and collected them with a micropipette into eppendorf tubes. Each neuron was studied by real-time PCR for the presence of mRNA for the vesicular transporter for GABA (VGAT), the vesicular transporter for Glutamte (VGLUT) and the housekeeping gene GADPh (as control). From 20 neurons analyzed 5 were negative to all mRNAs including the housekeeping control and were discarted. 7 of the remaining 15 neurons (46%) showed VGAT, VGLUT and GADPh mRNA, and the remaining 8 (54%) showed only VGLUT and GADPh but no VGAT mRNA. These results clearly indicate co-localization of both neurotransmitters in the same neuron. Together with the electrophysiological evidence provided in the adjoining poster indicate co-release of GABA and Glutamate in aPVT terminals projecting to the SCN. Suported by CONACyT and PAPIIT IN227107

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Asynchronous Neurons in the SCN of Adult VIP Knockout Mice Exhibit Preservation of Pacemaker Characteristics

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Vasoactive intestinal polypeptide (VIP) signaling is crucial for robust behavioral and SCN rhythms. Previous studies show that SCN from neonatal VIP knockout or VPAC2 knockout mice (Mus musculus) have depressed rhythmicity in clock gene expression, a lack of robust neural synchrony, and a marked reduction in the proportion of rhythmic to non-rhythmic neurons. To assess the role of VIP in adult SCN, VIP-KO mice were bred to short half-life Per1::GFP mice developed in our lab. Coronal slices (200u) of SCN were explanted from adult male mice and Per1::GFP rhythms were imaged for the first 90 hours in vitro with time-lapse laser-scanning confocal microscopy and then analyzed with MetaMorph (Molecular Devices). SCN neurons from VIP-KO mice were rhythmic, although lower in Per1::GFP expression than heterozygotes and wildtypes. SCN of VIP-KO mice (N = 15 animals; 578 neurons) exhibited significantly greater neuronal phase dispersion than heterozygous (N = 14 animals; 939 neurons) and wildtype (N =

12 animals; 625 neurons) siblings. The proportion of rhythmic to non-rhythmic neurons per animal was comparable among VIP-KO and sibling heterozygotes and wildtype mice. Characteristics of individual cell clocks—such as Per1::GFP risetime, peak width and peak-to-peak time—did not differ across genotypes. Taken together, these results suggest that whereas VIP-KO mice may exhibit reduced Per1 expression, they possess cellular clocks of similar number and function to wildtype mice. Thus, a primary role of VIP in the SCN is to mediate cellular synchrony that leads to robust behavioral output. This research was supported by NIH T32 MH064913 and NIH/NIMH F31 MH080547 to CMC, NIH/NINDS F32 NS051183 to KLG, and NIH/NIMH RO1 MH63341 to DGM.

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Simultaneous Release of Glutamate and Gaba in Suprachiasmatic Neurons Induced by Stimulation of the Anterior Paraventricular Thalamus

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The anterior paraventricular thalamus (aPVT) projects to the suprachiasmatic nuclei (SCN), and this projection is necessary to phase advance circadian rhythms by light pulses during late subjective night. We characterized the postsynaptic responses of SCN neurons to aPVT stimulation by means of whole cell patch clamp recordings using acute sagittal brain slices, in rats. SCN monosynaptic responses to aPVT stimulation followed low (5Hz) and high (20 Hz) frequencies. At a holding potential around -40 mV they showed both excitatory- (EPSP) and inhibitory-postsynaptic potentials (IPSP). Administration of glutamate receptor blockers APV and DNQX abolished the EPSP and isolated IPSPs. Bicuculline administration abolished IPSPs isolating the EPSPs. Similar results were founded by minimal stimulation; further strychnine application did not modified the SCN responses to aPVT. These responses were found at either subjective-day or subjective-night recordings. We conclude that aPVT terminals simultaneously release glutamate and GABA into SCN neurons. Supported by CONACyT and PAPIIT IN227107

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Impact of the Loss of VIP On Rhythmic Gene Expression in the SCN and Peripheral Organs

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The neuropeptide vasoactive intestinal peptide (VIP) is required for the normal functioning of the circadian system. The loss of VIP or its receptor, VPAC2R, produces profound deficits in circadian rhythms in wheel running activity. Work with VPAC2R-deficient mice has led to the suggestion that VIP/VPAC2R signaling is essential for the generation of circadian oscillations in gene expression within the SCN. In this study, we sought to examine how the loss of VIP affects rhythmic gene expression in the SCN. For these experiments, VIP-deficient and WT mice were sacrificed at CT 2, 6, 10, 16 and 23 as determined by locomotor activity. In situ hybridization was used to measure Per1, Per2, and Bmal1 mRNA within the SCN. We found no evidence of rhythmicity in the levels of Bmal1 in the SCN of the VIP-deficient mice. Per2 levels were blunted compared to WT and appeared to exhibit a bimodal expression pattern. Surprisingly, we found clear circadian oscillations in the levels of Per1 in the SCN although the amplitude of this rhythms was reduced compared to WT. In the peripheral organs (heart, liver, adrenals), we used quantitative RT-PCR to measure gene expression. The loss of VIP did not affect the rhythms in Per2 expression in any of these tissues. In contrast, the rhythms in Per1 were blunted in the adrenals and heart but not the liver of the mutant mice. These data demonstrate that the impact of the loss of VIP varies with

the tissue and the specific clock gene. Among other issues, future work will need to address how the loss of VIP has such a potent impact on rhythmicity in Bmal expression within the SCN circuit.

P90

In Vivo Monitoring of Circadian Timing in Freely Moving Mice

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In mammals, the principal circadian pacemaker driving daily physiology and behavioral rhythms is located in the suprachiasmatic nucleus (SCN) in the anterior hypothalamus. The neural output of SCN is essential in the circadian regulation of behavioral activity. Although remarkable progress has been made in revealing the molecular basis of circadian rhythm generation within the SCN, the output pathways by which the SCN exert control over circadian rhythms are not well understood. Most SCN efferents target the subparaventricular zone (SPZ), which resides just dorsal to the SCN. Because of its relationship with the SCN, this output pathway has been proposed as a major component involved in the outflow for circadian regulation, based on lesion studies in rats. We have examined the downstream pathway of the central clock by means of multi-unit neural activity (MUA) in freely moving mice. The results demonstrate that SCN neural activity is tightly coupled to environmental photic input and anticorrelated with MUA rhythm in the SPZ. In Clock mutant mice exhibiting attenuated circadian locomotor rhythmicity, MUA rhythmicity in the SCN and SPZ is similarly blunted. These results suggest that the SPZ plays a functional role in relaying circadian and photic signals to centers involved in generating behavioral activity.

P91

Tetrodotoxin Increase Responsiveness of the Suprachiasmatic Nucleus Circadian Pacemaker to External Perturbation

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The role of synaptic communication was examined in the functions of circadian pacemaker in the mouse suprachiasmatic nucleus (SCN) which is consisted of multiple oscillating cells and at least two regional pacemakers. The circadian rhythms in Per1 and Bmal1 expression in the cultured SCN were measured continuously by mean of bioluminescence reporters, and effects of tetrodotoxin (TTX), a sodium channel blocker, and subsequent medium exchange at various phases of the circadian rhythms were examined on the circadian periods, amplitude and phase-responsiveness. TTX shortened the circadian periods of both rhythms and reduced the amplitude of circadian rhythm in Per1 but not Bmal1 expression. TTX application at any circadian phases did not change the phase of circadian rhythm significantly. On the other hand, medium exchange performed five days after TTX application produced phase-dependent phase-shifts in both circadian rhythms. The extents of phase-shifts were significantly larger in the TTX pretreated SCN culture than those observed in the TTX non-treated SCN. The shape of phase response curve (PRC) for medium exchange was different between the two circadian rhythms, and only a phase-advance portion was detected in the Per1 expression rhythm, whereas both phase-advance and phase-delay portions similar to the light-pulse type PRC were observed in the Bmal1 expression rhythm. As a result, medium exchange at particular circadian phases produced internal desynchronization between them, suggesting different oscillatory systems underling the two circadian rhythms. These findings indicate that the synaptic communication in the SCN integrates a number of oscillating neurons and stabilizes the circadian oscillation in the SCN.

P92

Abnormal Circadian Rhythms in Mice Deficient for the Kv3.1 and Kv3.2 Channels

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The ability to generate intrinsic circadian rhythms in electrical activity appears to be a unique property of mammalian SCN neurons and one essential to the function of the circadian timing system. In previous work, we determined that a specific potassium current known as the fast delayed rectifier (fDR) has a diurnal rhythm in SCN neurons. We also demonstrated that the Kv3.1 and Kv3.2 channels that generate this current are expressed in the SCN. Moreover, pharmacological blockade of this current prevents circadian rhythms in neural activity. We concluded that the fDR is necessary for the circadian modulation of electrical activity in SCN neurons, and represents an important part of the ionic basis for the generation of rhythmic output. To test this model, we have generated mice deficient in both Kv3.1 and Kv3.2 channels and are evaluating the impact of the loss of these channels on circadian rhythms in wheel running and gene expression. These double mutant mice have striking deficiencies in wheel running activity, many of which exhibit bimodal activity patterns. Furthermore, exposure to light in early subjective night results in smaller phase delays in the double mutants. We also have preliminary data indicating a reduced amplitude of the rhythm in Period2 expression in the double mutant mice. Understanding the ionic mechanisms underlying the generation of circadian rhythms in electrical activity in SCN neurons is a critical issue and the fDR current is presently the best candidate for a specific current responsible for this rhythm in firing rate in mammals.

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Andrew Vosko, UCLA

In mammals, the precise circadian timing of many biological processes depends on the generation of oscillations in neural activity of pacemaker cells in the suprachiasmatic nucleus (SCN). The ionic mechanisms that underlie these rhythms are important for translating intracellular events to regulate circadian rhythms at a systems level. Previous work in the SCN has demonstrated that SCN neurons express A-type potassium currents. In other neurons, A-type currents are critical regulators of action potential frequency. Here, we demonstrate that the magnitude of A-type potassium currents exhibits a diurnal rhythm that peaks during the day in the dorsal region of the SCN. While ventral SCN neurons also express the A-type current, the magnitude does not vary with time of day. Importantly, this rhythm continues in constant darkness, providing an important demonstration of the circadian regulation of an intrinsic voltage-gated current in mammalian cells. Finally, we found that members of the Kv4-family of potassium channels are highly expressed within the SCN. Together with previous studies, the present work indicates that the A-type potassium current represents an important component of the ionic basis for the generation of rhythmic output from SCN neurons.

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BK Channels Regulate Spontaneous Action Potential Rhythmicity in the Suprachiasmatic Nucleus

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The basis for intrinsic rhythmicity is generally understood to rely on transcription factors encoded by "clock genes", but little is known about how the SCN consolidates and communicates a daily time signal. Action potentials in the SCN are necessary for behavioral rhythm expression, and individual SCN neurons modulate their spontaneous action potential firing patterns over the circadian cycle, suggesting that the

daily patterning of SCN activity is necessary for normal behavioral rhythm expression. The BK K+ channel plays an important role in suppressing spontaneous firing at night in SCN neurons. Deletion of the Kcnma1 gene, encoding the BK channel pore-forming subunit, causes degradation of circadian behavioral and physiological rhythms. To test the hypothesis that loss of robust behavioral rhythmicity in Kcnma1-/- mice is due to the disruption of SFR rhythms in the SCN, we used multi-electrode arrays to record extracellular action potentials from acute wild-type (WT) and Kcnma1-/- hypothalamic slices. Patterns of activity from neurons within the SCN were tracked simultaneously for up to 3 days, and the phase, period, and synchronization of SFR rhythms were examined. Loss of BK channels decreased the amplitude of the circadian variation in SFR and increased arrhythmicity. Unexpectedly, Kcnma1-/- SCNs also showed increased variability in the phase peaks of the SFR rhythm and a modest increase in period. These results suggest that BK channels control multiple aspects of the SFR rhythm, and the complex characteristics of circadian behavioral rhythms are generated and integrated at the level of the SCN.

P95

Transgenic Approach Reveals Efferent Target Sites of Prokineticin 2 Expressing Neurons from the Suprachiasmatic Nucleus

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The suprachiasmatic nucleus (SCN) in the hypothalamus is the predominant circadian clock in the mammals. Essential to the understanding of circadian rhythms is the knowledge about the intrinsic organization of the SCN and its efferent projection map in the brain. Prokineticin (PK2) has been shown as an output molecule of SCN clock. To further understanding the mechanism of PK2 signaling in SCN output, we studied the efferent projections of PK2-expressing neurons in the SCN of a transgenic mouse line, in which the green fluorescence protein (GFP) marker was driven by the promoter of PK2 gene. Interestingly, immunostaining and in situ hybridization demonstrated that GFP reporter only labeled a fraction of PK2-expressing neurons in the SCN. The connections of GFP+ neurons were examined by combining immunohistochemistry against GFP reporter with neuronal tracing method with wheat germ agglutinin. The data revealed that PK2-expressing neurons project to the sub-paraventricular zone, paraventricular nucleus, dorsal medial hypothalamus, lateral hypothalamus, paraventricular thalamus nucleus and periaqueductal gray, but not lateral septum. These observations were consistent with earlier observations that PK2 regulates diverse circadian physiology and behaviors.

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tPA and Related Fibrinolytic Proteins Are Expressed in the Mouse Suprachiasmatic Nucleus In Vitro

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Photic resetting of the suprachiasmatic (SCN) circadian clock involves mechanisms similar to those that underlie hippocampal long-term potentiation (LTP). Two secretory proteins, tissue plasminogen activator (tPA) and brain-derived neurotrophic factor (BDNF), in particular have been implicated in LTP. Through conversion of plasminogen to plasmin, tPA can generate mature BDNF (mBDNF), which is critical for LTP expression. We are investigating tPA actions in the SCN.

In vitro glutamate-induced phase delays of SCN neuronal activity are inhibited by the tPA inhibitor, plasminogen activator inhibitor-1 (PAI-1), an effect prevented by co-applying exogenous plasmin. The plasmin inhibitor, 2-antiplasmin, also blocks glutamate-induced phase shifts, and this inhibition is

prevented by co-applying mBDNF. PAI-1 does not inhibit glutamate phase shifts in mice lacking its stabilizing protein, vitronectin. If tPA is critical for glutamate phase resetting, it should be expressed in the SCN at night. In these experiments we used western-blot analyses to demonstrate the presence of VN, PAI-1, plasminogen and tPA in protein extracts from mouse SCN tissue collected at Zeitgeber time (ZT) 16. In separate experiments we maintained mouse SCN and hippocampal tissue in vitro, assaying for protein expression at varying ZTs and varying times after slice preparation. The data demonstrate that tPA levels in the SCN exhibit a rhythmic pattern when maintained in vitro, with high levels during late day and subjective night. Conversely, tPA protein levels in hippocampal tissue does not exhibit this pattern. These results strongly suggest that tPA modulates glutamate-induced phase shifts of the SCN circadian clock through plasmin-associated actions.

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Glutamate Increases Tissue Plasminogen Activator Protein Expression in Mouse Suprachiasmatic Nucleus Brain Slices

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Tissue plasminogen activator (tPA) is an extracellular protease shown to be critical for learning-associated plasticity in the hippocampus. In the hippocampus, tPA cleaves plasminogen to form plasmin, which in turn generates mature brain-derived neurotrophic factor (mBDNF). mBDNF, in turn, enhances glutamatergic signaling. Our data indicate that tPA and BDNF are important for glutamate-induced phase resetting of the suprachiasmatic nucleus (SCN) circadian clock in vitro. Inhibition of tPA or plasmin prevents glutamate-induced phase shifts, and this inhibition is reversed by application of exogenous plasmin or BDNF, respectively. Our data also indicate that tPA, plasminogen, and associated proteins are all expressed in the SCN in vitro. Here we investigated whether glutamate alters tPA protein levels in SCN tissue. For these experiments, brain slices containing only the SCN and underlying optic chiasm were prepared from adult, male C57BL/6J mice and maintained in vitro. At zeitgeber time (ZT) 16 slices were left untreated or were treated with bath-applied glutamate (10mM). After 10 min slices were removed, placed in extraction buffer, and frozen for western blot analysis. Protein extracts from single brain slices were analyzed for tPA and actin, and tPA levels were expressed relative to actin levels. Relative tPA levels within a single experiment were then normalized relative to control. Results indicate that tPA levels increased in glutamate-treated tissue to 3 times control levels (n=3). Thus, glutamate appears to increase tPA protein expression in the SCN. This increase in tPA may be part of the mechanism through which glutamate resets SCN clock phase.

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Expression of Tissue Plasminogen Activator Protein in the Immortalized SCN2.2 Cell Line

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Studies indicate that numerous extracellular proteases play a role in central nervous system function. Tissue plasminogen activator (tPA) cleaves plasminogen to form plasmin, which catalyzes formation of mature brain derived neurotrophic factor. tPA protein is abundant within the hippocampus and hypothalamus, however, only its role in hippocampal long-term potentiation (LTP) has been well studied. Given the similarities between LTP and photic phase-resetting in the suprachiasmatic nucleus (SCN), we are investigating the role of tPA in the circadian clock. We have adopted the immortalized SCN2.2 cell line

as a model for investigating tPA action in the SCN. Because tPA and its associated proteins are secreted into the extracellular space, this model allows us to visualize proteins microscopically, and to analyze separately the culture compartments—media, cells and extracellular matrix (ECM)—in order to study intracellular and extracellular protein functions and interactions. Western blot analysis confirms that we can successfully separate these components: media and ECM extracts are actin-free. Consistent with our data from mouse SCN brain slices, western blot analysis indicates that tPA is abundantly produced in SCN2.2 cells. Importantly, using this model, we have further determined that tPA is secreted into the media and binds to the ECM. Previous studies suggest that tPA acts extracellularly, however this is the first study demonstrating that tPA directly interacts with the ECM in neural tissue. This finding has strong implications for its mechanisms of action and for the protein-protein interactions that may underlie photic phase-resetting in the SCN. Additional studies are in progress.

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The Suprachiasmatic Nucleus (SCN) and the Subparaventricular Zone (LSPV) of Day- and Night-Active Spiny Mice (Acomys)

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The mechanisms determining whether animals exhibit diurnal or nocturnal activity patterns are currently unclear and there is a need for research into the circadian system of species with varying patterns of rhythmicity. One species currently under investigation is the golden spiny mouse (A. russatus). These animals are diurnal in their natural habitat but are primarily active at night, and display an unusual variety of rhythm patterns, in the laboratory. We have hypothesized that A. russatus is either a nocturnal rodent whose circadian system, or masking responses, give it an unusual degree of plasticity, or that they are nocturnal rodents in the process of evolving into diurnal ones. Here, we show that the basic organization of cells and fibers within the SCN of A. russatus is similar to that of the closely related, and strictly nocturnal, common spiny mouse (A. cahirinus). The SCN in these animals includes a core area with fibers containing serotonin and neuropeptide Y, as well as cells containing calbindin, vasoactive intestinal polypeptide and gastrin releasing peptide. Cells containing vasopressin were seen in the "shell". This pattern is very similar to that seen in other nocturnal and diurnal rodents. Numbers of Fos-IR cells in the SCN were rhythmic in both species, and peaked at ZT 8; there were no rhythms in the lower subparaventricular zone, a direct target of the SCN. These data provide further evidence that SCN structure and function are similar across species, including those that are quite closely related but have very different activity patterns. Supported by BSF grants (2003048 and 2005522), and a Lev Zion fellowship to RC.

P100

Circadian Patterns of Neuropeptide Release in the Scn Region of Freely-Behaving Hamsters: In Vivo Assessments of Avp, Grp, Npy and Vip

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The circadian clock of the suprachiasmatic nucleus (SCN) integrates photic and nonphotic inputs for stable pacemaker entrainment and rhythm generation. This integration involves the actions of various neuropeptides within the SCN. Four neuropeptides involved in this regulation are arginine vasopressin (AVP), gastrin-releasing peptide (GRP), neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP). GRP and VIP are implicated in photic signaling, NPY mediates nonphotic input, and AVP mediates signal output. Despite this information little is known about the in vivo activities of these

neuropeptides in the SCN. Male Syrian hamsters under LD 14:10 received a microdialysis probe stereotaxically aimed at the SCN (n=4-5/peptide). After 48 h of recovery, microdialysis was undertaken over a 48-72 h period. Neuropeptides were measured using ultra-sensitive radioimmunoassays. Daily patterns of release were apparent for all of the neuropeptides, with overall highest outputs during the day. AVP release, low from midday through late night, peaked in early morning (ZT3; 160% of the daily mean). GRP and NPY release similarly was low from midday through late night, and rose steadily from lights-on to peak levels later during the day (ZT3-4; 200% and 175% of the daily mean, respectively). Thereafter output of both peptides dropped abruptly to lowest levels (66% and 36% of the daily mean, respectively) from ZT6-10. VIP release was highest (250% of the daily mean) from midday to lights-off. These data indicate differential patterns of release of neuropeptides in the SCN, possibly related to the diversity of their roles in circadian timekeeping. NIH NH35229 JDG

P101

Persistent Neurophysiological Effects of GRP and VIP On SCN Neurons

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Circadian rhythms maintained by the primary mammalian pacemaker, the suprachiasmatic nucleus (SCN), are entrained by light-induced phase modulation. Two neuropeptides have been implicated in intra-SCN photic signaling: gastrin releasing peptide (GRP) and vasoactive intestinal polypeptide (VIP). Acutely, VIP and GRP differentially affect firing rate, which is suppressed by VIP and increased by GRP. We have recently shown that GRP increases neurophysiological activity of Per1-induced SCN cells from Per1::GFP mice, and that this effect is long-lasting. We have extended these findings to VIP, and show that although VIP induces an acute suppression of firing rate, late-night application of VIP persistently increases neurophysiological activity of Per1-expressing SCN neurons similar to GRP. SCN slice cultures from Per1::GFP mice were prepared at ZT 1-3 and maintained overnight. The following day, cultures received 1-hour treatments of VIP or vehicle at projected ZT 21. Loose patch extracellular recordings of Per1-fluorescent neurons from VIP-treated slices three hours after VIP treatment revealed a significantly higher action potential frequency than those from vehicle-treated slices. In order to identify the underlying mechanism for GRP-induced increases in neurophysiological activity, we used the same approach and recorded from Per1::GFP neurons using whole-cell patch clamp in current-clamp mode. Preliminary data suggest that GRP induced a persistent depolarization of membrane potential. Overall, the results of this study suggest that while GRP and VIP have different acute effects on SCN neurophysiology, the persistent effect of these intra-SCN photic signals is excitatory, which may be mediated by a depolarized membrane potential in the case of GRP. This work was supported by National Institutes of Health Grants, NS051183 (KLG) and MH63341 (DGM).

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PER1 Rhythms in Extra-SCN Regions of the Diurnal Grass Rat, Arvicanthis Niloticus

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Rhythms in expression of Per genes are very similar in the SCN of diurnal and nocturnal species in light-dark (LD) cycles and in constant darkness (DD). Data on clock gene expression in extra-SCN oscillators are relatively limited in diurnal mammals. In this study we evaluated the hypothesis that the coupling between molecular rhythms within and beyond the SCN are different in day- and night-active species. We examined rhythms in numbers of cells containing immunoreactive PER1 in the central nucleus of the

amygdala (CEA) and in the oval nucleus of the bed nucleus of the stria terminalis (BNST-OV) of diurnal male grass rats kept on a 12:12 LD cycle or in DD. Male grass rats were perfused at four hour intervals (ZT/CT 2, 6, 10, 14, 18, and 22; n=5/group) and their brains were processed for immunohistochemical detection of PER1. In LD, rhythms in numbers of labeled cells in both the BNST-OV and in the CEA peaked early in the light period. In DD, rhythms in both of these areas became bimodal with one peak in the middle of the subjective day and the second late in the subjective night. These results suggest that molecular oscillators exist in extra-SCN regions of the grass rat brain, and that coupling between molecular oscillators within and beyond the SCN may be quite different in nocturnal and diurnal species. The data also reveal a novel change in the waveform of the rhythm, from unimodal to bimodal, in animals transferred to DD from LD conditions. This research was supported by the National Institute of Mental Health (RO1 MHO53433).

P103

Plcβ4 Gene Expression Profile in Mouse Brain and Liver Tissue

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In addition to the SCN, peripheral tissues, such as the liver, exhibit endogenous daily rhythms. In an effort to understand the molecular signaling processes that occur in the liver, we have been investigating the role of phospholipase Cβ4 (PLCβ4) in mouse hepatocytes. In the cytoplasm, PLCβ4 is activated by G-protein binding to regulate intracellular calcium and protein kinase C activity. We have found that this protein oscillates in abundance in the SCN and the liver, with a particularly robust and unique profile in the liver. Specifically, PLCβ4 translocates from the cytoplasm to the nucleus over the course of the day in hepatocytes, similar to what has been shown for clock proteins. In order to gain insight into the regulatory mechanism underlying PLCβ4 protein expression, we are currently analyzing the temporal profile of *plcb4* RNA in both the SCN and liver. Using semi-quantitative RT-PCR, our current data indicate a circadian oscillation in the expression profile *of plcb4* from mice housed in constant darkness. In the brain, we have begun to investigate the effect of a light pulse on *plcb4* expression through in situ hybridization techniques. To date, these data suggest a possible down regulation of *plcb4* expression in response to a light pulse administered in the early night. These experiments are designed to gain insight into the mechanism that regulates the transcription of the *plcb4* gene in the SCN and liver of the mouse.

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The Suprachiasmatic Nucleus (Scn) Entrains the Oscillator Underlying Time Memory in Hamsters

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We have shown in three species of rodent and in a primate (the common marmoset), that performance on associative learning tasks depends on the match between the time of testing and the time of day of the prior training. On conditioned place preference (CPP) and place avoidance (CPA) paradigms, animals register the time of day that training occurs even when the task does not require this knowledge. Animals then express the learned context associations only at the training time.

The oscillator that underlies these performance rhythms appears to be dopamine dependent. Both amphetamine and haloperidol elicit rhythmic expression of place conditioning (CPP and CPA, respectively). Moreover, the peak performance time following CPA training can be reset by single injections of amphetamine administered in the animals' home cage. The rhythms do not require an intact SCN. We

now report that the SCN is a weak Zeitgeber for the CPA rhythm. Animals that are trained to avoid a foot shock at ZT11, will continue to exhibit optimal performance near that time. However, when trained at ZT3, the optimal time will be ZT3 for a few days, but will move to ZT11 after about two weeks. This is without further training. Also, if the LD cycle is phase advanced following training at ZT3, so that ZT3 now becomes ZT11, the peak performance remains at the "old ZT3". It appears the SCN can entrain the oscillator underlying time of day learning, and that dusk is a default time of peak performance on the CPA task, regardless of the circadian phase of training. Supported by the Natural Sciences and Engineering Research Council of Canada.

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Individual SCN Neurons Are Weak Oscillators with Changeable Circadian Phenotypes

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The suprachiasmatic nuclei (SCN) of the hypothalamus each contain approximately 10,000 neurons responsible for coordinating daily rhythms in physiology and behavior. It is unclear whether all SCN neurons have the ability to function as self-sustained oscillators. To test whether single SCN neurons are autonomous clocks and to characterize properties of their rhythms, we used two independent methods of isolating cells. First, we recorded PERIOD2-driven bioluminescence from single neurons in very low density cultures (<< 3,000 cells/mm2) and subsequently immunolabeled cells for arginine vasopressin (AVP) and vasoactive intestinal polypeptide (VIP). We found that only ~20% of SCN neurons (n = 376 of 1480 recorded in 14 cultures) were circadian, comprising multiple SCN cell types. In addition, we could induce rhythmic PER2 expression in previously non-circadian neurons by adding forskolin (10 μM). In complementary experiments, we recorded bioluminescence from PER2::LUC organotypic SCN slice cultures treated for 6 days with tetrodotoxin (TTX; 0.5 μM) to block voltage-gated sodium channels and reduce cell-cell communication. We found that 17% of recorded cells (range 11%-28%; n=4 applications) were circadian in PER2 expression in the presence of TTX; importantly, a subset of rhythmic and arrhythmic cells switched their phenotype during a subsequent TTX treatment. These results suggest that individual SCN neurons, including those expressing AVP, VIP or neither, change their circadian phenotypes depending on conditions. This is the first demonstration of heterogeneous pacemaking ability not only across SCN neurons, but within individual cells. Supported by NIH grant MH63104 (EDH), NSF Graduate Research Fellowship (ABW), and Arnold and Mabel Beckman Foundation, Beckman Scholars Program (NA).

P106

Three-Dimensional Imaging of Neural Networks in Brain: An Approach for Studying Localization, Interaction and Function of Specific Neurons in a Single Circuit

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Histological analyses such as in situ hybridization (ISH) and immunohistochemistry (IHC) have given insight into characteristics of individual neurons. However, for understanding total physiological events orchestrated by those neurons, a precise examination of neural connections among neurons is

crucial. To understand the neural networks, we have established a novel imaging technique, by which interactions of individual neurons expressing different proteins and/or their connections can be visualized. We distinguished different types of neurons stained by different colors using various fluorescent or bioluminescent markers, and constructed total images of the neuronal networks by a 3-D imaging software. The images clearly revealed spatial distribution of each type of neurons and raised possible connections among two or three distinct populations. The neural connection between the distinct populations was also confirmed by immunoelectron microscopy. This simple approach to visualize precise localization and interaction of specific neurons/proteins will further contribute to establish a "brain network map" for comprehensive understanding of the nervous system.

P107

Clock-to-Clock Coupling of SCN and Peripheral Cells by Diffusible Factors

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Loss of coordination between the central circadian clock, the SCN, and peripheral clocks has been implicated in increased incidences of metabolic disorders, cardiovascular disease, cancer, and sleep disorders. Studies of peripheral tissues cultured in isolation have revealed that in the SCN's absence, cellular rhythms continue but phase and period properties change in diverse tissues, including liver, lung, muscle, kidney and tail fibroblast. With decoupling, the various tissues lose temporal coherence as well as appropriate alignment to the daily cycle of sleep and wakefulness. Little is known about what couples an organism's circadian clocks, except that many can be entrained sufficiently by diffusible factor(s). Here we show that organotypic cultures of rat SCN brain slices are capable of entraining rhythms in fibroblasts when separated in co-culture by a MilliCell-CM membrane. Rhythms are entrained in both the stable mouse fibroblast NIH3T3 cell line and rat primary fibroblasts, as measured by expression of clock gene transcripts. Phasing of the peaks of Per2 and BMal expression is dependent on the phase of the SCN, as determined from spontaneous peak electrical activity of SCN brain slices prepared from alternate lighting schedules. Based on the results of these SCN:fibroblast co-cultures, we have undertaken peptidomic analysis of the releasate from SCN cultures. We have identified a number of known peptides including Arg-vasopresin and little SAAS, as well as several unidentified and putative peptides, released from the SCN. These provide a basis for discovering novel candidate coupling factors. This work has been supported by PHS P30 DA 018310.

Neural Clocks

P108

Circadian Control of Membrane Excitability in Drosophila Clock Neurons

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Drosophila circadian rhythms are governed by clock neurons, including pigment dispersing factor (PDF)-expressing lateral ventral neurons (LNvs), which are considered pacemakers of the fly circadian control system. Electrical hyperexcitation or silencing of LNvs disrupts fly circadian rhythms, suggesting LNv membrane excitability plays a critical role in circadian oscillation. To further refine our understanding of LNv membrane properties, we performed whole-cell patch-clamp recordings on LNvs in situ in adult fly whole-brain explants. We found that the resting membrane potential (RMP) and action potential

(AP) firing rate of large LNvs are modulated as a function of time-of-day. The lLNv RMP becomes more hyperpolarized as time progresses from early morning to dusk with a concomitant decrease in spontaneous AP firing rate in 12hr:12hr light:dark conditions (LD). In contrast, circadian defective per0 null mutant lLNv membrane excitability is nearly constant in LD. We also observed that WT lLNvs fire two distinct sizes of APs, large ones with amplitudes of 30-40mV and small ones of 15-20mV. Severing two hemispheres of the fly brain abolishes small APs, suggesting that small APs are generated in the contralateral optic lobe. Our results demonstrate that lLNv membrane excitability encodes time-of-day in LD via a cell-autonomous circadian clock-dependent mechanism. (Work in the laboratory of M.N.N. is supported in part by the Whitehall Foundation and NINDS (R01NS056443 and R01NS055035)).

P109

Cyclical Expression of Synaptic Proteins in the Visual System of Drosophila melanogaster

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The first optic neuropil (lamina) in fly's visual system exhibits several structural circadian rhythms. This includes oscillations in the number of tetrad synapses formed between the photoreceptor terminals (R1-R6) and L1, L2 monopolar interneurons that receive photic information from the photoreceptors. Looking for mechanisms involved in synaptic plasticity controlled by a circadian system we examined the expression of proteins important for assembling multiprotein complexes at synaptic contacts, a presynaptic active zone protein Bruchpilot (BRP) and a postsynaptic scaffolding protein Disc large (Dlg). Wild type Canton-S, per01 mutant, and transgenic GAL4/UAS lines of Drosophila were reared under light/dark (LD 12:12) and constant darkness (DD) regimes and decapitated at different times of the day and processed for confocal microscopy. Our results revealed different patterns of expression of BRP and Dlg within the lamina cartridge and of fluctuations of their abundance during the day and night. Dlg and BRP were most abundant at ZT13 (one hour after lights-off) and at ZT1 (one hour after lights-on), respectively. Interestingly, the oscillations in the level of expression of Dlg were more pronounced than in case of BRP. The observed changes indicate the cyclic remodeling of synaptic contacts in the lamina by a circadian input at the postsynaptic sites and direct light effect on the presynaptic protein BRP.

P110

Bioactivity of Membrane Tethered Pigment Dispersing Factor Neuropeptide Expressed in Drosophila Circadian Neurons

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In Drosophila, pigment dispersing factor (PDF), a neuropeptide that is secreted by the small and large ventral lateral neurons (LNv), plays a critical role in forming robust circadian rhythms of locomotor activity. To gain further insight into the cellular circuitry underlying the action of PDF, we have developed a membrane tethered form of PDF (tPDF) that is designed to activate PDF receptors in a cell autonomous manner. Consistent with the design, flies expressing tPDF with the timeless driver phenocopied the complex rhythm phenotype of flies overexpressing the endogenous form of the PDF gene, yet tPDF expressed in non-clock neurosecretory cells that project to the dorsal clock neurons do not affect locomotor rhythms. timeless-dependent tPDF expression excluding the LNvs also caused complex rhythms, suggesting that activation of PDF receptors present on non-PDF secreting clock neurons causes the phenotype. Interestingly, tPDF expression limited to the PDF secreting LNvs decreases the rhythmicity of locomotor rhythms in the presence of endogenous PDF, but partially rescues rhythmicity in pdf-null

flies, indicating the presence of PDF receptors on the PDF secreting neurons themselves and suggesting a complicated mode of PDF receptor activation for robust locomotor rhythms. We also provide evidence for the presence of PDF receptors expressing cells outside the central clock that regulate locomotor behavior. Our investigation demonstrates the usefulness of the tethered technology in understanding the complex cellular circuitry that underlies neuropeptide signaling. (Work in the laboratory of M.N.N. is supported by the Whitehall Foundation, NINDS (RO1NS055035, R01NS056443), and the Yale School of Medicine. C.C. is supported by NIGMS Institution Training Grant (T32GM007527))

P111

Internal Desynchronization between Groups of Circadian Pacemaker Neurons in the Fruit Fly Drosophila melanogaster

Charlotte Helfrich-Förster*, Corinna Wülbeck, Eva Grieshaber, Taishi Yoshii, Shobi Veleri, and Ralf Stanewsky, University of Regensburg

The activity rhythm of the fruit fly is controlled by a network of circadian pacemaker neurons that cyclically express the clock genes period, timeless and others. Most of these neurons appear to be functionally connected in the dorsal protocerebrum and in the accessory medulla—a small neuropil that houses the circadian pacemaker center in insects. We are interested in the question how the oscillations within different clock neurons are synchronized with each other to produce a common circadian output. The neuropeptide Pigment-Dispersing Factor (PDF) seems to play a major role in this synchronization. With help of the luciferase real reporter system for period, we show here that PDF most likely slows down the period of some neurons and accelerates that of others. This differential action of PDF on different clock cells may keep the oscillations in the different subsets of clock neurons slightly out of phase and may help to adapt wild-type flies to different environmental conditions. In mutants with extraordinary PDF-fibers in the dorsal brain, PDF causes internal desynchronization of the activity rhythm into two free-running components—one free-running with short period the other with long period. By immunostainings with antibodies against the clock proteins PERIOD and TIMELESS during the behaviorally desynchronized state we found that the molecular oscillations in the clock neurons are similar out of synchrony and we could identify the neurons that are slowed down and accelerated by PDF, respectively.

P112

Transcriptional Reporter Analysis to Understand PDF Receptor Expression Underlying the Generation of Behavioral Circadian Rhythms

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Daily rhythms of behavior of Drosophila are regulated by the circadian clock. The clock generates molecular oscillations of specific genes, such as period and timeless, and the phasic differences of these oscillations between different clock neurons are synchronized via neuronal interactions. Among ~150 clock neurons in an adult fly brain, only the 16 small and large LNvs (s-LNvs and l-LNvs) express the neuropeptide Pigment Dispersing Factor (PDF). PDF is required for normal synchrony among clock neurons, normal amplitude PER protein rhythms, and for normal behavioral rhythms. This suggests that PDF signaling must be important for the communication between clock neurons and perhaps also to downstream, non-clock cell outputs. A G-protein coupled receptor has been identified to be a PDF specific receptor (PDF-R), but the available antibody reagents have not yet clarified where it is expressed. Here we will report on two related approaches to map the expression of PDF-R.

First, we built transcriptional GAL4 constructs (pdfr-GAL4) capturing gDNA in the range of 0.6 ~ 2.5 kb of putative pdfr promoter regions. Various pdfr-GAL4 constructs are expressed in subsets of clock neurons, in subsets of neurons of the central complex, in subsets of neuroendocrine cells in the pars intercerebralis (PI), and in other brain regions. Second, to capture most if not all possible cis-regulatory elements, we built a recombineering construct of ~70 kb spanning the pdfr locus. A MYC epitope tag was inserted at the C terminus of the predicted open reading frame. Preliminary analysis suggests that, in the adult brain, PDFR-MYC is predominantly expressed in subset of clock neurons. These results provide support for the hypothesis that PDF is a synchronizing factor within the pacemaker clock network.

P113

Influence of the Neuropeptide Pigment-Dispersing Factor (PDF) on the Circadian Clock of Short- and Long-Periodic Rhythm Mutants of Drosophila melanogaster

Christa Kistenpfennig*, Cordula Mertens, Sinje Maruhn, Corinna Wülbeck, and Charlotte Helfrich-Förster

The Drosophila neuropeptide PDF is expressed in a subset of clock neurons (LNV) of the circadian pacemaker centre in the brain. PDF appears to be secreted in a circadian manner into the dorsal brain. Altered PDF levels in the dorsal brain disrupt behavioral rhythms: Flies lacking PDF exhibit weak rhythms with short periods and flies with elevated PDF levels have weak rhythms with long periods. These results suggest that PDF interferes with the molecular oscillations in clock neurons by altering their period.

It is yet unknown how PDF mediates these period changes; but PERIOD (PER) and TIMELESS (TIM), key proteins of the circadian cycle in Drosophila, are possible targets. Phosphorylation of clock proteins affects their stability, biological activity and the phase of their circadian oscillation. A hypothetical computer model from Petri and Stengl (2001) suggests that PDF influences the phosphorylation state of clock proteins mediated by the activation of kinases and/or phosphatases.

For this reasons we studied the effect of Pdf01 on the locomotor activity of different per and tim rhythm mutants. We found a clear interaction of the per-, tim- and Pdf01- mutations on the timing of the activity phases under light-dark cycles and on the free-running periods under constant conditions. To test whether PDF affects the phosphorylation state of PER and TIM we performed Western Blots on head extracts.

P114

Rhythmic Structural Plasticity in the Circuit Controlling Rest-Activity Cycles in Drosophila

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Clock output pathways are central to convey timing information from the circadian clock to a diversity of output systems, ranging from cell-autonomous processes to physiology and behavior. While the molecular mechanisms that generate and sustain rhythmicity at the cellular level are well understood, how this information is further structured to control specific behavioral outputs remains an open question. Rhythmic release of pigment dispersing factor (PDF) has been proposed to propagate the ¨time of day¨ information from core pacemaker cells to downstream targets underlying rhythmic locomotor activity. Indeed, such circadian changes in PDF intensity represent the only known mechanism through which the PDF circuit could communicate with its output. In this work we describe a novel circadian phenomenon involving extensive remodeling in the axonal terminals of the PDF circuit which display higher complexity during the day and significantly lower complexity at nighttime, both under daily cycles and constant

conditions. In support to its circadian nature, cycling is lost in bonafide clock-less mutants. We propose this clock-controlled structural plasticity as a candidate mechanism contributing to the transmission of the information downstream of pacemaker cells.

P115

Mosaic Analysis of Drosophila Circadian Pacemaker Neurons

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The core pacemaker network regulating circadian rhythmicity in *Drosophila melanogaster* is comprised of only eight circadian neurons (sLNvs-small ventral Lateral Neurons), and their circadian pacemaker function has been studied extensively. The high precision of pacemaker rhythms under constant conditions implies that individual pacemaker neurons are synchronized with each other. In spite of considerable interest in understanding the organization of cellular output integration and coupling of the pacemaker system both ipsilaterally and contralaterally, up to now it has been almost impossible to genetically isolate subsets of core neurons (sLNvs) to study the contribution they make to the the pacemaker network in vivo. With our growing ability to manipulate small subsets of cells, Drosophila has become an ideal system to reveal the operations of neural circuits. Among many other mosaic techniques, MARCM (Mosaic Analysis with a Repressible Cell Marker) offers the finest restriction of spatial expression of transgenes because it allows one to alter the activity of even a single neuron. With this in mind, we performed mosaic analyses to manipulate subsets of sLNvs using the MARCM technique. Through these manipulations, we initially altered the electrical state of subsets of sLNvs by hyperpolarizing these neurons. Our analyses indicate that the ipsilateral coupling of sLNvs is characterized by non-cell autonomous interactions whereas sLNvs in different hemispheres seem to act independently. Furthermore, the expression of distinct periods in pacemaker cells leads to complex output patterns in locomotor activity rhythms with multiple rhythmic components, suggesting an unpredicted lack of integration of cellular output signals.

P116

The Circadian Time-Dependent Effect of Pigment Dispersing Hormone on the Crayfish Visual Photoreceptor Excitability

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In crayfish, the visual photoreceptors are important structures in the organization of the circadian system responsible for the synchronization and expression of the amplitude of electroretinogram (ERG) rhythm. The pigment dispersing hormone (PDH) modulates the ERG amplitude because controls the amount of incoming light to retinular cells adjusting retinal distal pigment position to its light adapting state. The main propose of this research was demonstrate that PDH also affects the photoreceptors excitability in a manner which depends on the circadian time (CT)

Adult crayfish Procambarus clarkii in intermolt stage, previously adapted to light-dark cycles (LD 12:12) were employed. The isolated eyestalk was perfused in physiological solution plus 1nM of PDH and amplitude and duration of the electrical response to light, the receptor potential (RP) of retinular cells were determined. PDH produced a diminution in RP amplitude depending on CT application. The "amplitude response curve" is continuous with a minimum at 6CT. With regard to RP duration, increments and diminutions also depend on CT. The "duration response curve" has a breaking point in the range of 12-14 CT. Changes in the slopes of the depolarization and repolarization regimes could also been detected, supporting the idea that PDH modifies the ionic conductances underlying the RP in a CT dependent

manner. These results allow us to have a more complete idea about the importance and ubiquity of the PDH in the crayfish circadian system. This work was supported by IN200206 DGAPA grant.

P117

Migrating with Sex While Maintaining a Direction

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Eastern North American monarch butterflies (Danaus plexippus) undergo a spectacular fall migration to their overwintering grounds in Mexico. In contrast to summer butterflies, migrants are juvenile hormone (JH) deficient, which leads to reproductive diapause and increased longevity. Migrants also use time-compensated sun compass orientation to help them navigate in a south/southwesterly direction. Reproductive diapause persists at the wintering sites until spring, when the butterflies, by increasing JH levels, become reproductively competent, mate, and fly northward to lay fertilized eggs on newly emerged milkweed plants in the southern United States. It is unclear, however, whether JH deficiency and the accompanying reproductive diapause are required for the operation of navigational mechanisms in fall migrants. We tested this hypothesis by treating migratory butterflies with methoprene, a potent JH analog, or vehicle (control treatment). Both treatment groups were housed in either simulated outdoors light-dark conditions or a 6 hr-delayed light-dark cycle. Fourteen days later, butterflies were tethered and flight orientation was monitored outdoors in a flight simulator. Regardless of treatment (methoprene or vehicle), the migrants all manifested time-compensated flight orientation. The methoprene-treated migrants all had activated reproductive systems and exhibited marked reproductive behavior, while the vehicle-treated animals did not. These data show that migratory monarchs manifest robust time-compensated sun compass orientation even when their reproductive systems are turned on (at the morphological and behavioral levels) by JH analog treatment. Although JH deficiency may be involved in the induction of proper sun compass orientation, it is not required for its maintenance.

P118

Diurnal Rhythms in the Response to Natural Rewards in the Male Rat

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The impact of the circadian timing system upon behavior and physiology is pervasive and preliminary evidence suggests that there is a circadian modulation of drug-seeking behaviour and responsiveness to drugs of abuse (Baird & Gauvin, 2000; Abarca et al., 2002). To further characterize daily rhythmic variations in reward and to extend these observations to more natural reinforcers, young male rats were allowed to mate at varying times of day (ZT5, 11, 17 & 23) and the rewarding properties of these manipulations were assessed via a conditioned place preference apparatus. To ensure adequate performance at the conditioning stage, the rats were given sexual experience at varying time points prior to the behavioral trials. In agreement with previous results (e.g., Harlan et al., 1980), male sexual performance varied by time-of-day with a nadir near the light/dark transition and peak activity from the mid-dark to the mid-light period. By contrast, a significant sex-induced conditioned place preference was observed only during the mid-dark period. These results show for the first time that sexually-induced reward varies by time of day and indicate that the peak in sexual reward does not necessarily coincide with the zenith in sexual performance. Moreover, the observed peak of sex-induced place preference is in anti-phase to that reported previously for psychomotor stimulants (e.g., Kurtuncu et al., 2004). Thus, the rewarding properties of various stimuli may be differentially regulated by the circadian system. The results of further experiments utilizing other natural rewards will also be presented.

P119

Clock Gene Expression in Human BNST, Cingulate Cortex and Pineal Gland of Alzheimer's Disease Patients and Controls

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Circadian clock gene expression has been demonstrated in numerous human brain regions and rhythmically expressed in the pineal gland of controls, but not preclinical or clinical Alzheimer's Disease (AD) patients. Contrary results have also been reported. We sought to determine whether circadian clock gene expression could be detected in the brain and whether time of death was a factor in their level of expression. The bed nucleus of the stria terminalis (BNST), cingulate cortex, and pineal gland for AD patients and aged controls were obtained from the Douglas Mental Health University Institute Brain Bank. Total RNA was extracted, verified and relative expression levels of PERIOD1 (PER1), PERIOD2 (PER2), and BMAL1 were measured in relation to the non-rhythmic housekeeping gene CDK4 using quantitative real-time reverse transcription polymerase chain reaction. Samples were grouped according to time of death and analyses of variance (group x time of day) were performed for each brain region and gene. There was a significant effect of time for PER1 in the cingulate cortex and pineal gland, with post hoc comparisons revealing PER1 was significantly greater in the evening than at night for both brain regions. There was also a trend for a main effect of time for PER2 in all three brain regions. These results corroborate previous work suggesting a circadian variation of PER1 expression in the pineal and further suggest that rhythms of PER1 and possibly PER2 expression occur in the cingulate cortex and BNST, areas known to be involved in decision making and motivated behaviors.

P120

Circadian and Neurochemical Regulation of Neuronal Activity in the Lateral Habenula

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Recently the lateral habenula (LHb) has been implicated as a potential component of the mammalian circadian system. It is hypothesized that the master circadian pacemaker of the hypothalamic suprachiasmatic nuclei (SCN) sends neuropeptidergic signals to coordinate cellular activity in the LHb, which in turn projects to a number of regions involved in circadian function including the raphe nuclei, pineal gland and the preoptic nucleus. However, very little is known about the spontaneous electrical activity of LHb neurons, their responsiveness to putative SCN output signals or the endogenous time-keeping properties of this nucleus. In this study we examine the spontaneous electrical activity of rat and mouse LHb neurons in acute brain slices in vitro and investigate their responses to the SCN neurohormonal output factor arginine vasopressin (AVP). Further, using a luciferase reporter of PERIOD2 expression (PER2::LUC), we inspect molecular time-keeping of the LHb and greater habenular complex. LHb neurons displayed spontaneous firing of action potentials and were most active during mid-late day. Some individual cells displayed circadian rhythmicity in their firing activities. Bath applications of AVP evoked both activation and suppression responses in cellular activity from a subset of LHb neurons. Circadian variation in PER2::LUC expression was detected in acute habenula cultures for ~3days. These data demonstrate circadian rhythmicity of clock gene expression and electrical activity in the LHb and neuronal responsiveness to a known SCN output factor, AVP. Such findings support the idea that the LHb integrates SCN circadian information and relays this to downstream oscillators controlling physiology and behaviour. Supported by the BBSRC.

P121

Characterization of Circadian Gene Expression in the Brain of Xenopus Tropicalis

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The classical model of hierarchical organization of the mammalian circadian system deems a region of the hypothalamus of the brain, the suprachiasmatic nucleus (SCN), the "master clock." In some non-mammalian species, the master oscillator has been localized to tissues and organs other than the hypothalamus, including the retina of the eye, the parietal and pineal glands. In Xenopus laevis, Harada et al. (1998) showed that eye removal shortened the period of behavioral rhythms, but that only lesions directed to the hypothalamus caused arrhythmicity in behavior. The central oscillator in the brain of X. laevis or any Xenopus species has not been definitively localized. We are characterizing the spatial and temporal expression of clock genes in the brain of Xenopus tropicalis, a species closely related to X. laevis, in order to identify the neuronal structures that may be driving rhythmic behavior. Sequences to X. tropicalis per1, per2, bmal1, cry1, clock, AVP, VIP, and PACAP were amplified by RT-PCR. Using 35S-labeled riboprobes, in situ hybridization showed PACAP mRNA in the dorsal and lateral pallium, hypothalamic anterior preoptic area, SCN, ventral hypothalamus and thalamus, and regions in the hindbrain, as demonstrated previously in X. laevis by Hu et al. (2006). per1 mRNA signals were also found in the dorsal and lateral pallium, hypothalamic anterior preoptic area, SCN, ventral hypothalamus and thalamus, but no hindbrain labeling was observed. Characterization of the expression patterns of additional clock and neuropeptide genes is underway.

P122

Clock Gene Expression Patterns across the Mouse Brain

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It has been shown that the SCN is not the only brain area that exhibits robust circadian rhythms in expression of several clock genes. This suggests the idea of multiple clocks in the brain. In order to study the circadian gene expression patterns of clock genes throughout the mouse brain, we performed high-throughput in situ hybridization in the whole brain for the following genes: Bmal1, Per1, Per2, Cry2, Avp, Dbp, Csnk1e, Dec1, Dec2 and Prok2. For this purpose, three groups of male mice (C57BL/6JOlaHsd) were housed in running wheel cages. The first group was entrained under rectangular LD12:12 for 30 days. A second group received a rectangular LD 12:12 cycle for 27 days plus three days of DD to investigate endogenous circadian gene expression when there is no masking effect of light. A final group was exposed to a twilight LD 12:12 cycle for 30 days to investigate whether gene expression would differ in a more naturalistic light dark cycle. The mice were then sacrificed at six different time points (2, 6, 10, 14, 18, 22 ExT or InT) and spatiotemporal distribution of gene transcripts was analysed using high-throughput in-situ hybridization of mRNA. We reconstructed the brains in 4D (space and time dimension) and analysed qualitatively and semi-quantitatively gene expression throughout the brain. Preliminary results show that there may be different pattern of expression under rectangular and natural light regimes. For

instance, in the hippocampus and SCN Bmal1 and Per1 peak expression under twilight is advanced in the SCN in comparison with LD rectangular cycle and its pattern of gene expression is closer to the DD situation. Supported by BRAINTIME (EC QLG3-CT-2002-01829) and the 6th Framework Project EUCLOCK (No. 018741).

P123

Circadian Gene Regulation of the Neurotrophic Factor Purpurin in the Chicken, Gallus Gallus

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The purpurin gene encodes a small protein first identified in chickens as a cell-adhesion molecule with sequence and functional similarities to serum retinol-binding proteins. Retinol-binding proteins, including purpurin, are lipocalin proteins, which are a family of extracellular soluble proteins that transport small hydrophobic molecules. Originally, chick purpurin mRNA was found exclusively in both embryonic and adult retinas, and purported to function both as a neurotrophic factor and to increase nerve cell survival rates. Although the role and expression of purpurin during development has been documented, including a function in nerve cell regeneration, purpurin's role in matured cells is not well understood. As a result of our pineal gland transcriptional profiling we have further examined the expression and regulation of this molecule in the avian circadian system. Purpurin mRNA exhibits maximal mRNA abundance and robust circadian expression in the chicken pineal gland. Maximal levels of purpurin gene expression occur during the day in LD with near 20-fold amplitude; rhythmic mRNA expression peaks during the subjective day in free-running conditions with a 6-fold amplitude.

P124

Cameron Harris , Albion College

Research has shown that circadian rhythmicity has a significant impact on cognitive function (Folkard, Wever, & Wildgruber, 1983). Previous studies have shown time of day effects on memory, problem solving, and attention. Results usually show higher functioning during a person's optimal time of day than their non-optimal time of day. This study was designed to determine if there is a link between optimal time of day and two specific type of thinking; convergent and divergent thinking. Convergent thinking involves combining or joining different ideas together based on elements these ideas have in common. Divergent thinking on the other hand involves generating many different ideas about one item or topic. Participants' optimal time of day was determined using the Morningness Eveningness Questionnaire (Horne & Ostberg, 1976). Then, participants, either during their non-optimal or optimal time of day, were asked to complete the Remote Associates task, which measures convergent thinking, and the Creative Uses task which measures divergent thinking. Results showed no time of day effects on the Remote Associates Task ($t(74) = 0.29$, $p = 0.78$), indicating that convergent thinking is not affected by time of day. On the other hand, results for the creative uses task showed that participants are more creative during their optimal time of day compared to their non-optimal time of day ($t[74] = -2.00$, $p = .049$), indicating that divergent thinking is influenced by time of day. These findings indicate that convergent and divergent thinking are influenced differently by a person's optimal time of day.

P125

Circadian Modulation of Clock Genes, but Not Glutamate Uptake in Cultured Astrocytes

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Circadian clocks control daily rhythms in behavior, physiology, and metabolism. These clocks are based on intracellular transcription-translation feedback loops that sustain daily oscillations of gene expression in many cell types. Mammalian astrocytes display circadian rhythms in the expression of the clock genes Period1 (Per1) and Period2 (Per2). However, a functional role for circadian oscillations in astrocytes is unknown. Astrocytes play a critical role in the regulation of extrasynaptic glutamate levels via high-affinity glutamate transporters. Mutation in the clock gene Per2 was shown to reduce glutamate uptake by astrocytes (Spanagel R. et al, 2005). Because of this link between circadian gene expression and glutamate uptake by astrocytes, we asked whether glutamate uptake by glia is circadian. We monitored bioluminescence from pure cortical astrocytes cultured from transgenic rats carrying a bioluminescent reporter of Period1 transcription (Per1::luc) or from knock-in mice with a bioluminescent reporter of Period2 protein levels (Per2::luc). We also measured glutamate uptake as a function of time of day and glutamate concentration from bioluminescent cultures as well as from astrocytes derived form Clock/Clock mutant mice. We found that glutamate uptake and Period-driven bioluminescence levels depended on the Clock gene and on culture conditions, but not on circadian time. We conclude that, although maximal glutamate uptake by astrocytes is modulated by the mutant CLOCK protein and factors in culture media, the circadian clock does not confer rhythmicity to this physiological measure.

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Methamphetamine Lengthens and Reinstates Free-Running Periods of Locomotor Activity in Circadian Mutant Mice

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It is well-established that methamphetamine can lengthen circadian free-running periods in rats and mice. Interestingly, methamphetamine has been demonstrated to reinstate rhythmicity in Clock19 homozygote mice and, recently, in Cry1/Cry2 null mice. In the current study, we screened several circadian mutants, both intact and with lesions of their suprachiasmatic nuclei (SCN), to determine if there is a role for clock genes in the methamphetamine-sensitive circadian oscillator. The free-running locomotor periods of Npas2 knockout, Cry1/Cry2 double knockout, and Clock mutant mice were observed on clean water and while the animals were consuming methamphetamine-treated drinking water. Npas2 knockout mice displayed lengthened circadian periods when treated with methamphetamine. SCN-lesioned Npas2 deficient mice were arrhythmic in constant conditions; a long, stable free-running rhythm was observed in these animals following methamphetamine treatment. As expected, Cry1/Cry2 double knockout mice were arrhythmic in constant darkness; methamphetamine induced a long free-running rhythm in these animals. SCN-lesioned Cry1/Cry2 mice also exhibited robust rhythmicity in the presence of methamphetamine, with varied period lengths. Clock19 heterozygote and homozygote mice lengthened their circadian periods when treated with methamphetamine, confirming previous reports. The ability of methamphetamine to affect circadian outputs does not appear to depend on the SCN or many of the known, molecular components of the circadian clock. This suggests that methamphetamine is acting on a timing system of unknown location and molecular composition to influence circadian locomotor activity.

P127

Disruption of the Circadian Output Molecule Prokineticin 2 Results in Anxiolytic and Antidepressant-Like Effects in Mice

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Disrupted circadian rhythms are tightly associated with mood disorders. The suprachiasmatic nucleus (SCN) is the master pacemaker that drives circadian rhythms in mammals. However, the underlying molecular connections of circadian rhythm and mood disorders are still poorly understood. Prokineticin 2 (PK2) is a signaling molecule that is critical for transmitting the circadian rhythms from SCN. Previously, it is has been shown the receptor for PK2 is expressed in virtually all of the primary SCN target areas, many of which are also involved in the mood regulation. In the current study, we investigated the role of PK2 in the regulation of anxiety and depression-related behaviors. Intracerebroventricular (ICV) infusion of PK2 increased anxiety behavior, assessed by elevated plus maze and light/dark box. ICV delivery of PK2 also led to increased depression-like behaviors in the tests of forced swimming and learned helplessness. Conversely, mice lacking the PK2 gene (PK2-/- mice) displayed significantly reduced anxiety and depression-like behaviors. Furthermore, PK2-/- mice show impaired responses to exposal to new environments in terms of locomotor activity, arousal, body temperature and food intake. Our studies thus indicate that PK2 signaling plays a critical role in the stress-related traits in mice, and establish a possible molecular link between circadian rhythms and mood regulation.

P128

Involvement of the Mper2 Gene in the Development of Morphine-Induced Tolerance and Withdrawal

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Previously, our lab demonstrated the involvement of the clock gene Per2 in modulating several drug-induced behaviors (i.e. cocaine-induced sensitization or alcohol self-administration). The gene Per2 has also been shown to be specifically over-expressed in the frontal cortex of rats subjected to morphine withdrawal. In the present study, we therefore aimed at measuring the involvement of mPer2 in the development of morphine-induced tolerance and withdrawal. We therefore assessed in mPer2Brdm1 mutant mice and in their respective wild-type littermates (1) morphine-induced analgesia and tolerance, using the tail-immersion and the hot-plate tests, (2) naloxone-induced signs of physical withdrawal, and (3) naloxone-induced conditioned place aversion. In the tolerance tests, neither the pain thresholds nor the sensitivity to the acute analgesic effect of morphine were affected in mPer2Brdm1 mutant mice when compared to wild-type littermates. However, mPer2Brdm1 mutant mice showed significant decrease of the degree of tolerance to morphine and attenuated physical withdrawal signs. Interestingly, using a low dose of naloxone, mPer2Brdm1 mutant mice showed a stronger conditioned-place aversion than the controls. These results show the clear involvement of the mPer2 gene in the development of tolerance to the analgesic effects of morphine and in the expression of morphine withdrawal responses. In addition, they further suggest that mPer2 may act as a clock-output and modulate neurobiological systems (i.e. the opioidergic system) involved in morphine addiction. In order to identify these specific targets, qPCR analyses will be conducted for relevant receptors and transmitters in several brain areas in the above mentioned mutant and wild-type mice.

P129

Vasoactive Intestinal Peptide Is a Very Important Peptide for Heart Rate and Body Temperature Rhythms

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A variety of recent evidence indicates that the neuropeptide vasoactive intestinal peptide (VIP) is critical for normal functioning of the circadian system. SCN neurons containing VIP are part of the circuit through which the circadian system regulates autonomic function. Therefore, we sought to examine the impact of the loss of VIP on circadian rhythms focusing on physiological parameters highly dependent upon autonomic regulation. First, we characterized the circadian rhythms of heart rate (HR) and body temperature (Tb) in freely behaving mice using telemetry measurements. As expected, wildtype (WT) mice displayed diurnal changes with peaks in Tb and HR during the night. These rhythms continued when the WT mice were held in constant conditions with a period of approximately 23.6 hours. In contrast, the VIP-deficient mice did not exhibit significant day/night variation in Tb or HR. In LD conditions, the physiological outputs of the mutant mice were either arrhythmic or expressed low amplitude rhythms, in which the onset began during the first half of the day rather than in anticipation of the night. In DD conditions, some of the VIP-deficient mice exhibited low amplitude rhythms in Tb with a shortened free-running period of approximately 22.5 hours. None of the VIP-deficient mice exhibited rhythms in HR under these same conditions. In contrast, the loss of VIP did not appear to influence the acute autonomic regulation of HR. Overall, our data demonstrate that VIP is essential for the circadian regulation of cardiovascular function and body temperature.

P130

Circadian Variation of Spatial Memory in Mice Tested in the Morris Water Maze

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It has been suggested that hippocampus-dependent contextual learning and memory encodes circadian phase, such that optimal retrieval of information occurs at multiples of 24h after training (Holloway and Wansley 1973). Circadian modulation of memory on fear conditioning tests has also been reported, although the direction of the effect is inconsistent across studies (Valentinuzzi et al. 2001; Chaudhury and Colwell 2002). In this study we investigated the circadian modulation of spatial retention memory in two strains of mice (C57/Bl6 and 129 S1/SvImJ) using the Morris water maze. Mice in LD 12:12h were trained either during their inactive phase (ZT02) or their active phase (ZT14) over several consecutive days to learn a hidden platform location. Memory of this location was quantified by measures of quadrant preference and latency to finding the platform. Retention tests began 24h, 36h or 42h after the last training session and were then repeated every 6h up to 72h after the last training session. Overall, C57 mice had a better memory response in the first retention test 24h after the training in comparison to tests at 36h and 42h. Repeated retention tests indicated better performance at 24h intervals after training, but also a strong extinction of the previously learned location. Circadian variation of performance was reduced in mice that were over-trained, likely due to ceiling effects, and it was stronger in mice that were "good" learners. These results provide tentative support for the notion that time of day is encoded as a contextual cue modulating recall on a spatial memory task. Interestingly, 129 S1 mice when subsequently divided according to their learning performance revealed a different pattern of daily modulation of memory in comparison to C57 mice.

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P131

A Group of Dorsal Neurons Is Necessary for Rhythmic Locomotor Activity in Drosophila melanogaster

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The Drosophila circadian clock relies on the interaction of different groups of neurons, among them, the small ventral Lateral Neurons (s-LNvs) have been suggested as essential in controlling locomotor activity rhythms. In this study, we provide evidence that a group of Dorsal Neurons expressing CRYPTOCHROME at very low levels (DNs[CRY-]), is also critical for rhythmicity in constant darkness (DD). In particular, by expressing several transgenic constructs specifically in those neurons we were able to produce short, long and arrhythmic locomotor behaviour. We also show that the molecular functioning of the clock might be different in DNs[CRY-] and s-LNvs.

P132

The Localization of Neuropeptides and the Circadian Clock Protein PERIOD in the Circadian System of the Cockroach Leucophaea Maderae

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Lesion and transplantation studies located the circadian pacemaker center which controls locomotor activity rhythms in the cockroach Leucophaea maderae to the accessory medulla (AMe) of the brains´optic lobes. Associated with the AMe are 7 soma groups which can be distinguished according to soma size and location. The neuropil of the AMe can be further subdivided into a nodular-, an internodular-, and a shell region. As shown before with antibodies against different neuropeptides and neurotransmitters ventral neurons of the AMe stain with antibodies against PDH (8), FMRFamide (13), orcokinin (16), LMS (1), and allatotropin (3-4). In addition, the noduli of the AMe stained with antibodies against Mas-allatotropin, GABA, leucokinin, and more weakly against orcokinin and FMRFamide, while the internodular region of the AMe expressed immunoreactivity against PDH, FMRFamide, corazonin, orcokinin, Dip-allatostatin, and 5HT. The shell was stained with antisera against Dip-allatostatin, PDH, FMRFamide, orcokinin, corazonin, and 5HT. With antibodies against Leucophaea PERIOD (PER) antiPER immunoreactivity was localized in many neurons and glia in the brain. Depending on the fixation method antiPER staining was located in the nucleus and/ or cytoplasm. The PDH-ir neurons which belong to the ventral neurons of the AMe expressed PER-immunoreactivity in the nucleus. Current in situ hybridizations with probes against cockroach per examine the distribution of per mRNA in the cockroach brain. Altogether, the immunocytochemical examination of the AMe suggests that AMe neurons are circadian pacemaker cells which colocalize several different neuropeptides in local and output circuits of the circadian pacemaker center. [Supported by DFG STE531-18-1]

P133

The Behavioral Response of Paramecium tetraurelia Is Controlled by Both Lithium-Sensitive Ultradian and Circadian Rhythms

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The behavioral response, a change in swimming direction elicited by an action potential, is utilized by *Paramecium tetraurelia* as a means to locate optimal environments. This research has investigated the role of biological rhythms in the generation of directional changes. Individual cells (grown in a 12 hr dark:12 hour light cycle) were videotaped over a 24 hour period and the frequency of directional changes in one minute was measured every 5 minutes. It was discovered that throughout the 24 hour recording there was a recurring oscillation in frequency with a tau of 48.7 (+/- 2.34) minutes. These data indicate an ultradian clock that controls of the frequency of directional changes. However, when the data were measured over the entire 24 hour period, the number of directional changes increased during the afternoon and evening, before returning to the levels seen in the early morning. The tau for this change was 24 hours, indicating a circadian clock also influences the frequency of directional changes. Furthermore, the presence of 4 mM LiCl effected the two biological rhythms, but in the opposite direction. LiCl reduced the periodicity of the ultradian rhythms (the tau was 11.2 minutes), but increased the periodicity of the circadian rhythm (with a tau of 28.6 hours). Furthermore, growing cells in inositol inhibited the LiCl effect on the ultradian rhythm, but not for the circadian rhythm. These results will be discussed in terms of the overall regulation of the behavior and the generation of action potentials in Paramecium.

P134

Post-translational Regulation of TPH1 Is Responsible for the Nightly Surge of 5-HT Output in the Rat Pineal Gland

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Serotonin (5-hydroxytryptamine, 5-HT), a precursor for melatonin production, is produced abundantly in the pineal gland of all vertebrate animals. The synthesis of 5-HT is rate-limited by the tryptophan hydroxylase (TPH) whose activity displays two-fold increase at night in the rat pineal. Earlier studies from our laboratory demonstrate that pineal 5-HT output exhibits dynamic circadian rhythms with elevated levels at early night, which is controlled by the adrenergic signaling at night. In this study, we report that (1) 5-HT total output from the pineal gland and TPH1 protein levels both display diurnal rhythms with a 2-fold increase at night; (2) stimulation of cAMP signaling elevates 5-HT output in vivo; (3) 5-HT total output and TPH1 protein content in rat pineal gland are both sensitive to light exposures at night. Consistent with these in vivo findings, molecular analysis of TPH1 protein revealed that (1) TPH1 is phosphorylated at the serine 58 in vitro and in the night pineal gland; (2) phosphorylation of TPH1 at this residue is required for the cAMP-enhanced TPH1 protein stability; and (3) TPH1 is degraded by proteasomal pathway. These data support the model that the increased nocturnal 5-HT synthesis in the pineal gland is mediated by the phosphorylation of TPH1 at the serine 58, which elevates the TPH1 protein content and activity at night.

P135

Circadian Breast Cancer Whole Genomic Expression

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Circadian whole genome analyses demonstrate that 5-10% of the genes of each studied tissue are under circadian clock control. These genes temporally coordinate different tissue specific key pathways. We have shown that circadian clock gene and protein expression oscillate in a mouse breast cancer albeit, with damped amplitudes as compared to host liver tissue. Here, we investigate which breast cancer non-clock genes and pathways are under circadian clock control. Methods: Equal amounts of RNA from 2-4 breast cancers harvested at every four hours around the clock (6 time points) from the syngeneic female mice kept in 12:12 light-dark cycle and another group exposed to 48 hours of darkness before tumor harvest were pooled into each of 12 groups. Affymetrix whole mouse genome microarrays were performed. Results: Expression of 818 and 821 mouse breast cancer transcripts changed significantly and rhythmically across the 6 circadian time points under light-dark and dark-dark light cycle conditions, respectively. Moreover, expression of 218 of the identified transcripts was cyclic both in the light-dark and dark-dark cycles. These results demonstrate that expression of a substantial number of non-clock breast cancer genes are under circadian influence. Some of them are light or dark driven and others oscillate identically in the presence and absence of any light cues. Circadian clocked cancer growth and spread relevant pathways will be compared and contrasted. The temporal organization of pathways targeted therapeutically indicates when in the day those targeted agents may most effectively control cancer.

P136

Expression of Clock Genes in the Embryonic Chicken Pineal Gland

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Recent studies have shown in the avian pineal gland that during development, light-dark cycles are necessary for the generation of rhythmic patterns in the expression of Per2. To test if the whole molecular clockwork would need light to start "ticking" during ontogenesis, the changes in the 24 hour pattern of Clock and Cry1 gene expression were examined both in vivo and in vitro in the pineal gland of chicken embryos. Fertilized chicken eggs were incubated under constant darkness. Other environmental factors such as temperature, humidity and egg rocking were kept constant. Pineal glands were collected between the 13th and 19th embryonic day (ED13-19) in 4 hour intervals within experiments of various lengths. Clock and Cry1 mRNA contents of pineal extracts was determined using semi quantitative RT-PCR. The expression of clock genes Clock and Cry1 showed episodic alterations under constant darkness from ED13. After ED17, rhythmic pattern of clock gene expression appeared under our experimental conditions both in vivo and in vitro. We concluded that rhythmic environmental factors are not necessarily needed for the generation of circadian patterns of clock gene expression during development. This work was supported by the Hungarian National Science Research Fund (OTKA T-046256) and the Hungarian Medical Research Council (ETT 635/2003).

P137

Circadian Control of T Cell Proliferation

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The immune system orchestrates specific defense against pathogens through lymphocyte action. The initial step in the immune response is the presentation of antigen to T lymphocytes inside lymph nodes (LNs). T cells then proliferate rapidly, and amass in numbers that overcome and eliminate pathogen. Though advances have been made in identifying peripheral clocks, very little is known about timing of events in the immune system. We hypothesized that a circadian clock in LNs controls T cell function. The aims of this study were to identify clock gene expression in mouse LNs, investigate T cell proliferation rhythms, and examine LN rhythms in Clock mutant mice. Adult WT and Clock mutant mice were housed under a light:dark cycle and sacrificed every 4h over 24h. LNs were sampled and used to: i) Extract RNA and quantify clock gene expression by real-time PCR; ii) Measure T cell proliferation following T cell receptor stimulation. Our results show that T cells exhibit rhythmic clock gene expression. Interestingly, T cells display a robust circadian variation in proliferation after stimulation. Both clock gene and T cell proliferation rhythms are blunted in Clock mice, indicating that the circadian clock influences T cell response. This is the first evidence of a clock in LNs, and of control of the immune response by the molecular clockwork. Our results have uncovered a novel peripheral clock that regulates a key function of the immune system, and provide a basis for illuminating relationships between these systems. Research supported by the Canadian Institutes of Health Research.

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Proteomics of the Circadian Clock

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Circadian clocks are endogenous and cell-autonomous oscillators that regulate physiology and behavior. The molecular mechanism that controls circadian oscillations is established by transcriptional and translational feedback loops. Thus, clock core proteins regulate the transcription of their own mRNAs which will provide cycles of gene expression. In addition to transcriptional and translation mechanisms post-translation events have been shown to be critical for the correct timing of the system. Large scale transcriptional profiling has provided invaluable information on the molecular mechanisms controlling daily oscillations. However, post-transcriptional regulation is likely to play a key mechanistic role in the circadian regulation of protein oscillations in the cells. Circadian rhythms in protein expression and the underlying mechanisms could be revealed by a global and quantitative analysis of the cell proteome at different circadian time-points, especially when analyzed together with available data on the circadian transcriptome. To this purpose we propose to apply high resolution mass spectrometry, in combination with stable isotope labeling by amino acids in cell culture (SILAC), which allows quantification of proteins in complex mixtures. Our group has employed this method to analyze whole proteomes of cultured cells and identified and quantified more than 5000 proteins, the largest proteomes reported to date.

P139

Circadian Disruption in Host and Tumor along Experimental Cancer Progression

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Altered circadian rhythms predict for poor survival both in tumor-bearing mice and cancer patients. Here we investigated the host circadian physiology, circadian clock gene expression and cell cycle distribution along experimental tumor progression (Glasgow osteosarcoma—GOS).

Methods Mice aged 7 weeks were synchronized with LD 12:12. A telemetric sensor was implanted intraperitoneally for body temperature and locomotor activity monitoring. Two weeks later, a fragment of GOS was implanted subcutaneously. Blood and tumor were sampled at one of 6 circadian times (ZT 3, 7, 11, 15, 19 or 23) on days 9 and 10 (early stage) and on days 12 and 13 (advanced stage) after tumor inoculation for determination in corticosterone (RIA), clock gene expression (Rev-erb, Per2, Bmal1 by qPCR) and cell cycle distribution (flow cytometry). Results Circadian rhythms in temperature and activity as well as corticosterone gradually dampened along tumor progression. Conversely, the proportion of G2/M cells increased and apoptosis decreased in advanced stage tumors compared with early stage ones (G2/M: 33 ± 1% vs 29 ± 1%, $p = 0.002$; apoptosis: 15 ± 1% vs 10 ± 0.6%, $p < 0.001$). Significant circadian rhythms were found in early stage tumors for all 3 clock gene expressions and for the proportions of G1-, S- and G2/M-phase cells, while these rhythms were ablated in late stage tumors. Conclusions Tumor progression was associated with gradual disruption of the circadian timing system involving host physiology, tumor clock genes and circadian cell cycle gating. Such disruption could in turn favor further uncontrolled malignant growth. This work was supported by the European Union through the Network of Excellence BioSim, Contract No. LSHB-CT-2004-005137.

P140

Circadian Rhythms in Mouse Blood Coagulation: Evidence for an Overlapping Role of Clock and Npas2 Transcription Factors

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The mechanisms underlying the circadian control of gene expression in peripheral tissues, and influencing many biological pathways, are poorly defined. Factor VII (FVII), the protease triggering blood coagulation, represents a valuable model to address this issue in liver since its plasma levels oscillate in a circadian manner and its promoter contains E-boxes, putative DNA-binding sites for CLOCK:BMAL1 and NPAS2:BMAL1 heterodimers and hallmarks of circadian regulation. Peaks of FVII mRNA levels in liver of wild type mice preceded those in plasma, to indicate a transcriptional regulation, and were abolished in Clock-/-;Npas2-/- mutant mice, thus demonstrating a role for CLOCK and NPAS2 circadian transcription factors. The investigation in Npas2-/- and ClockD19/D19 mutant mice, which express functionally defective heterodimers, revealed robust rhythms of FVII expression in both animal models, suggesting a redundant role for NPAS2 and CLOCK. The molecular bases of these observations were established through reporter gene assays. FVII transactivation activity of the NPAS2:BMAL1 and CLOCK:BMAL1 heterodimers resulted to be i) comparable (4-fold increase), ii) dampened by the negative circadian regulators PER2 and CRY1, and iii) abolished upon E-box mutagenesis. Our data provide the first evidence in peripheral oscillators for an overlapping role of CLOCK and NPAS2 in the regulation of circadian controlled genes.

P141

Chronic Jet Lag as Hepatocarcinogenesis Promoter in Mice

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Circadian disruption through chronic jet-lag (CJL) favours genetic instability and cellular proliferation in liver. We investigated the role of CJL as tumor promoter in mice exposed to hepatic carcinogen, diethylnitrosamine (DEN). Male B6D2F1 mice received DEN 10 mg/kg/day i.p. (cumulative dose: 243 mg/kg), then were randomized to remain in LD12:12 or to be submitted to CJL (8-h advance of light every 2 days). Rest-activity and body temperature were monitored. Serum liver enzymes were determined repeatedly. After 250 days, mice were sacrificed and examined for neoplastic lesions.

DEN itself markedly disrupted both circadian outputs. Furthermore CJL lengthened the mean circadian periods of temperature (25.06 ± 0.1 h, $p<0.001$) and activity (24.9 ± 0.06 h, $p= 0.001$) with a 3-fold decrease in relative amplitude ($p < 0.001$). CJL increased mean serum ASAT, a marker of liver damage (386 ± 48 vs 224 ± 26, $p=0.006$). On day 250, the incidence of microscopic liver nodules was 92% (11/12 mice, 1-6 tumors/liver) in CJL and 85% (11/13 mice, 1-4 tumors/liver) in LD. Mean diameter of the largest tumor ~doubled in CJL vs LD (8.5 vs 4.4 mm, $p=0.027$). DEN induced hepatocellular carcinomas, cholangiocarcinomas, sarcomas and mixed tumors. In LD, a single histologic type per liver was observed. In CJL, up to 4 different types were associated in the same liver. Primary tumors were also found in lung (both groups) and in kidney (CJL only). CJL moderately promoted DEN-initiated carcinogenesis. Environmental conditions that disrupt circadian clock coordination significantly accelerate cancer development. Supported by ARTBC International, Villejuif, France and Université Paris-Sud, Orsay, France.

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Inactivation of Period2 Clock Gene Accelerates Intestinal Tumorigenesis

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Colorectal cancer (CRC) risk is increased in shift workers with presumed circadian disruption. Proliferation of intestinal cells is gated by the circadian clock. Period2 gene (Per2) is a key circadian clock gene. Mice deficient in Per2 (Per2m/m) display altered circadian behavioral rhythms, de-regulation of c-Myc and Cyclin D, tissue hyperplasias, and increased cancer susceptibility. A recent screen of all mutations in human cancers identified mutations in Per2. Mutations in the adenomatous polyposis coli (Apc) gene contribute to spontaneous and hereditary CRC. The ApcMin/+ mouse is a widely used model for colorectal cancer. Loss of Apc function leads to constitutive Wnt stimulation, increased catenin and downstream gene expression (c-Myc, Cyclin D), and adenoma formation. We crossed Per2m/m mice with ApcMin/+ mice. Per2m/mxApcMin/+ mice have twice the number of small intestinal polyps (83.9 vs 36.8, $p<0.05$), but no differences in colonic polyps compared to ApcMin/+ mice. Consistent with this higher tumor burden, Per2m/mxApcMin/+ mice are more anemic (hematocrits 40.6 vs 46.9%, $p<0.05$) with more pronounced splenomegaly (0.20 vs 0.11 grams, $p<0.05$), and greater splenic extra-medullary hematopoiesis than ApcMin/+ mice. ApcMin/+ mice with a heterozygous Per2 mutation (Per2m/+xApcMin/+) did not differ from ApcMin/+ mice. These results support the role of Per2 in suppressing tumorigenesis in the small intestine. We will provide evidence that Per2 exerts this effect through its clocking of intestinal epithelial cell proliferation. Per2 may be an effective target for prevention and therapy of CRC.

P143

Early Social Isolation of O. degus: An Animal Model for Depression and Eating Disorders

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This study assessed the effects of isolation on the development, cognition, eating behaviors, and circadian rhythms of young *Octodon degus*, a highly social and precocious rodent. Preliminary data suggest that isolation of young degus can result in behaviors resembling human depression and reduced food intake. Ten female weanling degus were obtained from a breeding colony at Albion College, four of which were individually isolated and the remaining six housed in pairs of two. We predicted that isolated animals would display more anxiety-related behavior, reduced spatial cognitive ability, and have lower weights. It is also predicted that isolated animals will have altered circadian rhythms relative to group-housed animals based on previous research indicating anxiety levels induced by the isolation and a lack of social cues affect these patterns. An open field task was used to measure anxiety and a 6-arm radial arm maze used to measure cognition. All animals were weighed weekly. Circadian activity data were collected with running wheels at regular intervals throughout the experiment. Comparisons between groups will be discussed. Data are discussed in terms of using degus as animal models for human depression and eating disorders.

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Time-Dependent Spatial Learning in O. degus with the Radial Arm Maze

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The diurnal rodent, *Octodon degus*, was trained to find food rewards with two different patterns of locations at two different times of day in a 6-arm radial maze. The location of the rewards was dependent on the time of day, morning or evening, so the animals were required to use their internal clocks to determine the location of the correct arms at a particular time of day. We predicted that degus would be able to perform this task because of the nature of their native environment and the necessity to find food sources at varying times of day and we predicted that males would learn the task faster than females. Results include discussion of the animals' ability to learn the location of rewards in the radial arm maze, the ability to learn the more complex time-dependent task and any significant sex differences.

P145

G-Protein–Coupled Receptor Kinase 2 Is Required for Rhythmic Olfactory Responses in Drosophila melanogaster

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The Drosophila circadian clock controls rhythms in the amplitude of odor-induced electrophysiological responses that peak during the middle of night. These rhythms are dependent on clocks in olfactory sensory neurons (OSNs), but how these clocks control olfactory responses is not understood. Here we show that the levels of G-protein coupled receptor kinase 2 (Gprk2) mRNA and protein cycle in a circadian clock-dependent manner with a peak around midnight in antennae. Gprk2 overexpression in OSNs of wild-type or cyc01 flies elicit constant high amplitude electroantennogram (EAG) responses to ethyl

acetate, whereas Gprk mutants produce constant low amplitude EAG responses to ethyl acetate in wild-type flies. Odorant receptors (ORs) accumulate to high levels in the dendrites of OSNs around mid-night, and this dendritic localization of ORs is enhanced by Gprk2 at times when ORs are primarily localized in the cell body. These results suggest that rhythmic Gprk2 expression controls EAG response rhythms by regulating OR accumulation. Studies to define the mechanisms by which GPRK2 controls OR trafficking and the circadian clock controls Gprk2 expression will be presented.

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Great efforts have been directed to the dissection of the cell-autonomous circadian oscillator in Drosophila. However, less information is available regarding how this oscillator controls rhythmic rest-activity cycles. We have identified a viable allele of roundabout, robohy, where the period of locomotor activity is shortened. From its role in axon pathfinding we anticipated developmental defects in circadian structures; however robohy produced minor defects in the architecture of the circuits essential for rhythmic behavior. The observation that ROBO is detected in the circadian network, coupled to its effect on rhythmicity when downregulated specifically within the PDF circuit in the adult strengthened the possibility of a novel role for ROBO at this postdevelopmental stage. Genetic interactions between pdf01 and robohy suggest that ROBO could alter the communication within different clusters of the circadian network, thus impinging on two basic properties, periodicity and / or rhythmicity. In particular, introducing a copy of robohy into pdf01 gave rise to over 80% rhythmic individuals and also rescued the morning anticipation, suggesting that PDF-independent factors could modulate this circadian behavior. Early translocation of PERIOD to the nucleus in robohy pacemaker cells were first detected in adult flies indicating that robohy could only affect the molecular oscillations when the adult circadian network is functional. We propose that distorted neuronal activity caused by defective synaptic contacts in any component of the circadian network could in turn impinge upon the molecular oscillations in the small LNvs.

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The Network of Clock-Controlled Genes in Cyanobacteria

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In the cyanobacterium *Synechococcus elongatus*, all promoter activities are under the control of the circadian clock in continuous light conditions. Previously, Kucho et al. used PCR-fragment-based DNA microarray to examine genome-wide transcription profiles in Synechocystis and identified 9% of all genes as clock-controlled genes. Liu et al. examined the genome-wide expression by 'promoter-trap' analyis in synechococcus in which amplitude and precision of circadian transcription rhythm are much higher. They reported all promoter activity of 800 randomly chosen genes showed circadian rhythm in LL conditions. However, microarray analysis has not been adopted for synchococcus as far.

We employed high-density oligonucleotide arrays (GeneChip) to examine genome-wide Synechococcus gene expression. Our analysis identified at least 30% of 2,538 possible ORFs as clock-controlled genes. We performed northern analysis for the clock-controlled genes. The result validated the accuracy of our microarray analysis. The cyclic genes were classified into two groups, one peaking at subjective dawn and the other peaking at subjective dusk. Such circadian control was nullified in kaiABC-null strains. These results are consistent with our previously suggested model (Tomita et al. 2005). Based on these results, we will discuss the genetic network among clock-related genes.

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Genetic Dissection of the Circadian Regulation of Transcription in Cyanobacterial Cells

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In the cyanobacterium Synechococcus elongatus PCC 7942, the temporal information from the KaiABC-based circadian oscillator is transmitted to the regulatory machinery for gene expression. A number of mutants that are deficient in circadian gene expression have been identified and analyzed. Among them, rpaA, sasA and labA are shown to play key roles in the output pathways from the circadian oscillator. It was proposed that RpaA acts as a transcriptional factor to regulate the circadian kaiBC expression by receiving both a positive signal from SasA and negative one from LabA. However, the labA/sasA double mutant did show a low-amplitude circadian kaiBC expression, suggesting that there are additional circadian output pathways to regulate the kaiBC expression. We performed further screening to identify those genes that are involved in the additional output pathways.

P149

Regulation of Immune System Function by the Circadian Clock

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The circadian clock is implicated in several aspects of physiology including regulation of the immune response. However, the mechanisms and pathways by which the circadian clock regulates immune system function are unclear. A candidate clock structure and signal for regulation of the immune responses are the pineal gland and its hormone melatonin. In chicken, Gallus gallus, the avian pineal gland serves as a valuable circadian model system. In addition to containing photoreceptors and autonomous circadian oscillators capable of generating rhythms of melatonin, the pineal gland is also possesses functional lymphocyte populations which undergo daily rhythms in proliferation. In this study, the mRNA abundance of several immune function genes were expressed in a rhythmic manner in pineal tissue in both LD and DD photoperiods. Rhythmic genes include proteins implicated in regulation of cytokine production, leukocyte development and proliferation, as well as apoptosis. Examination of clock and immune function gene expression in peripheral immune tissues yielded several interesting results including daily clock gene expression similar to phasing in the pineal gland. Further, genes of the melatonin biosynthesis pathway were also expressed in a rhythmic fashion in the spleen indicating localized source of melatonin production in a peripheral immune tissue. These data as well as the effects of pinealectomy upon peripheral immune tissue function are presented.

P150

Is Clock Involved in Murine Sperm Maturation? Rhythmic Expression of Clock and Output Genes in the Sperm Ducts and Prostate of Mice

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Circadian clocks regulate multiple rhythms in mammalian tissues. In most examined organs, core circadian clock gene expression is oscillatory, with negative clock components, Per and Cry, peaking in antiphase to BMAL1. A notable exception is the testis, in which clock genes seem to be non-rhythmic. Earlier studies in mammals, however, did not examine patterns of clock gene expression in accessory ductal tissue, required for the maturation and transport of sperm. Previous studies in insects demonstrated control of sperm maturation in the vas deferens by a local circadian system. Sperm duct epithelia express clock genes and display circadian changes in pH level controlled by rhythmic activities of vacuolar ATPase (VATPase) and carbonic anhydrase (CAII). It is not known whether rhythms involved in processing of sperm are conserved beyond insects. To address this question in mice, we examined temporal patterns of Per1 and BMAL1 gene expression and protein abundance in the epidydimis, vas deferens, seminal vesicles and prostate. Results demonstrate oscillations of mPer1 and BMAL1 at both mRNA and protein levels in segments of sperm ducts. Strikingly, mPer1 and BMAL1 mRNA oscillate in antiphase in the vas deferens, seminal vesicles and prostate, with a similar peak-trough pattern as that observed in SCN. Two output genes, V-ATPase and CAII, which are rhythmic in sperm ducts of moths, are also rhythmic in sperm ducts of mice. Our data suggest that the role of a peripheral circadian system in sperm maturation may be conserved from insects to mammals. Supported in part by MNiSzW Grant N30306831/2338

P151

Heterogeneous Circadian Timing System (CTS) Status in Cancer Patients Revealed by Complementary Assessment of Tumor Clock Proteins and Rest-Activity Rhythm (Circact)

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Purpose: CircAct and PER2 in primary tumor (TUM) were separately found to predict for survival in metastatic colorectal cancer (MCC) patients (Clin Cancer Res, 2000: 3038; ASCO 2005: #3553; ASCO 2008, submitted). Here we studied for the first time the relationships between CircAct and PER1, PER2 and PER3 expression in TUM and healthy colonic mucosa (MUC) in chemo-naïve MCC patients enrolled in an international Phase III trial (J Clin Oncol, 2006: 3562).

Methods: Wrist-actigraphy (Ambulatory-Monitoring, USA) for 72h provided the autocorrelation coefficient (r24) and the dichotomy index (I<O) before chemotherapy. PER1, PER2 and PER3 expression were assessed in 2mm carrots with tissue microarrays using C-20, N-19 and Q-16 goat polyclonal antibodies, respectively (S. Cruz Biotechnology, USA). TUM and MUC were positive if 1% cells expressed PER. The % labeled tumor cells were further categorized into tertiles. The relationship between CircAct and PERs were analyzed with non-parametric tests ($p<0.01$). The concomitant prognostic value of PERs and CircAct for survival was also explored.

Findings: Actigraphy time-series were available for 130 patients. PERs expression was evaluated in 162 MUC and 192 TUM. Concomitant CircAct and PERs in MUC and TUM were available for 52 and 58

patients, respectively. PER1, PER2 or PER3, in MUC or TUM, were not significantly related to r24 or I<O. Preliminary subgroup analyses revealed worst survival in patients with both poor CircAct and low PER2 in TUM.

Interpretation: CircAct and tumor PERs are unrelated biomarkers of the CTS of cancer patients. Their concomitant assessment could foster personalizing cancer chronotherapeutics.

Entrainment and Photoreception

P152

T-Cycle Entrainment of a Split Circadian Rhythm in Syrian Hamsters

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Understanding of the circadian pacemaker has benefited from the isolation and manipulation of its constitutive components. Here we exploit the fact that under 24 h LDLD cycles, rodents readily split locomotor activity, melatonin and body temperature rhythms into two components that entrain to their respective scotophases. Our prior work suggests that this splitting reflects temporal dissociation of component oscillators within the SCN. To gain insight into the functional properties of component oscillators and their interactions, entrainment was assessed under lengthening and shortening LDLD T cycles. Male Syrian hamsters were induced to split rhythms in LDLD8:4:8:4. The light phase was subsequently lengthened or shortened at intervals in 15 min increments, ultimately yielding conditions of LDLD11:4:11:4 (T30) and LDLD5:4:5:4 (T18). The split activity rhythm was preserved during these manipulations allowing calculation of phase angles of entrainment for both activity components. In both groups, day-to-day activity onsets became progressively more variable as T cycles became more extreme, with markedly more variability in shortening Ts. Contrary to entrainment theory, phase angle of activity onsets did not systematically advance and delay relative to the light cycle in lengthening and shortening Ts, respectively. The two activity bouts, moreover, showed no evidence of differential entrainment in either condition. These and other results are interpreted to suggest that 1) oscillator splitting facilitates flexible entrainment to extreme and exotic zeitgebers; 2) that component oscillators entrain differently than the integrated pacemaker; and 3) that the rhythms are not the result of masking. Supported by NICHD-036460.

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Running Wheel Activity and Plasma Melatonin Profile in Two Hamster Species under LDLD8:4:8:4 Photoperiod

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The bifurcation or "splitting" of running wheel activity into two entrained activity components under LDLD8:4:8:4 is assumed to reflect expression of oscillatory subunits contributing to the mulit-oscillator model of circadian rhythm generation. Running wheel activity and plasma melatonin profiles of Syrian and Siberian hamsters entrained for 2 weeks under LDLD8:4:8:4 were analyzed to determine whether elevated melatonin secretion occurred in one or both 4hr scotophases compared to LD20:4 controls. This allowed us to test whether the 2 oscillators inferred to mediate these activity bouts have similar or different effects on melatonin secretion. Blood plasma was collected 1, 2, 3, or 4hrs into each 4hr scotophase, or 1hr after lights on. Siberian hamsters showed significant melatonin elevation toward the end of each scotophase, with a sharp decrease in plasma melatonin 1hr after lights on. Siberian hamsters entrained in LD20:4 also exhibited elevated melatonin levels during the scotophase, with an abrupt decline observed 1hr after lights on. Syrian hamsters showed no significant increases in plasma melatonin concentrations approaching the

end of either subjective night in LDLD8:4:8:4 or LD20:4. However, significant increases were observed when acutely extending the night scotophase in LDLD8:4:8:4. Under standard laboratory photoperiods (e.g., LD14:10) plasma melatonin secretes as early as 2hrs after activity onset in the Siberian hamster, whereas the Syrian hamster takes on average 3.78hrs. It is likely the mechanism driving differences in phase angle for activity onset and melatonin secretion under normal entrainment between species is related to their contrasting melatonin profiles under LDLD8:4:8:4. Funded by NICHD grant 36460

P154

Phase Resetting of the Circadian System Involves Signaling through Cav2.1 Calcium Channels

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Mammalian circadian rhythms in physiology and behavior are driven by the circadian pacemaker of the suprachiasmatic nucleus (SCN) and are synchronized to the external 24h light-dark cycle. Disruption of the circadian timing system arises from shift work and traveling between time zones and is a risk factor for fatigue-related accidents. Overt circadian rhythms require several days to adjust to a new time schedule, particularly to advances. Inertia in resetting seems to arise from signaling from other parts of the central nervous system to the SCN, but the involved mechanisms are not known. We examined circadian resetting in knock-in mice carrying R192Q mutated CaV2.1 calcium channels, which results in increased presynaptic calcium influx and neurotransmitter release. These animals are a model for human migraine and show increased cortical spreading depression. With recordings of excitatory postsynaptic currents (EPSC's), we confirmed the presence of increased excitatory activity in the SCN. R192Q mice showed enhanced ability to shift their behavioral activity and sleep-wake (electroencephalogram) patterns after 6-h advances of the light-dark cycle. In vitro recordings of electrical impulse frequency in SCN slices revealed that neither the retinal input pathway nor the phase shifting capacity of the SCN itself is responsible for the observed enhancement of resetting behavior. In vivo recordings of electrical activity of SCN neurons showed enhanced shifts in R192Q as compared to wild-type mice. The results indicate that inertia in phase resetting is a systemic property which is established through neuronal Cav2.1-dependent signaling pathways from extra-SCN areas to the SCN.

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Role for Vesicular Glutamate Transporter 2 (VGLUT2) in Mediating Photic Input to the Circadian Pacemaker

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Abundant evidence indicates that glutamate transmits light stimuli from retinal ganglion cells (RGC) to the suprachiasmatic nucleus (SCN). However, conclusive interpretation of pharmacological results is complicated since other non-retinorecipient SCN neurons express glutamate receptors and other brain regions send afferent glutamatergic projections to the SCN. Moreover, the presence of other neuromodulators in RGCs further complicates assigning a conclusive role for glutamate. Since packaging of glutamate into synaptic vesicles is required for its function as a neurotransmitter, targeting vesicular glutamate transporters (VGLUT) is an excellent strategy for specifically disrupting glutamate neurotransmission. Genetic disruption of VGLUT isoform 1 (VGLUT1) impairs image formation while circadian entrainment remains intact, presumably due to continued retinal expression of another transporter isoform, VGLUT2. Here, adeno-associated virus containing cre-recombinase (AAV-Cre) was

injected intraocularly into mice conditional Cre-dependent knockout of the VGLUT2 gene. Biotelemetery transmitters recorded body temperature and activity for two weeks during a 12:12 hr light-dark cycle (LD). Subsequently, mice were released into constant darkness (DD) and a light pulse was administered at CT 21 on the second day of DD. Following the experiment, retinas were processed for cre and melanopsin expression by immunohistochemistry, and VGLUT2 by in situ hybridization. Coronal hypothalamic slices containing SCN were processed for c-Fos.

Preliminary data indicates that VGLUT2 conditional knockout mice sustaining bilateral intraocular injections of AAV-Cre expressed free-running circadian rhythms in LD and exhibited a highly attenuated response to a phase-shifting light pulse as compared with control VGLUT 2 conditional knockout mice receiving AAV containing Green Fluorescent Protein (GFP).

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The Ventrolateral SCN Responds to Light Independently of the Circadian Phase of the Dorsomedial SCN

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Rats housed in a 22-hour light-dark (LD) cycle exhibit two distinct circadian activity bouts simultaneously: one is entrained to the LD cycle and another free-runs at a period greater than 24 hours. These two activity bouts are associated with independent oscillations in clock gene expression in the ventrolateral (vl-) and dorsomedial (dm-) SCN, respectively. In the present study, we assessed the ability of these desynchronized subregions to respond to phase-resetting light pulses. Male Wistar rats were singly housed in a 22-hour LD cycle until activity rhythms desynchronized. Animals were then subjected to a 30-minute light pulse or control dark pulse, 4 hours after lights-off during the mid-subjective day or mid-subjective night as determined by the free-running activity bout. Animals were sacrificed 60 minutes after the start of the pulse, and brains were processed for per1 in situ hybridization. per1 mRNA was consistently elevated in the vlSCN of light-pulsed animals and was absent in the vlSCN of control-pulsed animals, with no effect of time of day. However, in the dmSCN per1 mRNA expression was elevated during the subjective day and low in the subjective night, with no effect of light treatment. We conclude that the vlSCN's ability to respond to photic signals at night does not depend on the phase of the dmSCN, but rather appears to be an intrinsic property of this subregion. Our results indicate that dual oscillators within the SCN can be dissociated from each other in an anatomically intact model, while maintaining at least some of their basic functional properties.

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The Per1 Response to Light within the Suprachiasmatic Nucleus of the Diurnal Octodon degus and the Nocturnal Rat

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Behavioral studies show that the circadian systems of diurnal mammals are sensitive to light throughout the day, whereas those of nocturnal mammals are unresponsive to midday light. We therefore hypothesized that the photosensitive clock gene Per1 would show an increased sensitivity to midday light in diurnal mammals as compared to nocturnal animals. To test this hypothesis, brains of the diurnal rodent *Octodon degus* and nocturnal rat were collected 2 hours following a light pulse at CT 4, 8, 12, 16, and 22 and compared to controls kept in constant darkness. Per1 mRNA expression levels of the suprachiasmatic nucleus (SCN) were analyzed using in situ hybridization. Total signal in the SCN of degus and rats were compared to detect whether the midday photic response of Per1 was related to the ability of each species

to respond to midday light by a behavioral phase shift. degus showed increased Per1 mRNA in response to light pulses during the subjective night similar to other species. Unexpectedly, we found that both degus and rats showed Per1 responses to midday light pulses.

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Selective Serotonin Reuptake Inhibitors and Raft Inhibitors Shorten the Period of Period1-Driven Circadian Bioluminescence Rhythms in Rat-1 Fibroblasts

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Alterations in circadian rhythm generation may be related to the development of mood disorders. Although it has been reported that the most popular antidepressant, selective serotonin reuptake inhibitors (SSRIs) affect circadian phase, no data are available that describe the effects SSRIs on other circadian parameters (period, amplitude and damping rate) in isolated cells. In the present study we used real time monitoring of bioluminescence in rat-1 fibroblasts expressing the Period1-luciferase transgene, and that in Period1-luciferase transgenic mouse suprachiasmatic nucleus (SCN) explants, in order to characterize the effects of the selective serotonin reuptake inhibitor (SSRI) on circadian clock function in vitro. We found that mRNA of the serotonin transporter (SERT), a target of SSRIs, was expressed in rat-1 fibroblasts. Sertraline, fluoxetine, fluvoxamine, citalopram and paroxetine all significantly shortened the period of Period1-bioluminescence rhythms in rat-1 fibroblasts. The effect of sertraline was dose-dependent, and it also shortened the circadian period in the SCN. SERT is associated with lipid microdomains, which are required for efficient SERT activity. Cholesterol chelating reagent -methylcyclodextrin significantly reduced the period in rat-1 fibroblasts. Furthermore, lipid binding reagent xylazine significantly reduced the period in rat-1 fibroblasts. In summary our data present evidence that SSRIs affect circadian rhythmicity. The action of SSRIs is likely mediated by suppression of SERT activity. A better understanding of the relationship between mental illness and biological timing may yield new insight into disease etiology and avenues for treatment.

P159

Altered Circadian Clock Function in Mice with Developmentally Damaged Serotonin Systems

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Serotonin plays an important role in circadian rhythms. The ETS transcription factor, Pet-1, is required for differentiation of serotonin neurons and proper development of the entire serotonin system. In Pet-1 knockout (KO) mice, most serotonin neurons fail to differentiate and those that remain are deficient in gene expression required for serotonin synthesis, storage, and reuptake. These experiments were designed to determine the effects of disruption of the entire serotonin system on circadian clock function. Pet-1 KO, heterozygote (HET), and wild-type (WT) mice were entrained to a 14:10 light-dark (LD) cycle prior to the start of the experiment. In LD, KO mice had altered activity profiles as compared to WT, with a reduced percentage of wheel-running activity during the early portion of the dark period and increased activity during the late dark period. HET animals were not significantly different from WT. Pet-1 KO mice showed longer circadian periods than HET and WT in constant dark (DD). No differences in period between WT and KO were seen in constant light; however, HET animals appear to have longer periods than KO animals. When maintained in DD for 70 days, WT animals showed a shortening of circadian

period over time, but KO animals maintained their long periods. These data suggest that a serotonin deficiency results in alterations in circadian clock function distinct from those produced in serotonin receptor KO mice.

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Resetting Effects of Serotonergic Drugs, Light and Their Combination on the Main Circadian Clock : New Light on the Serotonergic Paradox in the Rat

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The main circadian clock is localized in the suprachiasmatic nuclei (SCN) of the hypothalamus in mammals. This clock can be synchronized by many factors, among which light is the most powerful. The dense serotonergic pathway between raphe nuclei and SCN plays an important role in synchronization. In mice and hamsters, two nocturnal species, serotonergic agonists and serotonin reuptake inhibitors (e.g., fluoxetine) have a non-photic influence (i.e., shifting effects during the subjective day and attenuation of photic resetting during nighttime). A paradox concerning the rat, another nocturnal species, still remains, because serotonergic modulation of the rat's SCN in vivo shows photic-like features (i.e., shifting effects during nighttime). To delineate this paradox, i.p. injections of fluoxetine and serotonergic agonists have been performed at different circadian times (CT) with or without light pulses in rats kept in constant darkness. At CT6, fluoxetine induced non-photic-like phase-advances of the wheel-running activity rhythm in a dose-dependent manner.

At CT22, fluoxetine had no effect alone but decreased light-induced phase-advances. 5-HT3 (m-Chlorophenylbiguanide) and 5-HT7 (AS-19) receptor agonists injected alone at CT22 induced significant phase-advances and no phase-shift, respectively. Moreover, neither 5-HT3 nor 5-HT7 agonists significantly modified light-induced phase-advances. Therefore, in rats, stimulation of 5-HT3 (and probably 5-HT2C) receptors produces photic-like effects. When the rat's serotonergic system is activated in vivo by fluoxetine, this induces non photic-like responses comparable to those found in other nocturnal rodents, including phase-advances during the subjective day and a negative modulation of light resetting during late night.

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NAN-190 Potentiates the Circadian Response to Light and Speeds Re-Entrainment to Advanced Light Cycles

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Health problems can arise from de-synchrony between the external environment and the endogenous circadian rhythm, yet the circadian system is not able to quickly adjust to large, abrupt changes in the external daily cycle. In this study, we investigated the ability of the serotonin 1A receptor partial agonist, NAN-190, to potentiate the circadian rhythm response to light as measured by phase of behavioral activity rhythms. NAN-190 (5 mg/kg, i.p.) was able to significantly potentiate the response to light both in dark-adapted and entrained hamsters. This effect was dose-dependent. Furthermore, NAN-190 was effective even when administered up to 6 hours after light onset. Response to a light pulse was both greater in magnitude and involved fewer unstable transient cycles. Finally, NAN-190 was able to speed re-entrainment to a 6 h advance of the light: dark cycle by an average of 6 days when compared to vehicle-treated animals. This work suggests that compounds like NAN-190 may hold great potential as a pharmaceutical treatment for jetlag, shift work, and other circadian disorders.

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Short-term Constant Light Can Potentiate Serotonergic Phase-Resetting and Immediate Reentrainment of the Circadian Clock to Large (10-Hour) Jet Lag Shifts

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Recently we showed that the phase-resetting effects of nonphotic stimuli, including exogenous serotonin (5-HT) agonists are dramatically enhanced by brief (~2 day) constant light exposure (LLb). Here we determined: 1) if LLb potentiates phase-resetting effects of endogenous 5-HT activity stimulated by L-tryptophan (Trp) and/or fluoxetine (Flu); and 2) if LLb increases the rate of 5-HT agonist-aided reentrainment to simulated jet-lag. In both experiments Syrian hamsters under a 14:10 LD cycle (LD) were exposed to LLb for 2 days or kept under LD prior to treatment. In Expt. 1, hamsters received i.p. injection of Trp, Flu, Flu+Trp or DMSO vehicle at ZT0, then released to constant darkness to measure phase-shifts of the circadian activity rhythm. There were no shifting responses to any treatment in LD. In marked contrast, LLb-treated animals receiving L-tryptophan or fluoxetine+L-trytophan exhibited large (2.0-2.5 h) phase-advance shifts ($p<0.001$ vs. respective LD groups). In Expt. 2, animals under LD or exposed to LLb received i.p. injection of the 5-HT1A,7 agonist, 8-OH-DPAT (DPAT) or DMSO at ZT0, followed by a 10 h advance of their LD cycle. Most DPAT-treated animals (8/12) immediately reentrained to the LD shift (within 1 LD cycle), with some requiring a few days (group mean=4 days). None of the animals from any other group immediately reentrained (times averaging 11-16 days; all $p<0.03$ vs. LLb+DPAT). These data show that LLb significantly potentiates the phase-resetting effect of stimulated endogenous serotonergic activity and also markedly speeds reentrainment to large shifts of the LD cycle. NIH grant NH35229 to JDG.

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Molecular Underpinnings of Serotonergic Enhancement of Photic Phase Shifts

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The serotonin mixed agonist/antagonist NAN-190 has been shown to greatly potentiate photic phase shifts in hamsters, as well as decrease the amount of light-induced Fos expression in the SCN. However, the molecular mechanism through which NAN-190 potentiates photic phase shifts is still largely unknown. The goal of the present experiment was to examine the effect of NAN-190 on light-induced genes and proteins found within the SCN, as well as the phosphorylated form of proteins in the biochemical cascade to the nucleus which are expressed immediately following a light pulse. Adult male mice, housed in constant darkness, were given an i.p. injection of either NAN-190 (2.5mg/kg) or vehicle control (DMSO) at CT21, followed by a 15-minute light pulse at CT22. Tissue was labeled for one of the following: Fos, mPer1, mPer2, P-ERK, or P-CREB. Pre-treatment with NAN-190 resulted in a significant reduction in the amount of P-CREB and Fos expression observed, 15- and 90-minutes following the start of the light pulse, respectively. However, there was no effect found for the expression of either mPer1 or mPer2, nor for the amount of P-ERK observed within SCN cells. These results suggest that NAN-190 enhances photic phase shifts, at least partially, through its effect on the expression or phosphorylation of proteins involved in the early signaling pathways within the cells of the SCN following light exposure, but does not appear to adjust the expression of the clock genes in the time course we observed.

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Further Examination of Median Raphe Projections to the Suprachiasmatic Nucleus in Syrian Hamsters

Glenn R. Yamakawa* and Michael C. Antle

The raphe complex is the primary source of serotonin in the mammalian brain. Previous studies have shown that the median raphe nucleus (MRN) projects to the suprachiasmatic nucleus (SCN) and only about half of those projections are serotonergic. This SCN afferent is thought to modulate some photic and non-photic effects. A recent study found the presence of vesicular glutamate transporter 3 (VGLUT3) in the MRN of hamsters. We sought to determine if these VGLUT3 cells might project to the SCN. The retrograde tract tracer cholera toxin subunit (CTb) was applied to the SCN of Syrian hamsters. Triple label immunocytochemistry for CTb, serotonin and VGLUT3 was conducted in the MRN. Consistent with previous findings, many of the projections to the SCN were serotonergic. Also consistent with previous findings, many of these VGLUT3 cells double labeled for serotonin. Some VGLUT3 positive CTb labeled cells did not triple label for serotonin, however. This suggests that the non-serotonergic MRN cells projecting to the SCN may use glutamate as a neurotransmitter. Further studies will be needed to confirm this hypothesis.

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Specific Targeting of Melanopsin-Expressing Retinal Ganglion Cells with a Saporin Conjugate

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Intrinsically photosensitive retinal ganglion cells (ipRGCs) in mammals express melanopsin as their photopigment and project to brain areas involved in circadian entrainment. Saporin (SAP) is a type 1 ribosome-inactivating protein extracted from the seeds of the soapwort, Saponaria officinalis. We have developed a saporin-based immunotoxin to target ipRGCs in the mouse retina. UF008, an anti-melanopsin antibody raised against the 15 N-terminal amino acids of mouse melanopsin, has been conjugated to saporin (Advanced Targeting Systems, San Diego, CA). We intravitreously injected various doses of UF008-SAP conjugate into eyes of adult C57BL/6J mice and looked at melanopsin immunohistochemistry in flat mounted retinas. Death of ipRGCs was dose dependent. The lowest effective dose was determined to be 400 ng/eye. Consequently, this dosage was used to assess the time course of the toxin. A two-week postinjection interval was determined to be the earliest time point when the optimal targeting effects of UF008-SAP could be observed. Immunostaining of fixed retinal cryosections showed no non-specific effects to the retinas after the injections. We conclude the UF008-SAP targets melanopsin cells specifically, without causing any collateral damage to the rest of the retinal neurons. Hence, the UF008-SAP conjugate is a new tool for studying the effects of melanopsin cell loss in the fully developed adult retina on circadian behavior and other forms of non-visual photophysiology.

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Developmental Regulation of Melanopsin-Containing Retinal Ganglion Cells

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In mammals, the pathways by which rod and cone photoreceptors mediate vision have been known for some time. The roles that these cells play in photoentrainment, however, have been less clear. Phase-shifting studies on animals lacking rods and cones suggested the presence of an additional ocular photoreceptor because loss of these cells did not affect light entrainment. The discovery of intrinsically photosensitive retinal ganglion cells (ipRGCs) that express the photopigment melanopsin supported this idea. These cells project directly to the circadian clock, located in the suprachiasmatic nuclei (SCN) and thereby mediate light-induced behaviors. These findings suggested that rods and cones were not necessary for photoentrainment. Conflicting evidence arose, however, when attenuated phase-shifting responses were observed in the retinally degenerate CBA/J mouse, which exhibit an early onset form of retinal degeneration. The goal of this study was to examine the potential role of outer retina maturation in controlling the development of ipRGCs, and whether regulation at this level dictates the function of the adult circadian system. We confirm the phase-shifting differences seen between the CBA/J mice and their controls and characterize the time course of retinal degeneration. We report that dendritic stratification is unaltered in mice with early retinal degeneration, suggesting that photoreceptors are not necessary for this process. However, CBA/J mice have greater numbers of ipRGCs in the adult than the controls, implicating the importance of outer retina maturation in regulating developmental cell death. We conclude that photoreceptor regulation of ipRGCs during development may determine the overall function of the circadian system.

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Targeted Destruction of ipRGCs with a Saporin Conjugate Greatly Targeted Destruction of ipRGCs with a Saporin Conjugate Greatly Alters the Effects of Light on Mouse Circadian Rhythms

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Both classical and ipRGCs photoreceptors contribute to entrainment and several other activities. Here, the ribosome-inactivating protein, saporin (SAP), conjugated to UF008, an anti-melanopsin antibody, was injected intravitreously to test the effect of ipRGC loss. C57BL/6J mice were entrained to LD12:12 (light = 1 μW/cm2), then anesthetized and unilaterally enucleated; the other eye was later injected with 400 ng SAP/UF008 or 400 ng SAP/IgG conjugate in 2 μl saline . No animal lost entrainment. Under an exotic photoperiod in which light gradually diminished to zero over a 6 hr interval each day, all animals entrained, but SAP/UF008 mice assumed much earlier phase angles than controls (10.78 ±0.45 hr after midnight vs 16.95 ±0.26 hr) and received much larger doses of light energy per day (233.3 vs 122.5 Joules). In DD, circadian periods did not differ between groups. In LL, control period lengthened (0.7±0.05 hr) compared to no change (0.04±0.07 hr) for SAP/UF008 animals. Upon return to LD12:12, entrainment failed in 5 of 10 SAP/UF008 mice (vs 0/10 controls). Masking in LL or to a 1 hr light pulse was eliminated by SAP/UF008 treatment. Stainable ipRGCs were reduced by about 80%, although a variety of cell markers indicated that the remaining retina was apparently normal. Retinal innervation of the SCN was greatly reduced, revealing an unexpected topography in the remaining terminal field. Thus far, SAP/UF002 treatment has not prevented initial entrainment, possibly because the remaining cells provide the SCN with light information minimally sufficient for entrainment.

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The Role of Entrainment for the Circadian Response of the Proteome

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In order to accommodate and anticipate daily environmental rhythms, organisms in all phyla have developed circadian systems that regulate the temporal program, from gene expression to behavior. Entrainment in a natural environment depends upon environmental cues called zeitgebers. Temperature is one of the most important zeitgebers and plays a crucial role for organisms that are not permanently exposed to light. Such an organism is Neurospora crassa, which entrains more stable to temperature than to light. However, our knowledge concerning the underlying molecular features of this entrainment is still limited and specifically translational processes that may regulate the circadian system have to be studied in more detail. In a quantitative proteomics approach we are analyzing the changes in the proteome over the course of time. We are comparing the response to entrainment in Neurospora crassa by comparing the free-running rhythm with entrained rhythms. In this mass spectroscopy based approach, we can analyze both the differential protein expression and post-translational modifications at the circadian level. Preliminary data shows that almost all of the identified proteins seem to be oscillating over the course of 24h. In addition, the imposed zeitgeber rhythm seems to be reflected in the oscillations of the protein expression.

P169

Effects of Monochromatic Light on the Endogenous Pacemaker of Drosophila melanogaster

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The fruit fly uses several photoreceptors to entrain the endogenous clock: The compound eyes, the Hofbauer-Buchner eyelets, the ocelli and cryptochrome (CRY). Recently, we showed that the compound eyes are crucial for the correct seasonal adaptation of morning (M) and evening (E) activity peaks. This includes the sensing of dim light and the acceleration and deceleration of M and E oscillators upon constant light. In the cryb mutant background, constant white light provokes a simultaneous free-run of M- and E-oscillators with short and long periods, respectively. In a wild-type background such a differential action of constant light on M- and E-oscillators could hardly be seen, since CRY leads to degradation of TIM and stops the clock oscillations already at rather low irradiances. Here we show that this behavior is strongly dependent on the wavelength of light. Wavelengths in the range of 585-630nm did not cause arhythmia in wild-type flies even not at energies up to 4000 mW/cm2. At these wavelengths we observe period changes and internal desynchronization into the above mentioned two activity components in an energy dependent manner. In contrast, light of 540nm causes arhythmia at energies as low as 85 mW/cm2. This wavelength likely activates the blue light receptor CRY. We started a systematic test of monochromatic light to reveal which rhodopsin(s) of the compound eyes (Rh1, Rh3, Rh4, Rh5, Rh6) mediate the acceleration and deceleration of M- and E-oscillators, respectively. The first results will be presented.

P170

A Chicken Model of Blindness Reveals Functional Photoreceptors in the Inner Retina of Non-mammalian Vertebrates

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In mammals, photoreceptors (PRs) located in the inner retina convey photic information to the brain via a non-image forming (NIF) circuitry that regulates a number of non-visual functions. In non-mammalian vertebrates, the retina, deep brain PRs and the pineal organ may be photoreceptive; however, the study of the NIF circuitry has not been explored yet. Herein, we investigated light perception in the absence of functional cone and rod PRs by using GUCY1* chickens. These birds carry an autosomal recessive mutation that causes photoreceptor cell degeneration and blindness at hatch since null electroretinograms were obtained after light exposure. However, they showed light responses in both the pupillary light reflex (PLR) and the entrainment of feeding rhythms to a 12:12 h light dark (LD) cycle. Moreover, when the extraretinal photoperception was inhibited by head occlusion, daily rhythms in feeding were tightly entrained to diverse white and blue LD cycles whereas light responses were lost after enucleation. Blind animals displayed consensual PLRs to white and monochromatic light of 400-550 nm. An action spectrum for the PLRs demonstrated that this response is driven by a single opsin/vitamin A2-based photopigment with peak sensitivity around 484 nm. The results represent the first characterization of functional PRs in the chicken inner retina participating in the regulation of NIF activities. Supported by Fundación Florencio Fiorini, FONCyT, CONICET, SeCyT-UNC, and Agencia Córdoba Ciencia.

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Photoreaction Mechanism of the Circadian Blue-Light Receptor Drosophila Cryptochrome

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Cryptochromes (CRY) are flavoproteins, which belong to the cryptochrome/ photolyase family. Photolyases repair UV-damaged DNA with help of two chromophores, flavinadenindinucleotide (FAD) and either methenyltetrahydrofolate (MTHF) (most organisms) or 8-hydroxy-5-deazariboflavin (8-HDF). Despite their high sequence- and structural similarity to photolyases, the cryptochromes do not exhibit DNA-repair activities. Drosophila cryptochrome (dCRY) is a blue-light photoreceptor mediating light-synchronization of the circadian clock. To provide a better understanding of the biological functions of Drosophila cryptochrome, we have established a purification scheme that enables us to gain mg amounts of monomeric dCRY via baculovirus expression system. Using biochemical and biophysical methods we show that insect cell purified dCRY contains FAD in its oxidized state (FADox) and as the second chromophor MTHF which is converted to 10-formyldimethylhydrofolate (10-FDHF). Upon blue-light irradiation, dCRY undergoes a reversible absorption change, which is assigned to the conversion of FADox (ground state) to the anionic FAD°- radical (signalling state). This photoreaction mechanism is proposed to mediate resetting of the Drosophila circadian clock. Photo-cycle schemes of dCRY are discussed and some characteristic parameters are extracted like the quantum efficiency of signalling-state formation, the time-constant of photo-induced electron transfer, and the dark-state recovery time.

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Locomotor Activity Profiles of Wild-Type and Period Mutant Strains of Drosophila melanogaster under Different Photoperiods with Simulated Dawn and Dusk

Dirk Rieger* and Charlotte Helfrich-Förster, University of Regensburg

The fruit fly *Drosophila melanogaster* shows bimodal activity patterns with activity peaks in the morning (M) and evening (E). When dawn and dusk were simulated by logarithmically increasing/decreasing the light intensity during a 1.5 hour interval (LD 12:12), M and E peaks shift into early dawn and late dusk, respectively. For the wild-type strain CantonS, M and E peaks occurred at a light intensity of about 7.5 lux. In the present study we investigated the activity profiles of CantonS flies under long photoperiods with simulated dawn and dusk (day lengths: 14h, 16h, 18h and 20h). We found that the M peak occurred during early dawn at all photoperiods. For the E peak this was only true under moderate long days. Beyond the 16 h photoperiod, the E component remained at approximately the same position with respect to dawn and the M component. Thus, the phase relationship between M and E peaks (PsiM,E) did not increase beyond 16 h. We conclude that M and E oscillators are coupled and this coupling has a limited flexibility. To test the influence of fast and slow running clocks on the phases of M and E peaks, we studied pers and perl mutant flies. Besides significant differences in height and broadness of M and E peaks, we found that pers mutants reached a maximal PsiM,E of ~12 h, whereas perl mutants could increase PsiM,E to 20 h. The significance of these results will be discussed.

P173

Tracking Molecular Rhythms in the SCN and Peripheral Tissues during Chronic Jet Lag

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The mammalian circadian system can adapt to abrupt changes in the timing of the photoperiod, but several transient cycles are required to reestablish both a normal phase relationship with the environment and internal organization among the different oscillators within the organism. This desynchrony is thought to be one factor underlying jet-lag, the fatigue and discomfort associated with trans-meridian travel. Indeed, chronic jet-lag accelerates malignant growth and hastens death in aged mice and cardiomyopathic hamsters. While some data are available that describe the molecular resynchronization of the rodent circadian system to a single 6h phase advance, no data have addressed the multiple shift paradigms that have been shown to be harmful. In this study we tracked molecular rhythms in the SCN and some peripheral tissues after the 1st, 4th, 8th and 12th weekly 6 h phase-advance of the light schedule using the Per2-luc knock-in mouse. Preliminary results show that different organs entrain to the weekly-adjusted photoperiod at different rates, but that multiple shifts in the same direction were not more difficult to adjust to than a single shift. Interestingly, running wheel behavior seemed to follow the adjusted light schedule much better than the molecular rhythms of the SCN, perhaps due to the masking effects of light.

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Resynchronization of Per2-Luciferase in the Suprachiasmatic Nucleus and Peripheral Oscillators during Real-World Jet Lag

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During trans-meridian travel, the circadian clocks in the hypothalamic SCN and in the periphery are desynchronized with the local time at the destination and exhibit altered internal phase-relationships. Jet-lag symptoms arise from the disruption of circadian timing during this temporary period of adjustment. The physiological impact of chronic jet-lag (exposure to repeated shifts) has been associated with poor health including cardiovascular disease, cancer, and increased death in aged mice. All studies performed to date on molecular resynchronization have investigated a single phase-shift. We recently have started to investigate the effects of a 2 shift paradigm on molecular circadian rhythms in the SCN and in peripheral organs. Using the Per2-luc knock-in mouse, we followed the resynchronization of circadian rhythms in tissues of animals that were exposed to a 6 hr phase advance, followed 5 days later by a phase delay, mimicking a trip from the US Eastern coast to Europe and back. Peripheral organs show faster entrainment to a phase delay that follows a phase advance when compared to a phase delay alone. Data from the SCN indicates that the master clock is reticent to phase delay following a phase advance, suggesting a long-lasting effect of the previous advance (inertia). Since a 'return' phase delay (a delay following an advance) appears different from an identical but isolated phase delay, studies that only utilize an isolated shift paradigm must be interpreted with caution when applying the results to real-world travel.

P175

Aberrant Light Resetting Responses in Clock Null Mutant Mice

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The core circadian clock in the mouse consists of positive and negative autoregulatory transcriptional feedback loops. In the primary positive feedback loop, CLOCK and BMAL1 are central components of the molecular clock that act as key transcriptional regulators of the mammalian circadian system. To understand better the role of Clock and its transcriptional control of downstream clock genes, we sought to investigate the circadian system in the absence of CLOCK expression. To this end, we obtained ES cell clones from the German Genetrap Consortium and generated a mouse that harbors a genetrap upstream of the protein coding exons of the Clock gene. Mice harboring homozygous copies of the genetrap show elimination of Clock gene and protein expression throughout the mouse body. In concordance with previous reports, Clock genetrap null mutant mice show robust circadian rhythms in locomotor activity. However, we found abnormal phase-resetting responses that are distinctly different from conventional Clock knockouts, Clock mutants, and wild-type animals. Our results demonstrate that the light resetting response, a fundamental property of the circadian system, is abnormal and is not compensated by NPAS2 activity in Clock genetrap null mutant mice.

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Two Types of Melanopsin Retinal Ganglion Cell in the Mouse Retina Innervate the Hypothalamic Suprachiasmatic Nucleus and the Olivary Pretectal Nucleus

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Melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) innervate the suprachiasmatic nucleus (SCN) and the olivary pretectal nucleus (OPN) providing irradiance information for entrainment of circadian rhythms and for stimulating the pupillary light reflex. To investigate ipRGC projections, we used the heterozygous tau-lacZ mouse which expresses both melanopsin and -galactosidase. In tau-lacZ+/- mice, only 50% of melanopsin ipRGCs contain -galactosidase and these cells are specifically labeled with a C-terminus melanopsin antibody. Retrograde tracer injection into the SCN labels -galactosidase-expressing ipRGCs (termed M1) that comprise 80% of the SCN-projecting ipRGCs. M1 ipRGCs and an additional set of ipRGCs (termed M2) are labeled with a melanopsin antiserum targeted against the N-terminus of the melanopsin protein; M2 ipRGCs do not contain detectable -galactosidase and these cells make up the remainder of the SCN-projecting RGCs. Tracer injection into the OPN labeled non-melanopsin RGCs and both types of melanopsin ipRGC: 45% M1 and 55% M2. Infection of the iris with pseudorabies virus (PRV) results in retrograde transneuronal label of OPN projection neurons that innervate preganglionic parasympathetic neurons of the Edinger-Westphal nucleus; PRV-labeled cells were located almost exclusively within the terminal field of M1 ipRGCs in the periphery (shell) of the OPN. The OPN core receives retinal input and we hypothesize that the OPN core receives input from the M2 ipRGCs. Two subtypes of melanopsin ipRGCs project differentially to the SCN and OPN; the functional significance of ipRGCs subtypes is currently unknown.

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Circadian Light: 1+1<2

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The intrinsically photosensitive retinal ganglion cells (ipRGCs) play a central role in circadian regulation by light, but rods and cones also provide input to these cells via the outerplexiform layer of the retina. In humans, spectrally opponent blue versus yellow (b-y) bipolar cells lying distal to the ganglion cell layer has been hypothesized to provide direct input to the ipRGCs and therefore, the circadian system should exhibit subadditivity to some types of polychromatic light. Nine subjects participated in a within-subjects 3-night protocol. Three experimental conditions were employed that provided the same total irradiance at both eyes: 1) one unit of blue light (max = 450 nm, 0.077 W/m2) to the left eye plus one unit of green light (max = 525 nm, 0.211 W/m2) to the right eye, 2) one unit of blue light to the right eye plus one unit of green light to the left eye, and 3) ½ unit of blue light plus ½ unit of green light to both eyes. The unit amounts of light were calculated from a published model of human circadian phototransduction. Plasma melatonin levels were measured before and after 45-minute exposure to each lighting condition. Conditions 1 and 2 did not differ significantly in melatonin suppression while condition 3 resulted in significantly less melatonin suppression. Furthermore, the magnitudes of suppression were well predicted by the model. This study provides direct evidence that the human circadian system exhibits a subadditive response to light from spectrally opponent (b-y) neurons in the retina.

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Behavioral Responses of Vipr2-/- Mice to Light

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Vasoactive intestinal polypeptide and its receptor, VPAC2, play important roles in the functioning of the dominant circadian pacemaker, located in the hypothalamic suprachiasmatic nuclei (SCN). Mice lacking VPAC2 receptors (Vipr2-/-) show altered circadian rhythms and impaired synchronization to environmental lighting cues. However, light can increase phospho-protein and immediate early gene expression in the Vipr2-/- SCN demonstrating that the circadian clock is readily responsive to light in these mice. It is not clear whether these neurochemical responses to light can be transduced to behavioral changes as seen in wild-type (WT) animals. In this study we investigated the diurnal and circadian wheel-running profile of WT (C57BL/6J) and Vipr2-/- mice under a 12h light:12h complete darkness (LD) lighting schedule and in constant darkness (DD) and used 1h light pulses to shift the activity of mice in DD. Unlike WT mice, Vipr2-/- mice show grossly altered locomotor patterns making the analysis of behavioral responses to light problematic. However, analyses of both the onset and offset of locomotor activity reveal that in a subset of these mice light can reset the offset of behavioral rhythms during the subjective night. This suggests that the SCN clock of Vipr2-/- mice and the rhythms it generates are responsive to photic stimulation and that these responses can be integrated to whole animal behavioral changes.

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Hamsters without Jet Lag: Dimly Lit Nights Accelerate Re-Entrainment after a Shift in the Light:Dark Cycle

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Transmeridian travel produces a mismatch between internal time and the environmental light:dark cycle, resulting in a suite of cognitive and physiological maladies collectively known as jetlag. Most symptoms subside as the circadian clock resets to the new time zone, but re-entrainment can be a long process requiring many days to complete. Here we demonstrate in two hamster species, following a shift in the light:dark cycle, re-entrainment is accelerated by at least 40% when the scotophase is dimly lit (< 0.1 lux) rather than completely dark. Male hamsters were transferred to wheel-running cages and provided LD 14:10 with either completely dark or dimly lit nights. One week after transfer, the light:dark cycle was advanced and then delayed by an equivalent amount 2-3 weeks later. In Siberian hamsters, dim nighttime illumination accelerated re-entrainment after a 4 h shift in each direction in both young (20 wks) and aged animals (>80 wks). In Syrian hamsters, the number of days required to re-entrain after a 4 h shift in each direction was reduced when the nights were dimly lit rather than completely dark. Additionally, Syrian hamsters were exposed to 8 h shifts, and dim nighttime illumination again facilitated re-entrainment in each direction. Dim nighttime illumination may provide a promising alternative to current jetlag treatments since it does not require precise timing of a chronobiotic relative to an unknown phase of the clock. Further research is needed to determine whether this result generalizes to diurnal mammals and whether this procedure is safe and tolerable.

P180

Photic Re-setting Responses of the Tau Mutant Mouse and Hamster

Jake Lebiecki, Joita Dey, Julie Gibbs, Anne Venner, Andrew Coogan, Hugh Piggins, Rob Lucas and Andrew Loudon*, University of Manchester

The tau mutant hamster typically exhibits Type 0 re-setting responses to light after prolonged (49 day) periods in darkness (DD). To test whether such re-setting is associated with augmented clock gene expression, we defined the behavioural re-setting effects of a range of irradiances in tau and wild-type hamsters at CT14, and subsequently undertook in-situ determinations in the SCN for Per1. Our data reveal a linear relationship between Per1 induction and amplitude of phase shift, such that Type 0 resetting in taus was associated with augmentation of both Per1 induction and behavioural phase shifts. This suggests that the tau mutation alters molecular responses in the SCN to the re-setting effects of light. Tau mutant mice have a near identical circadian phenotype to tau hamsters, with a 3.7h reduction of period in homozygote taus of both species. In marked contrast, tau mutant mice do not exhibit Type 0 re-setting responses to light, even after 63 days in DD. Long duration light pulses (6h) do induce Type 0 re-setting, but these are not significantly different from WT mice. Finally, we have tested a range of sub-saturating irradiances on tau and WT mice and preliminary data suggest no significant differences. Thus, tau induces identical period-shortening effects in both species, but different re-setting responses to light following prolonged exposure to DD. The characteristic Type 0 re-setting of tau hamsters thus represents an important species difference in the phenotype of a mutation which exerts an identical effect on the accelerated circadian clock-work.

P181

Effects of Continuous Phase Shifts on Pregnancy and Offspring in O. degus

A.T. Tilot* and T.J. Jechura, Department of Psychology, Albion College

The effects of prenatal stress can be long-term; longitudinal studies have revealed cognitive deficits years into an animals lifespan. While the number of pharmacological and social stress studies is large, little research has been done regarding circadian rhythm disruption as a source of prenatal stress. The goal of the current experiment is to examine the effects of circadian disruption on pregnancy in Octodon degus, and track the development of the offspring in their first year. O. degus are social, diurnal rodents from Chile with a three-month gestation period; an excellent animal model for studies of human circadian rhythms. Baseline circadian rhythms were recorded with running wheels in 18 female O. degus, which were then mated with unrelated males. The light cycle of the experimental group was continually shifted to prevent full entrainment to a particular light cycle for more than a few days, causing circadian disruption in the animals similar to constant jet lag. The control animals were not subjected to these shifts, and activity data from the experimental and control groups were compared for differences in phase angle of entrainment, reentrainment rate and period length throughout the experiment. Research has demonstrated that shifting the internal clock is stressful, but the effect of this particular kind of stress on pregnancy and development is unclear and may have serious implications for populations at risk for chronic circadian disruption.

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The Effect of Alcohol and Olfactory Stimuli on the Reentrainment Rate of Octodon degus

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Although light is the most influential zeitgeber for many animals, nonphotic cues have also been shown to synchronize the clock in DD and improve the rate of reentrainment following a phase advance in the LD cycle. Research with female *Octodon degus* revealed links between a urine-based olfactory cue and accelerated reentrainment rate in the presence of light cues. Human females also demonstrate sensitivity to olfactory social cues when recovering from jetlag by accelerating reentrainment in the presence of particular pheromones. We tested the hypothesis that the steroid hormone-derived olfactory stimuli 4-16-androstadien-3-one and 1,3,5(10),16-estratetraen-3-ol would alter the rate of reentrainment in female degus. Here we show that only one of the hormone-derived olfactory cues successfully accelerated reentrainment rate after a 6-hour phase advance of the light cycle. We also examined the effect of alcohol on the rate of reentrainment in degus. Alcohol has been linked to the disruption of several circadian mechanisms. We investigated the effect of alcohol when consumed across the entire span of a 6-hour phase-advance in the degus. We hypothesized that alcohol would lengthen the time needed to reentrain after a 6-hour advance of the light:dark cycle. However, contrary to predictions, preliminary data suggest that alcohol reduced the length of reentrainment. Implications of these data and future research possibilities are discussed.

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Retinal Circuitry Underlying Acute Light Effects on Sleep

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The mammalian circadian pacemaker that is responsible for relaying time of day information to the rest of the organism is situated in the suprachiasmatic nucleus (SCN) of the hypothalamus and governs all daily rhythms. Light detected in the retina is transmitted via the retinohypothalamic tract to the SCN in order to synchronize its rhythm to the solar day leading to circadian photoentrainment of daily rhythms including sleep/wake cycles. However, independent of circadian rhythms, light can acutely suppress or augment these behaviors, a physiological response known as masking. We have recently determined that mice that lack melanopsin-containing retinal ganglion cells (ipRGCs) are unable to perform light-dependent subconscious functions including photoentrainment and masking. This indicates that the ipRGCs are responsible for transmitting all light information, including rod/cone input from the retina, to the non-image forming visual function centers. The circadian clock in the SCN is an integral part of sleep regulation leading to photoentrainment of this daily behavior. In order to determine the physiological pathways associated with the effects of acute light exposure on sleep/wake behavior, we investigated the sleep/wake transitions during 3-hour pulses of light administered at night in mice. We demonstrate that light induces sleep acutely when presented at night. Therefore, we asked the question: are ipRGCs the sole pathway for rod/cone light signal to acutely induce sleep, or do other conventional ganglion cells contribute to this function? To answer this question, we tested the acute effects of light on sleep in wild type animals and in mice in which the ipRGCs have been genetically ablated. Our results show that light exposure does not induce sleep in mice that lack ipRGCs.

P184

Blue-Enriched White Light in the Workplace Improves Self-Reported Alertness, Performance, and Sleep Quality

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Both light and heat can reset the Drosophila circadian clock. Although timeless protein (TIM) plays an important role in photic responses, this is mostly based on Western blots or histochemical experiments with light pulses in the late night. In the early night, there are fewer data. In addition, it is unknown whether light pulses cause TIM degradation preferentially in certain clock cells. Finally, the role of TIM in heat pulse phase shifting is unknown. These cause phase delays in the early night, with maximal values about half those of maximal light phase delays (ca. 2 vs ca. 4 hrs). To address these issues, we assayed TIM degradation by confocal microscopy immediately after a maximal light pulse at ZT15. The results indicate that there is no apparent TIM degradation in LNvs whereas TIM is rapidly and apparently completely degraded in all DN1, DN3, and most LNd neurons. A heat pulse as well as a lower intensity light pulse at ZT15 (to generate a comparable ca. 2 hr phase delay to heat) causes TIM degradation only in some of these same cells, resulting in a surprising "mosaic pattern." We suggest that the extent of phase shift is determined by the number of cells that respond to light or heat. The results further suggest that some cells belonging to the three groups are key timekeepers as well as phase shifting responders, which signal to the small LNvs—the cells that keep time in constant darkness. This view is consistent with recently published results.

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Identification and Characterization of Key Light-Sensitive Cells in the **Drosophila** *Circadian System*

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Both light and heat can reset the *Drosophila* circadian clock. Although *timeless* protein (TIM) plays an important role in photic responses, this is mostly based on Western blots or histochemical experiments with light pulses in the late night. In the early night, there are fewer data. In addition, it is unknown whether light pulses cause TIM degradation preferentially in certain clock cells. Finally, the role of TIM in heat pulse phase shifting is unknown. These cause phase delays in the early night, with maximal values about half those of maximal light phase delays (ca. 2 vs ca. 4 hrs). To address these issues, we assayed TIM degradation by confocal microscopy immediately after a maximal light pulse at ZT15. The results indicate that there is no apparent TIM degradation in LNvs whereas TIM is rapidly and apparently completely degraded in all DN1, DN3, and most LNd neurons. A heat pulse as well as a lower intensity light pulse at ZT15 (to generate a comparable ca. 2 hr phase delay to heat) causes TIM degradation only in some of these same cells, resulting in a surprising "mosaic pattern." We suggest that the extent of phase shift is determined by the number of cells that respond to light or heat. The results further suggest that some cells belonging to the three groups are key timekeepers as well as phase shifting responders, which signal to the small LNvs – the cells that keep time in constant darkness. This view is consistent with recently published results.

P186

Non-Parametric Temperature Entrainment of In Vitro Kaic Phosphorylation Rhythm

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Cyanobacteria are the simplest organisms known to exhibit circadian rhythms. Three clock proteins, KaiA, KaiB, and KaiC, have been identified as essential components for circadian clock of a cyanobacterium, Synechococcus elongatus PCC 7942. KaiC phosphorylation state oscillates with a 23-h period length when three recombinant KaiC proteins are incubated in vitro in the presence of ATP. This Kai-based chemical oscillation is thought to be the basic circadian pacemaker of Synechococcus. While KaiC phosphorylation rhythm persists with a temperature-compensated period, entrainment of the rhythm to the daily environmental cycles has not been demonstrated. Here, we tested whether temperature cycle could entrain the KaiC phosphorylation rhythm. We mixed the three proteins at 6-h intervals to obtain four preparations of different phase angles. Under the standard conditions at 30 ˚C, the four KaiC phosphorylation rhythms persisted with the period of 23-h with keeping the phase angle differences. In contrast, under a temperature cycle of 12-h 45 ˚C /12-h 30 ˚C (12H12L), the periods of the KaiC phosphorylation rhythms were entrained to 24 hours and the four rhythms were synchronized to each other. We found that the KaiC phosphorylation rhythm was entrained by temperature cycles of 10H10L, 12H12L and 14H14L, but failed to be entrained by 8H8L and 16H16L. As the period of the rhythm at 45 ˚C was similar to that at 30 ˚C, the observed entrainments of the KaiC phosphorylation rhythm can be explained by non-parametric model with phase shifting by the temperature steps.

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Nocte, a Novel Gene Involved in Temperature Synchronization of the Drosophila Circadian Clock

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A key feature of the circadian clock is its ability to entrain to environmental time cues (Zeitgebers). In addition to light that is the most powerful Zeitgeber, temperature cycles and high-temperature pulses can synchronize or reset, respectively, the clock mechanism. The Drosophila mutant nocte (no circadian temperature entrainment) is defective in molecular and behavioral synchronization to temperate- but not to light-dark cycles. The nocte mutation has been mapped to a single base-pair substitution in a gene encoding a large protein of unknown function. Presence of glutamine rich repetitions combined with lack of cysteines in the aminoacid sequence, suggest that Nocte is an intracellular protein, perhaps acting as a transactivator of transcription. The spatial expression pattern of nocte has been examined by real time PCR (RT-PCR) and by using a nocte-gal4 promoter fragment driving green fluorescence protein (GFP) expression. GFP signals were detected in hundreds of glial cells and in a number of neurons within the whole cephalic ganglion. These neurons included pigment dispersing hormone (PDH) immunoreactive cells showing that nocte is expressed at least in some of the clock neurons. Outside the brain, GFP expression was detected in the peripheral mechano- and chemoreceptors as well as in all internal tissues tested. RT-PCR analyses confirmed the widespread distribution of nocte, suggesting nocte has multiple roles. Indeed, downregulation (using nocte-RNAi), or overexpression (using UAS-nocte cDNA) demonstrates that nocte is essential for the survival of the fly.

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High Responsiveness to Medium Exchange of the Cultured Suprachiasmatic Nucleus in Pup Mice

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In the fetal and neonatal periods of rodents, the circadian clock in the suprachiasmatic nucleus of the hypothalamus (SCN) is able to entrain to non-photic maternal rhythms, but this capability disappears in later life. In order to understand the mechanism behind the non-photic entrainment in the early postnatal period, the phase response of Bmal1 expression rhythm to external stimuli was examined in the cultured SCN harvested at postnatal day 6. The SCN was obtained from the transgenic mice with a bioluminescence reporter for Bmal1 expression (Nishide et al., 2006). Phase-dependent phase shifts of circadian rhythm were detected in the pup as well as in the adult for culture medium exchange, but the amount of phase shift was significantly larger in the pup than in the adult SCN. The circadian period of Bmal1 expression rhythm was shorter and the variability of rhythmicity was larger in the pup SCN than in the adult. In addition, the amplitude of circadian rhythm was much lower in the pup than in the adult. It is concluded that the high responsiveness of cultured pup SCN to medium exchange is a possible mechanism for maternal entrainment.

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Differential Effects of Scheduled Exercise on Neuropeptide Deficient Mice

A. Power*, A.T.L. Hughes, S.M. Dupre, A.S.I. Loudon, R.E. Samuels, and H.D. Piggins, Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom

Vasoactive intestinal polypeptide (VIP) and its receptor, VPAC2, are abundant within the SCN. Mice deficient in VIP (VIP-/-) or lacking this receptor (Vipr2-/-) exhibit impaired synchronisation to photic cues and altered behavioral rhythms with a continuum of behavioral phenotype from arrhythmicity to weak, short period rhythms. Synchronisation of biological rhythms to non-photic stimuli is untested in these animals. We assessed the effects of scheduled exercise on locomotor and drinking rhythms in wildtype (WT), Vipr2-/- and VIP-/- mice in constant darkness (DD). Mice were released into DD and after 14 days, access to a running wheel was restricted to 6h/day for 21 days. Following this scheduled exercise, the majority of Vipr2-/- mice expressed a lengthened, near 24h period and 70% of the previously arrhythmic animals developed robust ~24h behavioral rhythms. In contrast, the behavioral rhythms of VIP-/- mice deteriorated in DD and could not be restored by scheduled exercise. Consequently, all 6 VIP-/- animals became arrhythmic. Analysis of c-Fos expression, a marker of neuronal activity, suggests that SCN cellular rhythms are not restored in entrained Vipr2-/- animals, despite restoration of behavioral rhythmicity. These data imply that a compensatory pathway or extra-SCN pacemaker is responsible for non-photic entrainment in Vipr2-/- mice. Using in situ hybridisation analysis of mBmal1, mPer1, mAVP and mPK2, we are currently examining several Vipr2-/- brain structures to establish whether restored rhythmic behavior is associated with novel patterns of gene expression.

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Phenobarbital Administration in the Early Afternoon Suppresses Expression of Period 1 but Not Period 2 Messenger Rna in the Suprachiasmatic Nucleus of Female Hamsters

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Phenobarbital administration to proestrous hamsters during the early afternoon blocks the luteinizing hormone (LH) surge and induces a phase advance in the circadian locomotor activity rhythm and the peak of the LH surge occurring on the next day. Many non-photic signals that advance the circadian pacemaker decrease expression of Period1 (Per1) and Period2 (Per2) in the suprachiasmatic nucleus (SCN) at the time of the peak in their daily rhythm. Furthermore, reduction of SCN Per1 peak expression by administration of antisense oligonucleotides induces a phase advance. Therefore, we hypothesized that decreases in SCN expression of Per1 and Per2 mediate phenobarbital-induced phase advances. To test this hypothesis, proestrous hamsters were injected with vehicle (ethanolic saline) or phenobarbital (100 mg/kg b.w.; N=8-9 each) in the early afternoon and euthanized 2 h later. In-situ hybridizations for Per1 and Per2 were conducted on coronal brain sections. Autoradiograms of the SCN and other regions (e.g., cingulate cortex and paraventricular thalamic nucleus [PVT]), serving as neuroanatomical controls, were analyzed by computer-assisted microdensitometry. The results demonstrated that phenobarbital significantly decreased SCN expression of Per1 mRNA but not Per2 mRNA. In the cingulate cortex, phenobarbital significantly lowered Per2 but not Per1 mRNA expression, while in the PVT, no effect of phenobarbital on the expression of either gene was observed. These findings partially support the hypothesis by showing that early afternoon administration of phenobarbital decreases SCN Per1 mRNA expression, similar to other non-photic signals, and differentially affects Per1 and Per2 mRNA expression in various brain regions.
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Serum Induction of Period1 Gene Expression Is Increased in Id2 Null Mouse Embryonic Fibroblasts: A Correlate for Enhanced Photic Phase Shifts in Id2 Null Mice

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Id2 is a helix-loop-helix transcription factor that is expressed in a circadian manner (Duffield et al., 2002), and whilst involved in regulation of development, has not been studied extensively in the context of the adult animal. Our previous studies have: 1) revealed a conserved rhythm of Id2 expression across multiple tissues, including SCN, with a phase-locked relationship with canonical clock genes; 2) identified circadian locomotor activity phenotypes, including enhanced rate of reentrainment and larger phase shifts under parametric entrainment conditions, and disruption of activity rhythms in DD; and 3) demonstrated a potent inhibitory effect of ID proteins upon CLOCK:BMAL1 transactivation of period1 and AVP gene activity. We have begun to explore the molecular mechanism by which these large phase shifts and rapid reentrainment are achieved in the Id2 null mouse. Serum stimulation of mouse embryonic fibroblasts (MEFs) was used as a model system to mimic light stimulation of the SCN. Id2-/- and Id2+/+ MEFs were generated, and following serum treatment of serum-starved confluent cells, RNA harvested at 15-30 min intervals over 8 hr, and gene expression analyzed by real-time RT-PCR. Treatment resulted in acute induction of c-fos, period1 and period2 at expected peak levels at 30 min, 75 min and 120 min, respectively. Whilst no consistent difference was observed between Id2+/+ and Id2-/- cells in the peak levels or duration

of elevated expression of per2, in 3 out of 5 independent experiments c-fos induction was higher, and in all experiments per1 was induced to an average 2-fold higher peak level ($P < 0.01$). As period1 is a state-variable of the circadian pacemaker, it is possible that increased induction of PERIOD1 is responsible for the entrainment phenotype described in the intact animal. These results are consistent with our previous findings and suggest a role for ID2 in the circadian clockwork in limiting the magnitude of phase responses.

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Phase Response Curve for Ramelteon in C3H/HeN Mice

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Ramelteon, a specific MT1/MT2 melatonin receptor agonist, promotes sleep and is used in the treatment of insomnia in humans. Here we assessed the effect of ramelteon on the phase of the onset of circadian rhythm of activity. C3H/NeH mice free running in constant darkness were treated every two hours with vehicle or ramelteon (90 mg/mouse, sc). The phase response curve (PRC) for ramelteon shows maximal phase advances during the subjective day [CT8: 0.84±0.16h (n=8); CT10: 1.13±0.14h (n=13); CT12: 0.9±0.21 (n=10)] and phase delays during the subjective night and early subjective day [CT18: -0.27±0.12h (n=6) to CT2: -0.94±0.14h (n=10)]. The advance portion of the PRC for ramelteon is broader (CT8-CT12) than for melatonin, which causes advances at CT10 only (Benloucif S & Dubocovich ML, 1996). However, maximal phase advances and delays for both melatonin and ramelteon occur at similar circadian times (CT10 for advances, CT2 for delays). The melatonin receptor(s) type involved in ramelteon-mediating phase advances at CT10 was assessed using melatonin receptor knockout (KO) mice. In C3H/NeH wild type and MT2-KO mice, ramelteon treatment at CT 10 phase advanced the onset of locomotor activity rhythm by 1.04+0.19h (n=5) and 1.18+0.25 (n=8), respectively. However, ramelteon did not affect the phase for the onset of locomotor activity rhythm in the MT1-KO or MT1/MT2-KO mice. The results suggest that ramelteon phase advances the onset of circadian rhythm of locomotor activity at CT 10 by activation of the MT1 receptor. Supported by Takeda Investigator-Sponsored Research Grant 06-016R to MLD

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Period 1 and Period 2 in Melatonin-Mediated Phase Advances in the Rat SCN

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The master circadian pacemaker resides in the suprachiasmatic nucleus (SCN) of the hypothalamus. Cells of the SCN have an intracellular clock based upon oscillations in clock genes and their products that approximate one day. Additionally, the SCN generates a near 24-h pattern of spontaneous neural activity that may communicate timing between cells of the SCN as well as in targets of SCN projections. The timing of these neural and molecular oscillations can be modulated by effectors that relay external and organismic information about environmental state to the circadian clock. Among these is the pineal hormone melatonin, a stimulus that advances the peak of neural activity when applied to an SCN brain slice at two distinct times of day—dusk (CT10) and dawn (CT 23) (McArthur et al., Endocrinology 138(2), 1997). Here we investigate the hypothesis that transcriptional regulation of clock genes is necessary for melatonin-mediated phase resetting in the rodent SCN. Using quantitative RT-PCR, we found that melatonin treatment of the SCN brain slice at CT 10 caused a significant induction of Per1 and Per2

transcripts 120 minutes following treatment. To test the necessity of these transcriptional events for phase resetting by melatonin, we used antisense oligodeoxynucleotides (ODNs) targeted to Per1 and Per2. ODNs to each of these targets effectively inhibited the phase advance by melatonin of neuronal rhythms in the SCN compared to controls. This study supports a link between melatonin signal transduction and pathways requiring Per1 and Per2 transcription in resetting the SCN circadian clock.

P194

From Bottle to Brain: The Impact of Drinking Patterns and Ethanol Pharmacokinetics on Circadian Photic Phase-Resetting in the Chronically-Drinking Hamster

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Circadian timing disruptions are major health problems for alcoholics, yet ethanol's effects on the SCN are unknown. The timing of the SCN is regulated primarily by photic input, and ethanol attenuates photic phase-advances. Here, we compared 24h locomotor and drinking patterns with systemic and SCN ethanol pharmacokinetics in chronically-drinking hamsters under 14L:10D, and examined drinking effects on photic phase-advances. Experiment 1: Microdialysis analysis of ethanol in the SCN of animals drinking 10% ethanol for 2 weeks (n=4) was undertaken over 24h. Higher SCN ethanol levels occurred early in the dark phase with lower levels for the remainder of the 24h. Experiment 2: Dual microdialysis analyses of subcutaneous and SCN ethanol concentrations were undertaken over 24h with simultaneous measurements of circadian locomotor activity and drinking. Subcutaneous and SCN ethanol peak levels were temporally closely aligned, with drinking bouts occurring ~40 minutes before ethanol peaks. SCN ethanol clearance was more rapid than subcutaneous clearance. Experiment 3: Animals drinking 20% ethanol (drinkers) or tap water for 2 wks received a 30 min light pulse (25 or 270 lux) at ZT18.5, and were released into constant darkness to assess phase-shifting. For the 25 lux stimulus, drinkers (n=5) showed attenuated responses relative to controls (phase-advances=0.32+0.07h and 1.19+0.16h, respectively; p=0.001). For the 270 lux stimulus, there was no difference between drinkers (n=6) and controls (n=5; phase-advances=1.41+0.19h and 1.68+0.21h, respectively; p=0.4). These data underscore the importance of considering time-of-day fluctuations in drinking and light intensity when assessing ethanol effects on photic phase-resetting. NIH AA015948 RAP and JDG.

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Maternal Control of the Fetal and Neonatal Rat Suprachiasmatic Nucleus

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The molecular clockwork within the suprachiasmatic nucleus (SCN) develops gradually during ontogenesis. The authors' previous work showed that rhythms in clock gene expression in the rat SCN were not detectable at embryonic day 19 (E)19, started to form at E20 and developed further via increasing amplitude until postnatal day (P)10. The aim of the present work was to address a question of whether the immature fetal and neonatal molecular SCN clockwork is reset by maternal cues. Phase of the maternal SCN clock was shifted by a 6-h delay of the external light-dark (LD) cycle at different stages of the fetal SCN development and phase of daily rhythms in spontaneous c-fos, Avp, Per1 and Per2 expression within the newborn SCN was studied by in situ hybridization. Results showed that exposure of pregnant rats to a 6-h delay of LD cycle at E18, but not at E20, phase-delayed the rhythm in c-fos and Avp expression in the

SCN of newborn pups at P0-1. The phase shift of LD cycle at E20 did, however, induce a phase-delay of the rhythms in Per1 and Per2 expression at P3 and P6. The results demonstrate a significant phase-shift of the SCN clock at the fetal developmental stage when no or very faint molecular oscillations were detected. This finding suggests that the maternal cues may drive rather than entrain the immature fetal rat SCN clock. Supported by the Grant Nos. 309080503, AV0Z50110509, LC554 and by the 6th FP EUCLOCK No. 018741.

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Surprisingly Weak Effect of Sex as a Zeitgeber in Male Rats

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Rodents can anticipate a daily mealtime by entrainment of a circadian oscillator that is anatomically distinct from the master light-entrainable pacemaker located in the suprachiasmatic nucleus. The location of food-entrainable oscillators driving food-anticipatory activity (FAA) rhythms is unknown. Recently, efforts have been made to determine whether FAA is a response to a metabolic challenge resulting from restricted feeding and/or a response to a reward component of feeding (Mendoza et al, 2005; Waddington Lamont et al, 2007). The current study examined whether or not a non-ingestive, rewarding stimulus is capable of generating anticipatory activity. In the first experiment male Sprague Dawley rats with adlib food and wheel access were provided restricted access to hormone primed estrous females for 1-h/day in the middle of the light period (ZT6-7). Following ~2 weeks of scheduled daily mating, increased general locomotor activity and wheel running were observed beginning 2 h prior to mate access, suggesting that rats can anticipate sex. However, increased wheel running and feeding were observed in the home cage immediately following mating, raising the possibility that anticipatory activity was related to ingestive behavior or activity rather than sex. In a follow-up experiment, male rats were provided access to estrous females for 1-h/day, food access was limited to lights-off (ZT12-24), and running wheels were locked from ZT6-12. Anticipatory activity was observed in this condition, but only in a minority of rats, and only after 4 weeks or more of scheduled mating. These results confirm previous findings (Mistlberger, 1992) that feeding behavior must be controlled to evaluate the efficacy of other rewards as zeitgebers for circadian anticipatory rhythms. These results also establish that non-ingestive stimuli are capable of generating anticipatory behaviour. Whether sex anticipation might be stronger in operant measures of behavior or if mating were scheduled at night remains to be determined. Supported by NSERC, Canada & MSFHR.

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Differential Effects of Scheduled Exercise on Neuropeptide Deficient Mice

A. Power*, A.T.L. Hughes, S.M. Dupre, R.E. Samuels, A.S.I. Loudon, and H.D. Piggins, Faculty of Life Sciences, University of Manchester

Vasoactive intestinal polypeptide (VIP) and its receptor, VPAC2, are abundant within the SCN. Mice deficient in VIP (VIP-/-) or lacking this receptor (Vipr2-/-) exhibit impaired synchronisation to photic cues and altered behavioral rhythms with a continuum of behavioral phenotype from arrhythmicity to weak, short period rhythms. Synchronisation of biological rhythms to non-photic stimuli is untested in these animals. We assessed the effects of scheduled exercise on locomotor and drinking rhythms in wildtype (WT), Vipr2-/- and VIP-/- mice in constant darkness (DD). Mice were released into DD and after 14 days, access to a running wheel was restricted to 6h/day for 21 days. Following this scheduled exercise, the majority of Vipr2-/- mice expressed a lengthened, near 24h period and 70% of the previously arrhythmic animals developed robust ~24h behavioral rhythms. In contrast, the behavioral rhythms of VIP-/- mice deteriorated in DD and could not be restored by scheduled exercise. Consequently, all 6

VIP-/- animals became arrhythmic. Analysis of c-Fos expression, a marker of neuronal activity, suggests that SCN cellular rhythms are not restored in entrained Vipr2-/- animals, despite restoration of behavioral rhythmicity. These data imply that a compensatory pathway or extra-SCN pacemaker is responsible for non-photic entrainment in Vipr2-/- mice. Using in situ hybridisation analysis of mBmal1, mPer1, mAVP and mPK2, we are currently examining several Vipr2-/- brain structures to establish whether restored rhythmic behavior is associated with novel patterns of gene expression. Supported by BBSRC. Behavior, molecular, locomotor, entrainment, Vipr2-/-

P198

Addictive Drugs Entrain Circadian Episodes of Activity in Rats

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There is increasing evidence that the biological systems which mediate endogenous circadian rhythms are involved in drug addiction. A series of drug-screening studies has revealed that addictive drugs act as zeitgebers to entrain locomotor, water drinking, and feeding activity rhythms to a daily drug administration time. Drug-induced circadian activity entrainment is based on four behavioral effects: (1) pre-injection anticipatory activity must be evident 1-2 hours prior to the administration time in the absence of any predictive cues; (2) the drug must produce a post-injection elicited activity effect; (3) on days when the drug is withheld and the rats are left undisturbed, entrained activity bouts should persist around the administration time for multiple days; (4) when the drug is administered on an infradian schedule that is longer than the range of circadian entrainment, ensuing activity is apparent approximately 24 hours after administration, and no pre-injection activity is evident in the 1-2 hours immediately prior the injection time. Addictive drug-induced entrainment of circadian activity rhythms has implications both for neuroanatomical and treatment models of drug addiction.

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Entrainment of Circadian Rhythm by Ambient Temperature Cycle in Mice

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The vast majority of studies of environmental control of circadian period (entrainment) focuses on photic stimuli (light-dark cycles). Of the relatively few studies of nonphotic entrainment, a small minority has examined the effects of environmental cycles of ambient temperature on the period and phase of circadian rhythms. The present study investigated the effects of environmental cycles of ambient temperature on the period and phase of the locomotor activity rhythm of domestic mice (CD-1 strain) maintained in constant darkness. Cycles of ambient temperature were obtained by the use of a commercial programmable refrigerated incubator, and locomotor activity was monitored in running wheels. A cooling cycle with 8 hours of cold (12 ˚C) and 16 hours of thermal neutrality (24 ˚C) per day produced relative coordination but not clear entrainment. A warming cycle with 8 hours of warmth (32 ˚C) and 16 hours of thermal neutrality (24 ˚C) was more effective than the cooling cycle but produced clear entrainment in only half of the animals. Although wider temperature cycles might yield more robust effects, the ranges used (24 ˚C to 12 ˚C, and 24 ˚C to 32 ˚C) are typical of the daily variation of ambient temperature in many regions of the earth inhabited by mice and should have been effective if entrainment by ambient temperature cycles were a natural phenomenon. Thus, cycles of ambient temperature can entrain circadian rhythms in mice, but they are relatively weak synchronizing stimuli.

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Expectancy for Food or Expectancy for Chocolate: Exclusive and Shared Timing Mechanisms in the Brain

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Restricted feeding schedules (RFS) are a potent entraining signal to induce anticipatory activity and to impose daily oscillations of c-Fos and clock proteins in brain structures in phase with meal time. Daily access to a palatable treat (chocolate) also has shown to elicit anticipatory activity and c-Fos expression mainly in corticolimbic structures. In the present study the influence of daily access to chocolate was explored on the oscillatory patterns of PER1 in hypothalamic and corticolimbic structures. Wistar rats were exposed to RFS or to daily access to 5g of chocolate for 3 weeks. Persistence of the food entrained rhythms was determined 7 days after interruption of the feeding protocols. Brain samples were obtained every 6 hours to complete a 24 h cycle.

RFS and chocolate produced a phase shift in PER1 daily cycles in corticolimbic structures setting peak values at ZT12 with higher amplitude in the chocolate group. Both RFS and chocolate groups also produced upregulation of PER1 in the SCN. RFS exclusively entrained hypothalamic structures. After 7 days in persistence food and chocolate entrained rhythms persisted in behavioral expectation and in PER1 expression in the dorsomedial nucleus, accumbens, prefrontal cortex and central amygdala.

The present data demonstrate the existence of different oscillatory systems, activated by metabolic stimuli or by reward and suggest the participation of PER1 in both entraining pathways. Persistence and amplification of PER1 oscillations in structures associated with reward suggest this oscillatory process to form part of food addictive behavior. This study was supported by grants DGAPA PAPIIT: IN-203803 and 203907, CONACYT 43950-M

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DNA Damage Resets the Mammalian Circadian Clock in Mouse Embryonic Fibroblasts

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An evolutionarily conserved molecular feedback loop regulates the circadian clock in organisms ranging from fungi to mammals. Environmental cues influence the timing of this system through the modification of components of the core oscillator. These include the negative regulators FRQ in the fungus Neurospora and the PER and CRY proteins in mammals. We have previously shown that DNA damage can reset the clock in a time of day dependent manner. DNA insult with the radiomimetic drug MMS in Neurospora during various times of the day leads to phase advances in the clock that change in magnitude depending on the time of day the drug is given; interestingly, only phase advances are evident. Maximal advances occur when FRQ is nascent and minimal advances occur when FRQ is mature. This is mediated by phosphorylation of FRQ by the checkpoint kinase PRD-4 (mammalian Chk2) which shifts hypo-phosphorylated FRQ to hyper-phosphorylated FRQ. This change in phosphorylation leads to FRQ degradation and thus shifts the phase of the clock. Here we show that the mammalian clock responds to DNA damage similarly. MMS treatment of serum-shocked mouse embryonic fibroblasts isolated from PER2::LUC transgenic mice that encode for a PER2/LUCIFERASE protein fusion results in phase advances that change in magnitude depending on when the drug is given. Furthermore, only phase advances are observed; the largest advances occur when PER2 is nascent and minimal advances occur when PER2 is mature. Our data, as well as other recent findings, suggest that this property of the clock is evolutionarily conserved.

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Potential GABA Receptor Involvement in Ethanol Actions on In Vitro Suprachiasmatic Nucleus Circadian Rhythms

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Ethanol (EtOH) affects circadian rhythms at least in part through direct actions within the suprachiasmatic nucleus (SCN). Acute EtOH inhibits photic/glutamate-induced phase shifts in hamsters and mice, in vivo and in vitro, respectively. Conversely, EtOH enhances daytime serotonergic phase shifts in vitro, an effect mimicked by compounds that inhibit glutamatergic signaling in the SCN. These in vitro effects of EtOH occur at low concentrations (10-20 mM), which are similar to those at which EtOH stimulates GABAA receptors containing the subunit. Here we investigate the ability of RO15-4513, a selective antagonist for GABAA receptors, to block EtOH actions. Coronal brain slices from C57BL/J6 mice were maintained in vitro. For daytime experiments, EtOH, RO15-4513, and/or the serotonin agonist, DPAT were bath applied to slices for 10 min at ZT6. The following day singleunit recordings were made to determine time of peak neuronal activity. DPAT (10uM) alone induces 3h phase advances, while DPAT+EtOH (20mM) induce 4.5h phase advances. EtOH, RO15-4513 (100nM) and RO15-4523+EtOH had no effect, and RO15-4523 did not alter DPAT-induced phase advances. However, RO15-4513 eliminated the enhancement of DPAT resetting induced by EtOH. Our preliminary nighttime experiments suggest that RO15-4513 alone or combined with EtOH (10mM) at ZT16 induces 3h phase delays, while EtOH alone has no effect. Additional experiments are needed to confirm and expand on these results. These data suggest that EtOH may stimulate GABAA receptors in the SCN, which may contribute to EtOH enhancement of non-photic phase resetting seen in vitro. NIH AA015948 RAP and JDG

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Phase Resetting of the Mammalian Circadian Clock by DNA Damage

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The circadian system has been associated with control over DNA damage and cell cycle response pathways. Conversely, little is known about the impact of genotoxic stress on the circadian clock. By using Rat-1 fibroblasts expressing an mPer2 promoter-driven luciferase reporter, we show that ionizing radiation exclusively phase advances circadian rhythms in a dose- and time-dependent manner. Notably, this in vitro finding translates to the living animal, because ionizing radiation also phase advance behavioral rhythms in mice. The underlying mechanism involves ATM-mediated damage signaling as radiation induced phase shifting was suppressed in caffeine or Ku-55933 (a specific ATM inhibitor) treated Rat-1 cells, as well as in fibroblasts from patients with the ionizing radiation-sensitive, cancer predisposing disorders ataxia telangiectasia (AT) and Nijmegen breakage syndrome (NBS). Ionizing radiation-induced phase shifting neither depends on up/downregulation of Per1, Per2, Cry1, Cry2, Bmal1, and Clock gene expression nor on de novo protein synthesis, and as such differs mechanistically from dexamethasone and forskolin provoked clock resetting. Interestingly, other type of DNA damage, such as, ultraviolet light and tert-butyl hydroperoxide also elicited a phase-advancing effect. Taken together, our data provide evidence that the mammalian circadian clock, like that of the lower eukaryote Neurospora, responds to DNA damage and suggest that clock resetting is a universal property of DNA damage. This work was supported by grants from The Netherlands Organization of Scientific Research (ZonMW Vici 918.36.619) and the European Community (EU-FP6 Integrated Projects "EUCLOCK" and "DNA repair").

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Organotypic Cultures of Mouse Peripheral Tissues Display Strong Phase Resetting in Response to Temperature Pulses within the Physiological Core Body Temperature Range

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The core body temperature of non-hibernating, common lab mice exhibits daily fluctuations between about 36C and 38.5C. The lowest body temperatures occur during the inactive light phase of a light:dark cycle, and the peak temperatures occur during the active dark phase. These rhythms of body temperature have been implicated in the synchronization of the circadian gene expression of peripheral tissues. While in vivo experiments are important for the understanding of entrainment and resetting properties of rhythms at the organismal level, it is extremely difficult, if not impossible, to separate the effects of activity, sleep, feeding, metabolic state, blood pressure, red-ox state, temperature, etc. By culturing tissues ex vivo we can measure the rhythms of luminescence of the Per2Luciferase reporter while manipulating only the temperature. In the present study mouse tissues were maintained in organotypic culture and were temperature pulsed within the normal temperature range of mouse core body temperature. Here we show that peripheral tissues such as pituitary, olfactory bulb, lung, liver, and kidney showed strong phase resetting properties when the temperature was changed from 36C to 38.5C for as little as 1 hour. In addition, large inductions in the peak to trough amplitudes of luminescence rhythms were apparent immediately after a pulse. Surprisingly, the SCN showed little and unpredictable shifts in response to pulses as long as six hours, and displayed no amplitude changes. These results indicate that temperature changes in the physiological range are sufficient to alter the phase and amplitude of the peripheral oscillators of mice.

Feeding and Metabolism

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Food—SCN Interaction: Meal Time at Zt9 Produces a Phase Advance of Locomotion

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The suprachiasmatic nucleus (SCN) is entrained by the light/dark cycle (LD), while feeding schedules selectively entrain peripherals oscillators. Under special conditions restricted feeding schedules modify the phase in the SCN (Abe et al, 2006; Castillo et al, 2004;), which suggests that the SCN is informed about feeding events. The aim of this study was to explore the interaction of feeding and the SCN dependent activity. Adult rats were maintained in a light /dark cycle, 12:12 (ZT0:lights on). Rats were randomly assigned to one of three groups for 3 weeks. Control group with food ad libitum; restricted feeding with food access for 2 hours at ZT9 (RF9); and double feeding scheduled group with two feeding events each day, for 1 hour each at Z T3 and ZT9 (RF3-9). The last day of the feeding protocol, lights were switched off in order to eliminate the influence of light and rats were left ad libitum for 3 days. The phase of activity onset was compared. RF3-9 produced a phase advance of the nocturnal activity towards (ZT9) under the LD cycle, whereas RF9 produced a phase advance, when the L/D cycle influence was eliminated. To determine whether the neuronal activity in the SCN was entrained to this feeding schedule, after the third day DD rats were perfused at ZT3, ZT6, ZT9, ZT12 or ZT15, Per1 and c-Fos expression were determined. Per1 was expressed similar as the controls in contrast the c-Fos pattern was changed with decreased values during the time points of food expectation. This project is supported by CONACYT 43950-M and PAPPIT UNAM IN-203907.

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Food Entrainment Modifies in a Differential Manner Clock Genes of Hypothalamic Nuclei

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Restricted food access becomes a potent zeitgeber for brain and peripheral oscillators; it induces behavioral activation and metabolic adjustments for the scheduled meal time. In order to uncover the possible anatomical substrate regulating food entrained oscillations, the present study proposes to identify in the hypothalamus neuronal groups that exhibit food entrained patterns in clock gene expression. Therefore we used in situ hybridization to identify food entrained temporal patterns in mPer1 and mPer2. Adult Wistar rats were kept in a 12:12h (light/dark) cycle with lights on at 6:00am (ZT0). Rats were submitted for 3 weeks to a restricted schedule with food access for 2hr daily (ZT6-ZT8). Control rats were maintained ad libitum. Animals were decapitated every three hours to complete a 24h cycle (n = 4 per time point), brains were removed and frozen for in situ hybridization.

The meal produced a phase advance of mPer1 in the SCN and induced a daily rhythm in the arcuate (ARC) and dorsomedial (DMH) nuclei with and anticipatory increase at ZT6 (meal time). The mPer2 rhythm was not changed in the SCN or ARC while in the DMH we observed a decrease in the rhythm. No changes of mPer1 or mPer2 patterns were observed in the ventromedial nucleus. The PVN showed a peak 6h after meal time exclusively in mPer2. Food entrainment induces differential rhythmicity of Per1 and Per2 expression in hypothalamic nuclei, including the SCN indicating a relevant role of the ARC and DMH anticipating the daily meal. Supported by ECOS NORD México-Francia, CONACYT 43950-M

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The Suprachiasmatic Nucleus Modifies FAA, C-FOS and PER1 Expression in Rats under Food Entrainment

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Restricted food access (RFA), induces food anticipatory activity (FAA), cellular activation and PER1 rhythmicity in several brain areas. It is well known that RFA does not entrain the suprachiasmatic nucleus (SCN) and animals with bilateral lesions of the SCN still exhibit strong FAA. However under constant darkness (D-D) long intervals under RFA as well as a palatable diet in ad libitum conditions produce entrainment of the SCN. The present study was aimed to determine whether the SCN participates in food entrainment, by testing the effects of SCN lesion on the activity and rhythmicity of hypothalamic nuclei involved with food entrainment and limbic system involve with motivational state, c-Fos Immunoreactivity was used as a marker of functional activation and PER1 as marker of rhythmicity. Intact rats and rats bearing bilateral lesions of SCN were food entrained for 3 weeks and the temporal profile of c-Fos-IR and Per1 expression in the hypothalamus and corticolimbic structures were compared. Both groups exhibited food entrained cellular patterns, however in SCN lesioned animals duration and amplitude of FAA was increased, and neuronal activation was of higher amplitude compared with intact animals in hypothalamic nuclei and in limbic system the activation was low. The PER1 expression the amplitude was increased in limbic structures. In conclusion SCN has a strong influence on FAA and neuronal response, and may be part of the oscillatory drive for corticolimbic structures. Supported by CONACYT 43950-M and DGAPA PAPPIT IN203907

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Do Circadian Clocks in the PVN and Arc Regulate Feeding Behavior and Energy Balance?

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The suprachiasmatic nucleus (SCN) of the hypothalamus is known to control circadian rhythms of locomotor behavior. However, comparatively little is known about the control of circadian rhythms of feeding behavior. Other brain regions outside the SCN express robust circadian rhythms of clock gene expression. Of these, the paraventricular (PVN) and arcuate (Arc) nuclei are particularly interesting, given their well-established roles in the control of feeding behavior, energy balance, and endocrine function. Further, mice lacking circadian clock function in all tissues have metabolic defects and arrhythmic feeding behavior. It is unclear if these defects are due to the lack of circadian clock function in the SCN, or in another tissue and/or brain region. We will generate mice in which elimination or rescue of circadian clock function is restricted to either the PVN or the POMC and/or NPY cells of the Arc. This will allow us to test the necessity and sufficiency of circadian clocks in the PVN and Arc for the generation of circadian rhythms of feeding behavior. Additionally, we will test the role of circadian clocks in the PVN and Arc in the regulation of glucose homeostasis and energy balance. In the case of the PVN, we can test the necessity and sufficiency of circadian clocks in the PVN for the generation of circadian rhythms of hypothalamic-pituitary-adrenal axis function.

P209

Feeding Cues Regulate the Abundance and Distribution of Phospholipase C 4 in the Mouse Liver

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Peripheral organs possess circadian clock machinery that can oscillate independently from the SCN. Although typically peripheral tissues are synchronized by the SCN, peripheral clock cells in the liver can be independently reset by a food stimulus which can override the SCN signals. Recently, we have found that in mice, the intracellular signaling enzyme, phospholipase C 4 undergoes a circadian oscillation in abundance and cellular distribution in the liver. In the present experiment, C57B6\J mice were exposed to a food restriction paradigm in order to evaluate possible effect of a food stimulus on PLC4. Food access was restricted to 4 hours a day for 2-3 weeks during which running wheel activity was continuously monitored. After entrainment to the food stimulus, animals were sacrificed at time points around the clock (ZT4, 5, 11, 12, 17, 20, and 23). Using Western blotting and immunohistochemical techniques, PLC4 protein levels and cellular distribution in the livers of the food restricted mice were compared to the livers of a control group that received food and water ad libitum. We found that unlike the control group, PLC4 in the food restricted mice was found to be nuclear and abundant during the day and cytoplasmic during the night. The opposite was found in the control group. To date, our data suggest that PLC4 is regulated by food restriction in a similar manner to that of known clock proteins and provide insight into the mechanism by which PLC4 is regulated in the mouse liver.

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Importance of the Interaction between the Dorsomedial Hipothalamic Nucleus and the Suprachiasmatic Nucleus for the Expression of the Food Anticipatory Activity

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The suprachiasmatic nucleus (SCN) shows during the (subjective) light period an increment of neural activity in its ventral part, which diminishes during the dark period. During food entrainment it is observed that animals increment their activity two or three hours before food has arrived (food anticipatory activity (FAA). Neuronal activity of the SCN can be visualized as c-Fos immunoreactivity in SCN neurons. Recently we observed that FAA during the light period coincides with a reduction of c-Fos in the SCN. Following retrograde tracer injections in the ventral SCN we observed neurons labeled in the dorsomedial nucleus of the hypothalamus (DMH). These neurons projecting to the SCN were shown to demonstrate c-Fos during food anticipation indicating their activation during FAA. Lesioning this area of the DMH resulted in a pronounced decrease of FAA and a simultaneous increase in c-Fos activity in the SCN. This result indicates that c-Fos activity in the SCN is negatively associated with expression of FAA. This was demonstrated by subsequent lesioning of the SCN, animals that first lost their FAA after DMH lesion recovered their FAA following lesioning of the SCN. These results indicate that the DMH is not the food entrained oscillator but interacts with the SCN to allow FAA. The question remains which is the origin of the FAA signal since this anticipatory behavior is only enhanced after lesioning DMH and SCN. This study was supported by DGAPA proyecto nr IN215308

P211

Shift Work Simulation in Mice

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The daily organization of internal biological rhythms has evolved to adapt physiology to the night-day rhythms of the environment in many organisms including human. Modern society challenges this adaptation in many ways such as artificial light, long distance travelling. Shift work can be considered as the most profound perturbation to internal rhythmical synchronization leading to abnormal physiological responses and reduced cognitive function. The aim of our project is to develop a mouse model to describe desynchronizations caused by shift work. To simulate shift work we use two methods: limitation of water-availability and limitation of food-availability to certain restricted phases of the light dark cycle. The mice can be motivated to be active at specific times of the day simulating early, late and normal shift work. In the current project we force the mice (C57Bl6 and CBA) to be active by performing a task where the mice have to open a particular door during a certain time period in order to get access to drinking water or to run in running wheel in order to obtain a certain food amount. Furthermore, we investigate the effect of shift work on the daily melatonin profile in CBA mice using urine samples collected for 24 hours on a filter paper.

P212

Predictability of a Meal and the Time of Day the Meal Arrives Both Modulate Period2 Expression in the Limbic Forebrain

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In rats, the expression of the clock protein Period2 (PER2) exhibits a circadian oscillation in the suprachiasmatic nucleus (SCN), as well as several areas of the limbic forebrain. The oval nucleus of the bed nucleus of the stria terminalis (BNSTov), the central nucleus of the amygdala (CEA), the basolateral amygdala (BLA) and the dentate gyrus (DG) all exhibit robust oscillations in PER2 expression and are areas of the brain that are important in the control of diverse emotional, homeostatic and motivational processes, including feeding. However, the role of clock gene expression in the limbic forebrain remains largely unknown. The main objective of this work was to study how changes in behavioral and metabolic rhythms (triggered by manipulations of food availability), influence the expression patterns of the clock protein PER2 outside of the SCN. By providing a 2-hour meal each day for 10 days at variable and unpredictable mealtimes, reliable food 'anticipation' was impossible. The arrival time of the 2-hour meal was varied either within the light phase, within the dark phase or across the 24-hour cycle. It was observed that PER2 expression in the BNSTov and CEA was blunted in all variable feeding conditions. In contrast, PER2 expression in the BLA and DG was disrupted principally by daytime feeding. In conclusion, 'anticipation' of predictable mealtimes appears to be necessary for coherent shifts of PER2 expression in the BNSTov and CEA, while PER2 expression in the BLA and DG appears to be linked to the time of day meals are presented.

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In Addition to the Arcuate Nucleus VGF Is Also Upregulated in the SCN under Negative Metabolic Conditions

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Several anatomical and physiological connections between the SCN, and other hypothalamic nuclei involved in energy homeostasis exist. Conditions that affect circadian rhythmicity have many consequences on the metabolic profile, including increase in adiposity and loss of rhythm for some metabolic parameters. Moreover, high caloric diet and food restriction affect sleep duration. Within the hypothalamus, the ARC has a special role in catching metabolic information from the circulation and communicating that to other areas including the SCN. These second-order centers receive metabolic information by means of ARC neurotransmitters. VGF is highly expressed in the ARC, its deletion causes loss of body weight and white adipose tissue, overexpression of NPY and decrease of -MSH, a status typical for fasted animals. To investigate the role of VGF in metabolism, rats were fasted for 48 hours, or fasted and re-fed; the VGF staining intensity in each condition has been analyzed by immunohistochemistry. The results indicate a strong increase in VGF production both in ARC, and SCN, but also in the PVN, The PVN is a center of integration of different inputs, the origin of central autonomic output and is innervated by both ARC and SCN. VGF innervation arising from the ARC is analyzed by using confocal laser scanning microscopy analysis of co-localization of NPY with VGF or SH with VGF. In addition the anterograde tracer PHAL is injected in the ARC while its projections are analyzed for VGF. The results show that PVN is target of both SCN and ARC VGF terminations. This study was supported in part by: Programma Master and Back, Regione Sardegna, Italy, and by DGAPA proyecto nr IN215308

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Night-Time Restricted Feeding Prevents Internal Desinchronization in a Model of Nightwork in Rats

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Nocturnal work produces internal desynchronization (ID). We have developed a model of night work, by placing rats in slowly rotating drums and forcing them to be active during their resting phase (light phase) for 8 hours daily. In this model activity and feeding patterns shift towards "working" hours, metabolic rhythms are disturbed, but SCN rhythms remain in phase with the LD cycle. We have hypothesized that the combination of activity and feeding during the light phase may promote desynchrony. In consequence restricting food access to the night and not combined with forced work may prevent ID., Rats were forced to be active with the rotating drums during their rest period (in the light phase), rats were either allowed to feed ad libitum, to eat only during their working hours (FD) or in their home cage at night (FN). General activity, temperature and food intake were continuously recorded; during the last week blood glucose and triacylglycerol were determined every 3 h. The FD rats had a significant decrement of the nocturnal activity and showed arrhythmic activity during the weekends, the peak of temperature and metabolic rhythms were disturbed. In contrast, FN rats did not present alterations in activity and metabolic rhythms with regards to non-working rats. These results demonstrate that feeding during the nocturnal work can be the cause of ID and that regulating feeding schedules can be a good strategy to normalize the ID caused by nocturnal work. Supported by Conacyt M-43950, DGAPA IN-203907.

P215

Restricting Food Availability to the Daytime Shifts the Expression of the Circadian Transcriptome in Mouse Liver

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Circadian clocks orchestrate behavior and metabolism in mammals mainly through changes in gene expression. It is known that 10% of the liver transcriptome in mice is rhythmically expressed. Using Affymetrix MOE 430_2 high density arrays in a high temporal resolution over 24 hours we show that restricting food availability to subjective daytime (CT1-CT9) shifts the peak expression of the majority of rhythmically expressed genes in mouse liver by more than 8 hours. We also show that target genes of transcriptionfactors linked to metabolism show a similar expression pattern among each other, thereby suggesting a rhythmic activity of their effectors. The peak expression of these targets is shifted by the imposed restricted feeding schedule to maintain the same temporal distance to the onset of feeding. Further pathway analysis revealed that rhythmic transcriptional coregulation of metabolic pathways is responsive to the time of foodintake.

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Feeding Synchronizes Nocturnin Expression in the Liver

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Nocturnin is a rhythmically expressed deadenylase that peaks in many tissues during the early night. Although mice lacking Nocturnin have normal circadian functions, they are resistant to diet-induced

obesity, indicating that Nocturnin plays a role in regulating the output of the clock. In addition to a rhythmic expression pattern, Nocturnin is also acutely induced by various stimuli in fibroblasts, suggesting that Nocturnin could also play a role in cellular responses to environmental signals. Kornmann et. al. (2007) showed that when the clock is specifically knocked down in the liver, Nocturnin and mPer2 are among a small group of genes that retain rhythmicity suggesting that in vivo, the oscillations of mPer2 and Nocturnin can be driven by a systemic cue. Peripheral oscillators are sensitive to feeding, and we examined whether feeding effects Nocturnin's expression in the liver. To investigate this, male mice were placed on a restricted feeding schedule from ZT3 to ZT9. After food entrainment, peripheral tissues were collected every three hours for a 24 hour period. Gene expression profiles for Nocturnin and mPer2 were measured using real-time PCR. The peak expression of Nocturnin and mPer2 shifted from early night to midday. Further studies showed that these expression profiles were not an acute response to food, but were rather driven by a food-entrained circadian oscillator. Presumably, the timely shift of Nocturnin expression to the start of a feeding cycle allows the deadenylase to regulate transcripts associated with nutrient uptake and utilization.

P217

Daily Rhythms of Food Anticipatory Activity (FAA) Do Not Require a Functional Circadian Clock

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When daily food access is restricted to a time window where rodents normally don't feed, they exhibit increased locomotor activity prior to food presentation. It was suggested that this behavior is driven by a 24-hour pacemaker, largely based on the fact that FAA onset timing shows little interday variation and that FAA is not triggered by exogenous cues. Based on SCN lesion studies it was further proposed that FAA is driven by a circadian oscillator residing outside the SCN proper. We tested this hypothesis by subjecting Bmal1-/- mice that lack functional circadian oscillators throughout the body to temporally restricted feeding. Under a regular light:dark cycle with daily food access restricted to a 3-hr window during the light phase, Bmal1-/- mice exhibited robust FAA paralleling wild-type behavior. FAA invariant from wild-type littermates was also observed in Per1-/-, Per2-/- double mutants that similarly to Bmal1-/- lack a functional circadian pacemaker. Furthermore, we did not detect compromised FAA in Per2-/- mice. We conclude that mammalian FAA does not rely on the established circadian timing system.

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Role of Incretins in Circadian Clock Mutant Mice

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Molecular clocks genes have recently been implicated in the coordinate regulation of behavior, energy balance, and metabolism. Mice harboring a mutation in the core circadian clock gene Clock develop not only dysregulation of sleep and locomotor activity rhythms, but also symptoms of the metabolic syndrome, including obesity, hyperphagia, hyperleptinemia, hypoinsulinemic hyperglycemia, and altered expression of hypothalamic genes that regulate appetite. Interestingly, Clock mutant animals consume increased number of meals during their normal rest period, suggesting a defect in satiety that compounds their dysregulation of long-term energy homeostasis. Additionally, Clock mutant mice have defects in beta cell function that give rise to post-prandial hyperglycemia. Together, these results suggest that altered gut-brain satiety and insulinotropic signaling may contribute to the metabolic defects in Clock mutant mice.

To test the hypothesis that the production and response to potent gastrointestinal signaling molecules involved in the "incretin" response might be disrupted in these circadian mutant animals, we have examined the effects of the Glp-1 incretin mimetic exenatide on circadian behavior and energy balance in Clock mutant mice. Here, we report that Clock mutant mice displayed exaggerated weight loss compared to controls in response to exogenous exenatide, as well as a greater rebound weight gain following cessation of exenatide delivery. Furthermore, exenatide induced phase-shifts in the circadian rhythm of locomotor activity in wild-type animals maintained in constant darkness. Together, our data suggests that loss of signaling within the gut-CNS axis may represent an important cause of the weight-gain and behavioral dysregulation in the Clock mutant animals.

P219

Daily Changes in the Sensitivity of the Arcuate Nucleus to Hypoglycemia

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Glucose, the most important fuel for the brain, shows in the blood a circadian rhythm which is controlled by the suprachiasmatic nucleus (SCN). The arcuate nucleus (ARC) is considered as an area of the brain that senses the concentration of metabolites and hormones in the general circulation. The ARC has reciprocal connections with the SCN which play a role in transmitting information about the concentration of hormones such as ghrelin to the SCN (Yi et al 2006). The reciprocity of the connections indicates that also the SCN may play a role in changing the sensitivity of the ARC to circulating hormones and metabolites. We propose that the SCN may influence the sensitivity of the ARC for circulating glucose and investigated the response of the ARC to hypoglycemia. In order to address this objective, Wistar rats were canulated in the jugular vein and intracerebroventricularly and received 2-deoxyglucose at two different time points, during the day. Bloodsamples were analyzed for plasma glucose and corticosterone. While the brains of animals perfused with fixative two hours after the 2-deoxyglucose challenge were analyzed for c-Fos. Our results indicate that the ARC responds differently to hypoglycemia at the two different time points, with a major expression of c-fos during the beginning of the night. This activation is exclusive to the ventromedial area of the ARC where mainly NPY containing neurons are activated by the hypoglycemic challenge. The mechanisms by which the SCN is controlling the sensitivity of the ARC are investigated. Financed by DGAPA project IN215308

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Loss of Nocturnin, a Circadian Deadenylase, Causes Altered Absorption of Dietary Lipid

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Nocturnin is a deadenylase that controls mRNA expression in a circadian manner by degrading the poly-A tail of target RNAs, leading to mRNA turnover or translational silencing. Previously we reported that a mouse lacking Nocturnin was resistant to diet-induced obesity and heptatic steatosis. The lean phenotype was not due to increased activity, decreased food intake or a higher metabolic rate. Transcript analysis in liver showed alterations in genes associated with lipid uptake and utilization. In order to investigate the mechanism behind this phenotype, we exposed WT and KO mice to various dietary challenges and also examined aspects of digestive tract function.

When subjected to dietary challenges including either a ketogenic diet, or food restriction, our KO mice lost more weight than their WT counterparts. When on a standard mouse chow, the KO mouse exhibited lower circulating beta-hydroxybutyrate—a finding consistent with altered lipid availability in the KO. Moreover, this latter discrepancy in the KO is not due to hepatocyte malfunction as hepatocyte analysis showed normal rates of both lipid uptake and beta-oxidation.

Research into digestive tract function showed a faster transit time for lipid, but not water in the KO in a gut motility assay. We propose that Nocturnin has a role in the absorption of dietary lipid in bowel, presumably by altering genes necessary for metabolism or digestion through circadian post-transcriptional modifications of targeted transcripts.

P221

Impact of Circadian Gene Transcription Factors in Pancreatic Beta Cell Function and Diabetes

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Circadian clock genes control 24-hour cycles of behavioral and physiological processes and impact metabolic systems that regulate energy utilization, feeding, carbohydrate turnover, and lipid metabolism. Our previous studies using Clock mutant animals revealed that mutation of the core Clock gene in mammals leads to obesity and signs of the metabolic syndrome, including hyperlipidemia, hyperleptinemia, and age-dependent hyperglycemia. We further found that 24-hour levels of glucose were increased in Clock mutant animals with hypoinsulinemia that was pronounced during the dark period. In wild-type mice we observed robust cell autonomous circadian gene transcription and protein oscillation in living isolated pancreatic islets of Langerhans. In comparison to islets of wild-type mice, islets isolated from Clock mutant animals were smaller in size, exhibited decreased proliferation, and had impaired glucose-responsive insulin secretion. Microarray analysis revealed that at basal glucose levels Clock mutant islets showed downregulation in expression of transcripts involved in proliferation, cell cycle, molecular transport, and post-translational protein modification in comparison to islets from wild type animals. 24-hour transcriptional analyses indicated a phase shift in the expression patterns of broad classes of RNAs involved in glucose sensing and insulin secretion in Clock islets compared to wild type. Remarkably, islets isolated from the Bmal1 knockout animals similarly displayed reduced islet size and glucose-stimulated insulin secretion. Collectively, our studies demonstrate a cell-autonomous role of CLOCK and BMAL1 in beta-cell proliferation and glucose-stimulated insulin secretion, and uncover an unforeseen role for the circadian transcription network in the onset and progression of type 2 diabetes mellitus.

P222

Inhibitor of DNA Binding 2 (Id2) is a Circadian Rhythm Expressed Gene Required for Circadian Clock Output in the Mouse Liver

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Id2 is a helix-loop-helix transcription factor expressed in a circadian manner (Duffield et al., 2002, Current Biology 12: 551-557), and whilst involved in regulation of development, has not been studied extensively in the context of the adult animal. Our previous studies have: 1) revealed a conserved rhythm of Id2 expression across multiple tissues in a phase-locked relationship with canonical clock genes; 2) identified circadian phenotypes, including larger phase shifts under parametric entrainment conditions, and disruption of activity rhythms in DD; and 3) demonstrated a potent inhibitory effect of ID proteins upon CLOCK:BMAL1 transactivation of period1 and AVP gene activity. We have now begun to explore the potential role that ID2 may play in regulating clock output. Time-of-day specific liver gene expression in Id2+/+ and Id2-/- mice under circadian conditions was studied using DNA microarray analysis and real-time RT-PCR. Results of these analyses revealed that loss of ID2 does not disrupt normal rhythmic clock gene expression (period1, period 2, bmal1). Whilst pacemaker function appears intact, various genes normally under clock control are dysregulated in a time-specific manner: A cohort of different functional groups were identified, including genes associated with glucose and lipid metabolism. We have begun to investigate the possible physiological repercussions of disrupting ID2 on clock control of metabolism. We have observed both a reduction of gonadal fat pad in 75% of Id2-/- mice, and 38% reduction in quantity of lipid deposition in the liver ($P < 0.05$), as compared to Id2+/+ mice. These studies highlight lipid metabolism as one physiological clock output that may be disturbed in a time-specific manner in the absence of ID2. In conclusion, a major role for ID2 in the circadian clockwork appears to be to regulate a subset of physiological clock outputs.

P223

Functional Activity of the Liver Circadian Clock Revealed by Assessing Pentobarbital Sleep Time

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Drugs ranging from chemotherapeutics to anesthetics and analgesics have different efficacy and toxicity at different times of day. Pentobarbital (PB) has a time-of-day dependent hypnotic effect ("sleep time") when mice are housed in a light-dark cycle. PB is metabolized exclusively in the liver by members of the cytochrome (CYP) P450 gene family. Recently, DBP, HLF, and TEF were shown to modulate hepatic CYP activity, indicating these clock-controlled genes are important regulators of xenobiotic metabolism (Gachon et al., 2006). We report here that circadian control of PB sleep time (PBST) is dependent on a functional liver clock. There is a 2-fold PBST rhythm in wild-type mice, with peak and trough sleep times at circadian time (CT) 02 and CT14, respectively. Disruption of core clock genes disrupted the rhythm in PBST. Specifically, PBST did not differ between CT 02 and CT14 in either Per1/Per2/Per3 triple-deficient or in Clock-/- mice. The overall response of these mutant lines differed, however: PBST was short in Per1/Per2/Per3 triple-deficient mice, and long in Clock-/- mice at both injection times. Furthermore,

liver-specific CLOCK-deficient mice (Clockflox/flox;Albumin-Cre+/-) were similar to Clock-/- mice in having long PBST at both injection times. Since Clock-/- livers do not show organ-autonomous rhythms in vitro (DeBruyne et al., 2007), these results indicate that the PBST rhythm is dependent on a liver-autonomous clock.

P224

Cholesterogenic Output Genes: Regulation by Lipids, cAMP and the Circadian Rhythm

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Lipid homeostasis is maintained by different signaling pathways, such as the lipid (cholesterol and fatty acids) feedback loops, cAMP-signaling and the circadian rhythm. These pathways require a high level co-ordination at every physiological condition. To understand molecular mechanisms underlying cholesterol homeostasis we investigate the influence of the diet, circadian transcription factors and the cAMP signaling on the two model output genes from cholesterol synthesis, Hmgcr and Cyp51. LIVER: in mouse hepatoma cells cholesterol (SREBP-2 pathway) represses Hmgcr and Cyp51 but linoloeic acid (SREBP-1 pathway) represses only Hmgcr. The circadian expression of Cyp51 in the liver shows a maximum at CT 20-24, minimum at CT 12-16, while deletion of Crem erases the circadian expression. Hepatic Hmgcr peaks at CT20, deletion of Crem causes an 8h phase advance that is accompanied with a 4h phase advance of the liver cholesterol content. EMBRYONIC FIBROBLASTS (MEF): linoleic acid represses Cyp51 and Hmgcr in wild type MEFs but not in Clock mutant or Cry1, 2 defficient cells. Surprisingly, cholesterol has no effect on Cyp51 and Hmgcr in wild type MEFs. Mutation of Clock restores the cholesterol feedback while deletion of Cry1, 2 has no effect. CONCLUSIONS / HYPOTHESES: in the liver where cholesterol mediates the feedback, Crem isoforms are essential for the circadian expression of Cyp51 and Hmgcr by binding to proximal promoter CRE elements. In MEFs, Clock prevents the cholesterol feedback regulation of Cyp51 and Hmgcr, while it seems to be required for a functional linoleic acid feedback regulation, together with Cry1 and Cry2.

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Rhythmic Expression of a Subset of Genes in the Murine Colon Suggests a Role for Clock Genes in the Regulation of Biological Rhythms in the Gastrointestinal Tract

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Background. Disruption of biological rhythms such as time zone traveling has been associated with gastrointestinal symptoms. These observations suggest a functional correlation between biological rhythms and gastrointestinal physiology. The aim of this study was to determine (1) whether clock gene expression occurs in the gastrointestinal tract (2) whether clock genes can shift their expression patterns in response to restricted daytime feeding and if so, whether this is dependent- or independent on the central clock and to (3) identify a cohort of rhythmically expressed genes.

Methods. Using qPCR, Western, immunohistochemistry and microarrays, analyses were performed on colonic RNA and protein isolated from C57BL/6J mice q4 hours for 24 hours on a regular light/dark cycle. Results. Clock immunoreactivity was observed in the myenteric plexus and epithelial cells. Clock genes were expressed rhythmically throughout the gastrointestinal tract. Timed feeding shifted clock gene expression at RNA and protein level but did not shift clock gene expression in the central clock.

Significant differential gene expression profiles were detected in approximately 3.7% of colonic genes. Most rhythmically expressed genes were involved in cell signaling, differentiation and proliferation. 7% of genes (62/906) have been associated with colorectal cancer formation, 1.3% with inflammatory bowel disease (12/906) and 0.5% (4/906) with gastrointestinal motility. Conclusion. The presence of clock genes and the rhythmic expression of a subset of genes in the colon have potential significant physiological implications with respect to the pathogenesis of colorectal cancer formation (ie shiftworkers: Nurses Health Study), inflammatory bowel disease and gastrointestinal dysmotility.

Physiology

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"It's Just a Phase": Delays in Circadian Phase during Puberty in the Nocturnal Rat and the Diurnal Octodon degus

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Cross-culturally, adolescents are driven to go to sleep later than children or adults. This developmental change in the timing of sleep is likely to represent an overall delay in the phase of their circadian clock because the timing of other physiological rhythms is similarly delayed. We hypothesize that delayed circadian phase during adolescence is caused by gonadal hormone effects on the circadian system because it correlates with secondary sex development. To test this hypothesis, we developed two animal models for pubertal changes in circadian phase: the slow-developing, diurnal rodent, the Octodon degus (degu) and the fast-developing, nocturnal Rattus norvegicus (rat). Our data demonstrate that males and females of both species show delayed activity rhythms during puberty, as indicated by the timing of activity onset or peak activity. In the rat, these rhythms are most delayed in males during the 10 days prior to first preputial opening, and in females during the week prior to first vaginal opening. In the degu, rhythms are most delayed in males during the month encompassing penis spike completion and in females during the month surrounding the development of first vaginal opening. To examine the gonadal dependence of these circadian changes, we gonadectomized males and females and compared their within-subjects change in circadian phase to intact siblings. Preliminary data indicate that developmental changes in circadian phase are eliminated by pre-pubertal gonadectomy in degus. Rat data regarding gonadal dependence is pending.

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Circadian Rhythms in C. elegans: Locomotor Activity, Stress Tolerance and Metabolism

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C. elegans is a model organism not well characterized in chronobiological studies. We have recently designed an automated system to track individual nematodes and demonstrated circadian activity rhythms in both LD (light : dark, 12 h : 12 h) and DD (constant darkness) conditions (DD period 24,2 ± 1,6 h, n=80, p<0,05). Phototaxis data which showed a phototaxis index of 0,698 ± 0,096 towards the green wavelength of light. In addition, circadian rhythms were temperature compensated, with a Q10 of 1.09. Another approach to uncover rhythmic outputs was the study of stress tolerance behaviors. We found that *C. elegans* showed rhythmic stress tolerance patterns for oxidative and osmotic stress with peaks at ZT 12

(lights on) and ZT 0 (lights off), respectively. Stress-related gene expresssion was determined by sqRT-PCR: gpdh-1 and gpx showed a significant diurnal variation.

We have also studied circadian rhythms in metabolic variables, such as food consumption, whose rate (determined by decreasing OD600 of OP50 E. coli) was rhythmic and peaked at ZT 21 (ANOVA, p<0,05). Finally, we have measured aaNAT activity and melatonin levels, also as a possible circadian output signal in *C. elegans*. Our results show that aaNAT activity peaks at ZT 0. In summary, our results show that several different circadian outputs can be recorded in *C. elegans*, and the circadian rhythm of locomotor activity cn be entrained to environmental signals. These data might be a basis for the screening of putative circadian mutants in this species.

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Altered Locomotor Activity Rhythm in the Mouse Model of Mucopolysaccharidosis IIIB

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Mucopolysaccharidosis (MPS) IIIB or Sanfilippo Syndrome type B is a disease that results from a deficiency of -N-acetylglucosaminidase, a lysosomal enzyme required for the complete degradation of proteoglycans. Children affected with MPS IIIB appear normal in the first few years of life, but become hyperactive, develop sleep disturbances and have severe developmental delays as they age, and finally die. Similarly to the human disease, alterations at the balance, hearing and vision levels have been observed in the MPS IIIB mouse model. However, the effects on the rest-activity rhythm and on overall activity levels are still unclear. Our aim was to characterise the circadian rhythm of locomotor activity under different light conditions. The spontaneous rhythm of locomotor activity of young (2-4 months old) and old (7-9 months old) MPS IIIB mice kept under light-dark cycles (LD) was compared to that of wild-type mice. The free-running rhythm under constant darkness (DD) and the response to a light pulse were also examined, to further test circadian clock function. Differences in the circadian rhythm of locomotor activity between MPS IIIB and wild-type mice were observed both in LD and in DD conditions. Interestingly, in LD MPS IIIB mice showed a lengthening of the activity phase, which caused an increase in the amount of activity during the light phase. This finding is consistent with the increased night phase activity showed by human patients. In conclusion, MPS IIIB mice may prove a good model of mucopolysaccaridosis IIIB in humans, both at the histological and behavioural level.

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Disruption of Circadian Rhythms on the Days Following General (Propofol) Anesthesia in Both Rats and Human Patients

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It has been shown that general propofol anesthesia can reset the circadian rest-activity rhythm in rats free-running in constant darkness. Firstly, we examined propofol anesthesia effects on rest-activity and body temperature rhythms in rats exposed to normal light-dark conditions. On the day following anesthesia, propofol (120 mg/kg i.p) induced a phase-advance shift of acrophase of both rhythms studied (60-80 min). Phase-shift of body temperature acrophase (45-60 min) was still observed on the second day post-anesthesia whereas phase-shift of rest-activity acrophase was minor. Amplitude of both rhythms studied was decreased on the same post-anesthesia days. Mesor of rest-activity was decreased on the day following anesthesia whereas mesor of body temperature was not modified.

Secondly, we examined general anesthesia effects without surgical aggression on rest-activity rhythm in humans in real life conditions. 28 healthy patients scheduled for ambulatory colonoscopy (propofol 1.7-2.0 mg/kg i.v) were included. We investigated rest-activity rhythm using wrist actigraphy. On the days following anesthesia, diurnal rest was increased (40 min to 2 h; depending on the day considered) whereas nocturnal sleep was unchanged on the days following anesthesia. Parametric and non-parametric analyses showed a decrease of the strength of coupling rhythm to stable environmental Zeitgebers, and phase-shifts of rest-activity acrophase after anesthesia. Our results extend and emphasize the resetting effect of propofol anesthesia on the main circadian clock in rats, this effect persisting under naturalistic light conditions. General anesthesia also reset the rest-activity rhythm in patients; such an effect could underlie in part the post-operative sleep disorders.

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Circadian Regulation of Odor Discrimination

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Multiple mammalian cell types can express circadian rhythms in gene expression. How these molecular oscillations affect physiology has been addressed in the suprachiasmatic nucleus (SCN), but if or how extra-SCN circadian oscillators regulate physiology or behavior is unknown. We have reported that the main olfactory bulb (OB) contains a circadian clock which modulates neuronal firing and Period1 gene expression in vitro and responsivity to odors in vivo. To understand better the mechanisms by which this extra-SCN circadian pacemaker controls olfactory behavior, we have developed an assay to measure olfactory sensitivity and discriminability across circadian time. We trained male C57Bl/6 mice to discriminate between air and odor (air+vanilla). Mice performed for a water reward and discriminated vanilla dilutions from 1:10 to 1:1000,000000. Under constant darkness conditions, we tested mice at six circadian times (CT 4, 8, 12, 16, 20 and 24) for olfactory discrimination. From the percent correct (correctly noted air or odor) and percent false alarms (incorrectly responded odor present to air trials) we calculated d' (an index of olfactory discrimination). We also measured the time that animals spent sniffing the stimuli (sniff time) and the reaction time required to get a water reward (latency of response). We found a circadian rhythm in d' with higher values during the active phase (peaking around CT16-20 and lower values around CT8) in all mice (n= 3). Sniff time, but not latency of response, also showed a circadian rhythm with peak times that varied between mice. These results suggest that the OB clock increases olfactory discrimination during the early night. Supported by NIMH grant 63104.

P231

Asymmetry of Clock Gene Expression in Split Hamsters: A Tool to Assess Neural Control of the Phase of Peripheral Oscillators

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Lateralization of efferent SCN output in Syrian hamsters that split their circadian rhythms in LL may be used to study pathways by which the pacemaker entrains peripheral oscillators. In order to test the role of neuronal signals, we examined Per1 and Bmal1 expression in paired organs of animals that were transferred to LL. Locomotor rhythms split after ~7wks, and hamsters were killed 9wks later at projected activity onset (pAO), or at 3, 6 or 9hrs prior to pAO (n=5/group). Controls displaying a single circadian activity bout were killed 3hrs prior to pAO. In these unsplit animals, clock gene expression was bilaterally symmetrical in SCN, adrenal medulla and cortex, lung and kidney. In contrast, asymmetrical Per1 expression in rostral

and middle-SCN of split animals occurred at all phases and peaked at 6hrs before pAO. In every split animal (5/5) killed at this phase, Per1 expression in the adrenal medulla and cortex was higher on the side contralateral to the SCN side with higher Per1 levels. The incidence of asymmetry of Per1 mRNA was also greatest (3/5 animals) in lung and kidney of split animals at 6hrs before pAO. Nevertheless, mean asymmetry of clock gene expression in adrenal cortex, medulla, kidney and lung of split hamsters was generally similar to that in controls. Non-lateralized SCN dependent neural signals and/or humoral input are likely to contribute to the phase of peripheral oscillators, since none of the peripheral organs exhibit the dramatic asymmetry observed in the SCN. Supported by NIH RO1MH070019.

P232

Withdrawal from Chronic Ethanol Disrupts Circadian Activity Rhythms in the Mouse

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Withdrawal from chronic ethanol results in severe circadian disturbances, including disruption of the sleep-wake cycle. To study this, we assessed circadian activity in C57BL/6J mice undergoing withdrawal following chronic, long-term ethanol administration. Male mice that received 10% ethanol in their drinking water for 2 months (n=3) were introduced to water while controls (n=3) were maintained on water. During ethanol withdrawal, the mice were maintained under constant darkness and entrained to a 1 min light pulse (25 lux) administered at their initial ZT 6. General circadian locomotor activity was measured using infrared motion detectors interfaced with a computerized data acquisition system. Activity measurements were averaged over a 10 day period beginning immediately after ethanol withdrawal. Bouts of activity were defined as periods of activity separated by at least 10 minutes of non-activity. Total bouts averaged over the 24 h circadian day were significantly different between the ethanol-withdrawn group vs. water controls (15.43 +/-0.35 and 16.67 +/-0.47, respectively; $p<0.05$). Activity bouts for the ethanol-withdrawn group vs. water controls during the subjective day averaged 9.77+/-0.57 vs. 9.17+/-0.55, respectively ($p<0.4$), and activity bouts during the subjective night averaged 5.67+/-0.57 vs. 7.5+/-.055, respectively ($p<0.03$). A fragmented sleep/wake cycle in humans is problematic with withdrawal from alcohol. Our analysis revealed that decreased sporadic activity during the subjective night and throughout the 24 h circadian day is associated with ethanol withdrawal. Therefore, this animal model is useful for studying the effects of ethanol withdrawal on the circadian sleep-wake cycle. NIH grant AA015948 RAP and JDG.

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Changes in Circadian Rhythms across Reproductive State in Female Grass Rats (Arvicanthis niloticus)

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Circadian rhythms are typically examined in constant conditions, but in a variety of real world circumstances adaptive plasticity is evident. In this study, we characterized changes in the circadian rhythms of activity and core body temperature (Tb) in female diurnal Nile grass rats (Arvicanthis niloticus) as they went through a series of reproductive states: virgin non-pregnant, pregnant (P), pregnant and lactating (PL), lactating only (L), and post-weaning. There were no changes in the phases of either of

the two rhythms as a function of reproductive state. However, effects of reproductive state on amplitude were evident in both rhythms. The amplitude of the activity rhythm increased from early to late P and PL, and females were more nocturnal during early than late PL and L. The amplitude of the Tb rhythm significantly dropped by mid-P and remained low all the way into mid-L due to rises in Tb during the troughs seen in the mid-light and mid-dark phases of the LD cycle. Effects of pregnancy and lactation on the rhythms studied were not additive. The changes we observed could be due to masking effects emerging from processes associated with the demands of pregnancy and lactation or to a re-organization of the circadian mechanisms regulating these rhythms as females transition from one reproductive state to another. This research was supported by NSF grant IOS-0130977

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Gonadotropin-Releasing Hormone Neurons in the Female Hamster Brain Express Period 1 Protein in a Time of Day Dependent Manner

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The pre-ovulatory lutenizing hormone (LH) surge is under circadian control and is mediated by gonadotropin-releasing hormone (GnRH) neurons located in the medial septum-diagonal band-rostral preoptic area of the brain. Serum-stimulated murine GnRH GT1-7 cells exhibit circadian rhythms of expression of many clock genes in vitro. Furthermore, GnRH neurons in vivo have been shown to receive direct or proximal connection from the master circadian clock, the suprachiasmatic nucleus (SCN), as well as from the anteroventral periventricular nucleus, which is innervated by the SCN. Thus, GnRH neurons present themselves as candidate oscillators to mediate circadian timing of the LH surge. The purpose of this study was to determine if a clock gene, Period 1, is expressed by GnRH neurons in vivo, and, if so, whether this expression varies throughout the day. Female hamsters exposed to an alternating light (L):dark (D) cycle (14L:10D) were anesthetized and transcardially perfused with 4% PFA at zeitgeber times ZT0, ZT5, ZT9, and ZT12 (ZT12 corresponding to the time of lights off). Coronal sections through the hypothalamus were subjected to double label immunocytochemistry using antibodies to the clock protein PERIOD 1 (PER1) and to GnRH. The results showed that approximately 20-45% of GnRH neurons express nuclear PER1. Furthermore, the percentage of GnRH neurons containing PER1 was significantly higher at ZT12 than at the other times studied, indicating a daily rhythm of expression. These data suggest that a population of GnRH neurons may be circadian oscillators that participate in the temporal regulation of the LH surge. Support: AG013418 (MJD).

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Dualcolor Luciferase System Revealed That Diffusible Signals from the Adrenal Gland Regulate Circadian Rhythmicity in Cultured Fibroblasts

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Dualcolor luciferase systems enable long-term and non-invading monitoring of gene expressions in two components simultaneously. Although the North Americcan firefly (P.hotinus pyralis) luciferase is the most frequently used reporter gene, this luciferase is not suitable to dualcolor luciferase system because of the spectral shift by pH conditions. To apply the dualcolor luciferase assay in living organism, we constructed transgenic mice which express green light-emitting luciferases (max = 540 nm) from Pyrearinus termitilluminans (ELuc) under the control of mBmal1 promoter (Bmal1-ELuc mice). Robust circadian rhythms of bioluminescence from cultured suprachiasmatic nucleus slices and peripheral tissue,

such as lung, liver, adrenal gland, were detected. To study the circadian synchronizer around the body, we cocultured some tissues of this mouse and Rat-1 cells which stably express red light-emitting luciferase (max=630 nm) from Phrixothrix hirtus (SLR) under the control of mBmal1 promoter (Bmal1-SLR cells). The green and red light emission from Bmal1-ELuc mice and Bmal1-SLR cells were successfully separated by an optical filter. The amplitudes and phases of Bmal1-SLR cells were affected by the coculture with adrenal glands, but were not altered by lungs. Together with the former findings that glucocorticoid reset the circadian rhythms of the Rat-1 cells, these results suggest that the adrenal gland reset the other peripheral cells by diffusible factor, such as glucocorticoid.

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Circadian Rhythms of Clock Genes and NFAT in Mouse Skeletal Muscle

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The synchronous expression of clock genes among cells in peripheral tissues has been shown to rely on both humoral and neuronal signals emanating from the SCN, in addition to nutritional signals resulting from food availability. Studies investigating peripheral tissues have shown that clock-controlled outputs are essential for the temporally coordinated execution of many tissue-specific functions. Very few studies have investigated circadian rhythms in skeletal muscle, a peripheral tissue which accounts for the majority of daily energy consumption in mammals. We are currently investigating the signaling pathways involved in the entrainment of circadian rhythms in skeletal muscle in vivo by focusing on the expression of clock genes and possible clock-controlled genes in skeletal muscle after motor denervation, sympathectomy or a restricted feeding schedule. Our results suggest that in skeletal muscle individual clock genes respond to specific signals, and the coordinated cycling of clock genes in muscle fibers is a consequence of the integration of these various signals. In addition, muscle fiber type appears to be a relevant factor for the amplitude of mRNA fluctuation of particular clock genes. Motor neuron activity plays a major role in muscle fiber type specification, and as we and others have shown, the calcineurin-NFAT signaling pathway mediates these effects. We are also looking at whether the daily oscillating conditions of skeletal muscles influence the nuclear translocation of different NFAT isoforms and transcription of NFAT target genes. Our results suggest that both the level of NFAT nuclear localization and transcriptional activity closely follow circadian rhythms of locomotor activity.

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Evidence for Specific Cell Types Involved in Circadian Timing in the Lung: a Role for Clara Cells

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Like other peripheral tissues, there are strong circadian variations in lung physiology (i.e. bronchial hyper-responsiveness and asthma). This study was undertaken to identify specific pulmonary cell types involved in circadian timing and to assess the influence of glucocorticoids (widely used in the treatment of asthma) on the lung clock. Agar-perfused lung slices (275μm) were prepared from PER2::Luc mice, placed under PMTs to measure bioluminescence output and robust circadian rhythms in PER2 expression identified (24.9±0.19h). Phasic application of the glucocorticoid analogue (dexamethasone) revealed a PRC with strong phase advance and delays, and no "dead-zone". Immunohistochemical localization of PER2 and CLOCK revealed expression predominantly within epithelial cells lining bronchioles. The glucocorticoid receptor (GR) was detected ubiquitously throughout the lung. Dual immunofluorescence co-localised PER2, CLOCK and GR with a marker for Clara cells (Clara Cell Secretory Protein), a specialized

population of bronchiolar epithelial cells which act as progenitor stem cells involved in the metabolism of xenobiotics. Treatment of PER2::LUC mice with naphthalene destroyed these cells and ablated circadian rhythms of lung slices. Next, we purified a culture of Clara cells from PER2::Luc mice, and identified robust cell-specific circadian patterns of PER2 expression, with a period of 24.2 h. Our studies thus reveal a key sub-set of cells (Clara cells) involved in circadian rhythmicity in the lung and their responsiveness to glucocorticoids. Since these cells are central to both bronchiolar re-modelling and xenobioitic insult, our data imply that these critical processes may be circadian timed in the lung.

Aging

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Activity Onsets as Circadian Phase Markers: What Time Is It Really?

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Although activity onset is commonly used as a phase marker for the beginning of subjective night in nocturnal animals, onsets may vary depending on running-wheel access (Aschoff, 1973). Here, we used passive infrared (IR) sensors combined with running-wheel sensors (WS) to determine: 1) how wheel access alters activity onset; 2) whether wheel access affects phase-resetting (using the 5-HT1A,7 agonist, 8-OH-DPAT) and 3) associated effects of ageing. Young (2-4 months) and old (19-22 months) hamsters were housed with an overhead IR sensor and unlocked wheel+WS (runners) or locked wheel (nonrunners). Under LD 14:10, young runner activity onset relative to lights-out measured by WS was delayed vs. that measured by IR (19+2 min vs. 1+3 min; $p<0.001$); there was no IR onset difference between runners and nonrunners. In old runners there was no WS vs. IR activity onset difference; however, runner onsets measured by WS were delayed vs. nonrunner onsets measured by IR (37+3 vs. 18+6 min vs.; $p<0.02$). Under constant darkness (DD), there was no WS vs. IR onset difference within young or old runners. There also were no wheel/sensor-related differences in phase-advances elicited by i.p. 8-OH-DPAT administered at CT 6 under DD within age groups. However, shifting magnitude was greatly reduced in old runners vs. young runners (-18+30 min vs. 84+18 min respectively; $p<0.01$), but was not significantly reduced in old nonrunners (49+30 min; $p<0.3$ vs. young runners). Thus, wheel access and method of activity measurement are important considerations when assessing chronotypic treatment effects within and across age groups. NIH NH35229 JDG.

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Aging Affects Resetting of Central and Peripheral Oscillators in PER2::luciferase Transgenic Mice Following a Six-Hour Advance of the L:D Cycle

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Rotating shift work and jet-lag have been associated with an increased risk of severe conditions including cancer, cardiovascular disease and sleep disorders. These conditions are often exacerbated by years of exposure, reflecting cumulative damage of repeated shifts or a declining capacity to cope with perturbations. We have reported a substantial rise in the mortality rate of aged mice following repeated phase advances. We have also shown an effect of aging on the response of central and peripheral clocks to a 6h phase-advance of the L:D cycle in Per1-luciferase rats. We examined the response of central and peripheral tissues from young (2-6 months old) and aged (24-27 months old) PER2::luciferase transgenic mice (PER2::LUC) to a 6h advance of the L:D cycle. Animals were euthanized one day before (baseline) or 1 and 5 days after a 6h advance of the L:D cycle and luminescence was recorded from cultures of spleen, esophagus, liver, pituitary, kidney, lung, thymus, cornea and SCN. Additional SCN cultures were

imaged with an intensified CCD camera. The peak of PER2::LUC expression on the first cycle in vitro was determined for each tissue, and for individual SCN neurons. Tissues from aged mice responded more slowly than did tissues from young mice and did not shift completely, even after 5 days in the new photoperiod. Together, these data suggest that the capacity to respond to phase-shifts, induced by jet-lag or shift work, is compromised in the aged animal.

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Early Circadian Abnormalities Are Predictive of Future Alzheimer's Disease Pathology

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Individuals diagnosed with Alzheimer's disease (AD) typically exhibit several circadian abnormalities early on in the progression of the disease, such as increased daytime napping, disrupted nighttime sleep, and sundowning. This study examined whether any circadian changes are present in a novel mouse model of AD (3xTg), pre- and post-AD pathology. No differences in photic-phase shifting ability at CT16 or CT22 were observed when mice were examined prior to or following plaque formation. However, significant changes were found in daytime activity levels prior to the time of plaque development. When compared to age-matched controls, AD mice exhibit greater overall activity levels, with much higher activity observed both during the subjective day and the subjective night, as AD pathology progresses. A reverse in activity levels was observed in female mice exhibiting both plaque and tangle pathology, with general activity dropping dramatically when compared to pre-tangle formation as well as age-matched controls. These findings correspond to the irregular sleep/wake patterns of patients in the early stages of AD. This model demonstrates the potential for circadian abnormalities to act as a predictive tool for diagnosing the development of AD pathology, and in studying the neurological changes associated with circadian dysfunction in individuals with AD.

P241

Age-Related Changes in Pineal Melatonin Content and Reproduction in the Marsh Rice Rat (Oryzomys palustris)

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Pineal melatonin production significantly declines with age in many species. In male and female rice rats, young and middle-age groups had the highest mid-dark levels of melatonin on 16L:8D compared to the oldest age group. Aged males and females continued to exhibit reproductive sensitivity to the effects of a short photoperiod (12L:12D), but pineal melatonin content was not altered at mid-dark in either gender regardless of photoperiod. Continuous-release melatonin implants significantly reduced reproductive organ mass in aged males, but not aged females. Pineal melatonin content at mid-dark was not altered by melatonin implants in either gender. Aged males responded to a daytime injection of the beta adrenergic receptor agonist isoproterenol (ISO) with significantly elevated pineal melatonin content within one hour after injection, but not to an ISO injection at the time of lights off suggesting a change in sensitivity to the effects of ISO. Lastly, a 20 minute acute light pulse (~ 685 lux), beginning three hours after lights off on 16L:8D (a time when rice rats have significantly elevated levels of melatonin), failed to suppress pineal melatonin content relative to dark-exposed controls in both sexes. Thus, the pineal gland of the aged rice rat is relatively insensitive to light at a time of increased melatonin production. These results show that age affects the ability of the pineal gland to synthesize melatonin in rice rats and that reproductive effects are influenced by photoperiod and melatonin. (Supported by funds from Indiana University Southeast to KEE.)

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Poor Melatonin Synthesis, Aging Sleep and Melatonin Replacement: Three-Year Follow-Up Study

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Sleep problems become ordinary with advancing age. Despite that the physiological meanings of melatonin on human sleep remain controversial, numerous number of elderly insomniacs receive melatonin replacement for months or years. The present 3-year follow up study in the 81 elderly people (mean age, 69 years) revealed a diverse inter-individual variety of innate sleep quality and melatonin secretion levels in the present subjects and that the magnitude of decline in nocturnal melatonin secretion volume, but not the absolute value of melatonin secretion at each measure point, contributed to the deteriorated sleep quality. To examine the effectiveness of melatonin replacement for sleep maintenance deterioration in the present subjects, thirty-eight out of the study subjects received 1mg sustained release melatonin 1 hour before bedtime for 4 weeks on a single-blind placebo-controlled crossover basis. Melatonin replacement trial showed that the magnitude of decline in nocturnal melatonin secretion volume could also predict the responsiveness to melatonin replacement. The present study suggests the possible role of diminished melatonin secretion for age-related changes in sleep quality, but also the difficulty to clinically predict the candidates for melatonin replacement referring to the cross-sectionally determinable parameters before treatment. At least a part of the subjects with poor melatonin synthesis could have developed compensative function against the low melatonin signals from their earlier years. These findings raise a caution to place an excessive expectation to the long-term supplementation of melatonin for elderly insomniacs without objective sleep estimation even when they show poor melatonin synthesis.

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Differential Responses to Short Wavelength Light in Older Individuals

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Reduced sensitivity to short wavelength light with age has been revealed for the acute non-image forming response of melatonin suppression. The current study aimed to determine if a similar age-related reduction in response to short wavelength light occurs for circadian phase shifting and subjective alertness. Eleven young (23.0 ± 2.9 years) and 15 older (65.8 ± 5.0 years) healthy males participated in two laboratory sessions that included a 2 h intermittent monochromatic light pulse (~6x1013 photons/cm2/sec), individually timed to begin 8.5–10.5 h after their DLMO determined in a prior visit. Subjects were exposed to short wavelength light (456 nm) in one session and medium wavelength light (548 nm) in another; five older subjects participated in only a short wavelength light condition. The magnitude of phase advance was assessed as the difference in plasma melatonin onset and offset phase markers pre- and post-light exposure. Subjective alertness was rated on a 9-point scale during and for 5 h after light exposure. In the phase advancing response the older group exhibited similar wavelength sensitivity to the young group (456 > 548 nm) but with smaller phase advances (non significant). By contrast, the alertness response to short wavelength light was significantly reduced in the older group ($F_{1, 24} = 23.6$, $p<0.0001$). The findings show that whilst there is reduced responsiveness to the acute effects of short wavelength light in older

people (melatonin suppression, alertness) the response to phase shifting is not significantly impaired. Supported by EU Marie Curie RTN grant (MCRTN-CT-2004-512362) and the 6th Framework Project EUCLOCK (018471).

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Effects of Aging and Alzheimer's Disease on Scale-Invariant Activity Regulation

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In humans and other animals there exist scale-invariant patterns in activity. Lesions studies in rats have suggested that the circadian pacemaker (suprachiasmatic nucleus; SCN) is a central node in the control network responsible for this scale-invariant activity pattern over a wide time-scale range (from ~4 to 24 hours). Aging and Alzheimer's disease (AD) are associated with functional disturbances of the SCN. Therefore, we hypothesized that the scale-invariant activity regulation in humans changes with aging and AD. We studied 13 young control subjects (mean±SD: 25.5±6.1yrs), 13 elderly control subjects (68.6±6.1yrs), 13 early-stage AD (68.5±6.1yrs), and 14 late-stage AD (83.9±6.7yrs). Activity was continuously recorded using wrist-actigraphy over at least one week, while subjects maintained habitual sleep/wake schedules. Using detrended fluctuation analysis, scale-invariant patterns were characterized by a scaling exponent : =1.00 indicating complex ('healthy') feedback activity control, and =0.50 indicating white noise without feedback control. In young controls, activity fluctuations during the daytime exhibited robust scale-invariant patterns at time scales from minutes to 10 hours (=0.93±0.02; mean±SE). The scale-invariant patterns were significantly altered at time scales >2 hours in elderly controls (=0.82±0.03; p=0.0018), and were altered further in early-stage AD (=0.80±0.03; p=0.0003) and late-stage AD groups (=0.68±0.02; p<0.0001). These results are consistent with the hypotheses that: (i) the reduction of the scale-invariant activity regulation in the elderly and AD reflects the SCN-related functional disturbances; and (ii) in humans, like in rats, the SCN is a central node in the control network responsible for the scale-invariant activity patterns over a wide time-scale range.

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The Circadian Phase of Bedtime as a Predictor of Time in Bed and Total Sleep Time in Elderly Men and Women

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It is possible that part of the poorer quality and shorter sleep duration characteristic of the elderly is the result of an earlier phasing circadian pacemaker, and thus an inappropriate phase angle between bedtime and pacemaker. One approach is to delay the phase of the pacemaker by applying bright evening lights. Unfortunately, though, seniors do not like doing this. Alternatively, one can advance bedtime. In an earlier archival diary-based study of the relation between spontaneous changes in bedtime and the amount of sleep obtained (128 healthy seniors each studied for 7d), we observed that earlier bedtimes were significantly associated with more time in bed (TIB) and more total sleep time (TST). This led to an intervention study in which the bedtimes of ten healthy seniors (9f, 1m, 70y-82y) were delayed and advanced by 2h in a time-isolation laboratory. Sleep was measured by polysomnography, circadian rhythms by rectal temperature. Each subject attended three ~120h sessions conducted in temporal isolation. Each session started with 2 nights at habitual bedtime and rise-time, followed by 3 nights for which only bedtime was specified:

control = habitual bedtime (HBT); advance = HBT-2h; delay = HBT+2h. The design thus allowed TIB to become a dependent variable in a time-isolated situation, and TST to be essentially ad-lib. In terms of both TIB and TST, the order was advance > control > delay, with the effect more pronounced in TIB than in TST. Possible mechanisms for this effect, based upon circadian phase and phase angle, are discussed.

Seasonal

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Seasonal Behaviour in Drosophila melanogaster and norpA

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Drosophila melanogaster locomotor activity responds to seasonal conditions by a modulation of the "evening" activity component. During simulated winters of cold temperature and short days an advanced evening locomotor peak occurs with more daytime locomotor activity; on the other hand long photoperiods and warm temperatures gives a delay in the evening peak resulting in more nocturnal activity, and thereby avoids a possible desiccation during the hottest hours of the day (the "siesta"). This pattern of activity is related to a thermosensitive splicing event that occurs in a 3' intron in the period transcript with a higher level of splicing and earlier accumulation of PERIOD protein at short days and low temperatures. In this regard, a mutation in norpA gene which encodes for a phospholipase-C, generates a high level of per splicing at warmer temperature, so that mutants behave as if is colder than it actually is. In addition, norpA mutants show an impaired capability to synchronize to temperature cycles, indicating a role in the temperature entrainment pathways. We have dissected the role of norpA in period expressing neurons to further study thermal entrainment.

P247

Naturally Occurring Variation in Cryptochrome in European Populations of Drosophila melanogaster

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Circadian clock genes are target for natural selection that drives the emergence of new alleles, resulting in molecular adaptations to various environmental conditions. Previous studies in natural Drosophila populations revealed molecular polymorphisms in the period and timeless genes that follow a latitudinal cline and allow the circadian pacemaker to adapt to different temperatures and light conditions. To explore natural variation in cryptochrome (cry), the Drosophila circadian-dedicated photoreceptor, we have sequenced the complete coding DNA of 18 cry alleles. We have identified a large number of single nucleotide polymorphisms including seven non-synonymous changes. The SNP frequency spectra and linkage disequilibrium suggests that this variation is maintained by balancing selection. A non-conservative SNP in the FAD domain involving a leucine-histidine replacement (L232H) appear to segregate in all populations tested. Pseudo congenic lines, harbouring either the L232 or the H232 alleles show significantly different light response, a difference, which seems to be recapitulated in transgenic flies. We have tested the frequency of the two alleles in 13 populations across Europe. While neither of these alleles follows a latitudinal cline, the level of heterozygosity does; the frequency of homozygous flies increases in Northern latitudes. We are testing the possibility that different activity profiles of flies carrying different alleles facilitate assortative mating, a process that is enhanced in northern latitudes due to longer days during the reproductive season.

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The Responses of Circadian Gene Expression to Skeleton Photoperiod in a Short-Day Plant Lemna Paucicostata 6746

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The day length provides an environmental cue that allows plants to flower in response to the changing seasons. *L. paucicostata 6746* (duckweed) is an obligatory and sensitive short-day plant. The floating, tiny plant bodies, rapid growth rates, and strictly controllable environments under aseptic culture conditions are some of the traits that make this plant experimentally attractive. Hillman's experiments (1964) analyzing "skeleton" photoperiods using *L. paucicostata 6746* have given the concept of photoperiodic time measurement by use of an endogenous circadian clock. "Skeleton" photoperiod is the term named by Pittendrigh to describe schedules that are given two short light pulses in the 24 h day. Flowering responses under skeleton photoperiods depend strongly on the timing of the first pulse in the dark period. For example, in an experiment using the 11:13 schedule (15-minite light followed by 10.5 hr of darkness followed by 15-minute light followed by 13 hr of darkness), cultures that received 24 hr darkness gave low flowering, while 15 hr darkness gave a much higher value. Recently, we developed a convenient method to monitor the circadian rhythm using a bioluminescent reporter gene introduced into the Lemna plants by particle bomberdment. We monitored the bioluminescent rhythms under various skeleton photoperiods in detail, and examined the phase response curve to understand the phase relationship between rhythm and light pulse. We discuss how the circadian clock is entrained under skeleton photoperiods.

P249

Characterisation of Cry1-Associated Genes Rhythmically Driven by Melatonin, in the Sheep Pars Tuberalis

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The nocturnal melatonin (Mel) signal acts on the pars tuberalis (PT), located at the interface of the hypothalamus and pituitary, and is involved in seasonal prolactin secretion. The PT expresses Mel receptors, and here clock genes Per1 and Cry1 are rhythmically expressed, with Per1 tracking light onset/melatonin decline, and Cry1 directly induced by melatonin and night-onset. Changes in Per/Cry phasing have been proposed as an internal co-incidence timer for photoperiodic time measurement. Using cDNA microarrays we characterized a subset of genes up-regulated at the onset of the night in the PT of short-day housed sheep. Among this Cry1 associated cluster, we identified the phosphoribosyltransferase Pbef/Nampt. Pbef/Nampt is the rate limiting enzyme in the NAD salvage pathway in cells and is involved in cellular energy sensing. To test whether Pbef/nampt is melatonin-induced, we implanted sheep with sc melatonin and killed animals at +1, +2 and +6h. Subsequent in situ hybridization experiments revealed strong induction in the PT within 1h of treatment. These studies demonstrate that cry1 is not the only melatonin-induced gene in the PT and suggest that melatonin rhythmically regulates a key cellular metabolic pathway. We also show that Pbef/nampt is strongly expressed in the SCN of the Siberian hamster, while previous studies reveal strong rhythmic expression in adipose tissue. Published data show a strong link between energy sensing through the NAD cycle and regulation of circadian clock genes in the CNS. Our study now suggests that these pathways may also be direct targets for melatonin action.

P250

Per1-Luciferase Expression in SCN and Pineal Explants Varies with Day Length in Photoperiodic Rats

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Many mammals use annual changes in day length, or photoperiod, to adjust their physiological state with changes in season. The central pacemaker in the suprachiasmatic nuclei (SCN) and melatonin from the pineal gland play critical roles in directing photoperiodic responses in hamsters. Since most laboratory rats and mice are not photoperiodic and it is technically challenging to create transgenic hamsters, it has been difficult to examine the encoding of day length in isolated tissues from photoperiodic species. To investigate photoperiod encoding in ex vivo tissues, we have generated a novel photoperiodic animal model by backcrossing the photoperiodic Fischer 344 (F344) strain with non-photoperiodic transgenic Per1-luciferase (Per1-luc) rats. Per1-luc/F344 rats in a short photoperiod (8L:16D) had reduced body weight, testes weight, and estimated testes volume compared to rats in a long photoperiod (16L:8D). Bioluminescence imaging of horizontal SCN slices showed regional differences in the phase of the Per1-luc expression rhythm. Furthermore, the phase distribution in the SCN varied depending on day length. After the first cycle, the phase distribution within the SCN changed, suggesting that the in vivo situation was best represented by the first peak of Per1-luc expression. By measuring luminescence with photomultiplier tubes, we found that the duration of elevated Per1-luc expression was compressed in pineal glands explanted from rats in a long photoperiod compared to rats in a short photoperiod. Taken together, our results suggest that Per1-luc/F344 rats are an appropriate model for investigating how temporal changes in day length result in alterations in behavior and physiology.

P251

Global Transcriptional Response to Different Photoperiods within the Rostral and Caudal SCN

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Before the elucidation of the molecular clock a 'morning' and an 'evening' oscillator was posited as a mechanism which could explain how animals living in non-equatorial latitudes could adapt there daily timing to track dawn and dusk. The principles of this model have been demonstrated recently in flies, where two distinct groups of pace maker neurons are attributed specific roles as morning and evening oscillators, tracking dawn and dusk respectively. More recently, rostral and caudal sub-regions of the mammalian master pacemaker, the suprachiasmatic nucleus (SCN), have been suggested as morning and evening oscillators. To further investigate the role of rostral and caudal SCN we examined transcription profiles of these regions during long (LD=18:6 hrs), equinoxal (LD=12:12 hrs) and short (LD=6:18 hrs) day-length. A highly accurate laser catapult microdissection and sensitive single cycle amplification approach has provided global insight into rostral and caudal SCN gene expression patterns through out a full circadian cycle. We see more marked transcriptional contrast between SCN regions than is observed between different day-lengths within either rostral or caudal SCN. Next we interrogated the data for oscillations based on harmonic regression combined with the F-test test for significance. We find: 1) oscillating mRNAs common to all regions under all day length's, including bmal1, period and cryptochrome genes, 2) unique oscillating mRNAs per region and per day-length and per both region and day-length, and 3)

specific clusters of genes within both rostral and caudal SCN expression of which tracks mid-night and mid-day during all photoperiods. We are currently further investigating candidate genes and biological processes which may underpin morning and evening roles within sub-regions of the SCN.

P252

Entrainment to Shifted Light Cycles Involves Rapid Synchronization of a Subset of SCN Neurons

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The suprachiasmatic nuclei (SCN) contain a circadian pacemaker that is entrained to the environmental light-dark cycle. Previous real-time electrical-activity measurements in SCN slices revealed bimodal peaks, with one shifted and one unshifted component, following a 6 hour delay of the light-dark cycle. It appeared that the ventral part of the SCN corresponded with the rapidly shifting component while the dorsal region corresponded with the unshifted component. The aim of the present study was to provide a quantitative analysis of the observed bimodal electrical activity pattern. We exposed a total of 22 animals to a 6 h delay of the light cycle, prepared hypothalamic slices, and performed recordings of electrical impulse frequency with stationary electrodes. We observed 10 double peaks, 9 single peaks, and 3 peaks that exhibited an intermediate waveform following the shift. Analysis of the bimodal activity records shows that the unshifted component is relatively broad (6.1 h ± 2.0 h) and the shifted component more narrow (2.2 h ± 1.2 h). Computer studies, including curve fitting analysis, showed that the number of action potentials that contribute to the shifted component is about 10 % of those that contribute to the unshifted component. Subpopulation analysis confirmed these findings, and showed strong synchronization in peak phase in the shifted component but not in the unshifted component. We conclude that there are substantial quantitative differences between the unshifted and shifted component in the SCN following a delay of the light cycle.

P253

Regulation of Onset and Cessation of Behavioral Activity by Electrical Impulse Frequency in the SCN

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The suprachiasmatic nuclei (SCN) of the hypothalamus contain a major pacemaker responsible for generating circadian rhythms in behavior and physiology. The SCN also measure the changing duration of daily light exposure over the seasons and is important for seasonal rhythms. Molecular and electrophysiological studies have shown that the circadian waveform of the SCN rhythm is affected by photoperiod. Besides the effect on the waveform of SCN rhythms, changes in photoperiod may also influence the sensitivity of downstream brain areas to the SCN signal. In this scenario, a change in downstream sensitivity leads to the perception of a change in day length. To investigate whether changes in sensitivity occur and contribute to photoperiodic encoding, we implanted mice that were kept under a short (LD 8:16) or long (LD 16:8) photoperiod with stationary micro-electrodes and simultaneously recorded SCN firing rate and drinking activity. The results show that both on- and offsets of drinking behavior occur when SCN firing rate is between 50% and 60% of maximal firing rate. These levels are not significantly affected by photoperiod. We also did not observe changes in these levels following release of the animals into constant darkness. We conclude from these results that fixed levels of neural activity trigger the onset and cessation of behavioral activity, both in long and in short photoperiods. Modification of the waveform of the SCN signal appears to be the primary mechanism responsible for the effect of photoperiod on the duration of daytime behavioral activity.

P254

Effect of Photoperiod on Locomotor Activity, Adrenal Steroid Rhythms and Gene Expression in Nonhuman Primates

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Although human behavior and physiology are seasonally modulated, the specific influence of day-length is poorly defined. To address this issue, we sequentially exposed female rhesus macaques to the following lighting regimens: 12L:12D, 8L:16D (short days), 12L:12D and 16L:8D (long days), in order to mimic the photoperiods associated with fall, winter, spring and summer, respectively. We analyzed the rhythms of locomotor activity and adrenocortical hormones, and performed genome-wide expression profiling of the adrenal gland, an organ that is timely regulated throughout the day/night cycle. Analysis of locomotor rhythms revealed higher levels of nocturnal activity in short days ($P<0.005$) as well as phase-advancement of activity onset and acrophase, whereas exposure to long days was associated with a phase-delay in those parameters. Day length had no effect on either the mean plasma level or amplitude of the 24-hour cortisol or DHEAS rhythms. Interestingly, however, we detected a phase-advancement of both hormone rhythms in short days. In the case of cortisol, maximum hormone levels were attained during the night, several hours before lights on. In addition, the ascending phase of plasma corticosteroid rhythms was longer under 8L:16D, and shorter under 16L:8D. Analysis of the adrenal gland transcriptome from monkeys kept in short days, 12L:12D and long days, revealed differences in the expression of genes involved in development, lipid synthesis and metabolism, and immune function. Taken together the results indicate that, in primates, changes in day length can affect the dynamics of endogenous rhythms and exert an effect on gene expression in the adrenal gland.

P255

Wavelength Dependence for Effects of Dim Scotophase Illumination on Circadian Waveform and Re-entrainment in Siberian Hamsters

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Compared to totally dark nights, dim scotophase illumination (DSI), promotes rapid expansion of activity time () in 10L:14D and increased rhythm splitting on LDLD. Here we report influences of DSI irradiance and wavelength on the above responses using a new 28 night assay protocol: on the 5th night of recording in 14L:10D, the scotophase is increased symmetrically to yield 10L:14D for 10 nights during which animals receive darkness or DSI. Next DSI is extinguished and the photoperiod is symmetrically increased to yield 16L:8D. Finally, after four 8D nights, DSI is turned on while imposing a 4D:8L:4D:8L cycle for 10 split nights. A first experiment using 560 nm DSI included 3 groups: DIM1 with DSI irradiance at 5.30 x 10-6 Wcm-2, DIM2 (1.34 x 10-6 Wcm-2, n=8), and DARK (n=20, 0). Compared to DARK, transfer to 10L:14D resulted in larger in DIM1 and DIM2 ($p<0.001$). LDLD resulted in split rhythms in 12/12 on DIM1, and 7/18 in DARK ($p<0.001$, 2 animals excluded for insufficient wheel counts). In comparison, 5/8 split in DIM2 ($p< 0.022$ vs. DIM1; $p =0.264$ vs. DARK). Following multiple repeats of this protocol with more DSI irradiance groups per wavelength, we have fit dose response curves with estimated EC50s of 2.388 x 10e+9 for 560 nm, compared to 1.313 x 10e+11 photons/cm2/s for 695 nm DSI. Data for DSI at 590 and 470 nm is accumulating. Ultimately, adding more wavelengths, we aim to determine action spectra to help identify the contributing photopigment(s). [Supported by NSF-IBN-034639, NICHD-36460].

P256

Dose-Response to Short Wavelength Light for Phase Resetting in Syrian Hamsters: Influence of Photoperiodic History and Background Illumination

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In Syrian hamsters, bright light-induced phase shifts are greater after entrainment to short versus long photoperiods. Moreover, entrainment in this species is markedly affected by whether scotophases are completely dark or dimly illuminated. Two separate experiments describe fluence-response curves for phase advances and delays by short wavelength light, following entrainment to LD14:10 versus LD10:14 and dark versus dim light background. The phase advance experiment employed a 21-day Aschoff Type II protocol. After 11 days under LD, animals were released into constant dim (DIM) or dark (DD) conditions. On the first night of DIM or DD, a light pulse was administered. Animals remained under DIM or DD for 10 additional days and were reentrained for 3 weeks before the next pulse. The phase delay protocol consisted of four, three-week waves of release into DIM or DD for five days followed by three light pulses at CT14, each separated by 5 days. Re-entrainment to the assigned photoperiodic condition occurred between each wave. In both experiments, 15-minute pulses consisted of a range of progressively increasing irradiances of 480 nm light at 0.003, 0.03, 0.25, 1.31, 4.86, 68.03 uW/cm2 and a no pulse control. Phase shifts, activity duration, and free-running period were determined for each condition. Preliminary data show a dose-response relationship, with larger phase shifts as irradiance is increased for each condition. Data on half-maximum and saturation levels are currently being obtained. Understanding the influence of photic history on circadian rhythms may have mechanistic as well as practical implications. Support: NICHD-36460.

P257

Season, Estrous, and Circadian Cycle Timing of Breast Cancer Resection Interact to Determine Post Resection Breast Cancer Metastatic Recurrence Risk

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Surgery remains the primary treatment for breast cancer. Surgical wounding and associated tissue injury and repair mechanisms can potentially support metastatic cancer growth. There is a 26% better chance of 10-year disease-free survival in young women whose breast cancers are resected in the luteal, versus the follicular, stage of their menstrual cycle. Breast cancer incidence, histopathologic grade, and invasiveness are each modulated by season. We, therefore, evaluated interactions between seasonal, fertility and circadian cycles in 1,214 C3HeB/FeJ female mice, kept in constant 12:12 lighting conditions from birth until death, whose breast cancers were resected for cure at known estrous cycle stages, either during usual sleep or activity phases of the circadian cycle, in one of 22 consecutive similar experiments, each conducted at one of four seasons from 1992 to 2006. Average breast cancer growth rates and the risk of post resection metastatic spread each depend, complexly, upon the time of day, estrous cycle stage and season of cancer resection. Mice resected near the autumnal equinox are most frequently cured. Beneficial effects of estrus or metestrus (luteal) phase resection are most prominent in spring and winter, less prominent in autumn, and absent in summer. Resection in the late sleep phase is associated with the largest estrous cycle dependence. The optimal circadian resection timing is reversed in summer and winter. Cancer outcomes are meaningfully and robustly affected by each of these three important biological cycles. These temporal patterns are exploitable to improve breast cancer prevention, screening, diagnosis and therapy.

P258

Circannual Rhythm of Global Breast Cancer Incidence

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There is growing evidence that breast cancer is a seasonally modulated disease. Because of the paucity of data supporting a biological basis of breast cancer seasonality, these time structures have been attributed to cultural and behavioral variations among cancer patients and medical care delivery patterns. We hypothesize that both the host and the cancer, and therefore the host-cancer balance, as represented by the timing of peak breast cancer incidence, are inherently seasonal. We collected and analyzed breast cancer incidence data from 65 regions around the world to determine whether a global seasonal distribution exists. We recorded the peak timing and amplitudes of breast cancer incidence annual pattern in relation to global latitude of the population. In general, breast cancer incidence shows bi-annual (major/minor) peaks in the Northern Hemisphere and a single, annual peak in the Southern Hemisphere. The annual breast cancer incidence rhythm is damped near the Equator. Phase is relatively stable, across the globe, but, the timing of the major Northern Hemisphere peak inverts when the Equator is crossed. The latitudinal breast cancer incidence behavior supports the likelihood that biological causes contribute meaningfully to human breast cancer seasonality. This interpretation is strongly supported by our findings of prominent breast cancer growth rate and post resection breast cancer metastatic spread annual rhythms among mice who are kept in constant (12L/12D) conditions from birth until death.

P259

Science Publishing in the 21st Century

Bora Zivkovic, Public Library of Science, San Francisco CA

The Internet is rapidly changing the way information—including scientific information—is getting published and disseminated. Views on who owns the information are also changing, with a marked generational difference—the Facebook generation has difficulties comprehending the concept of intellectual property in the old, 20th century sense. The inexorable move towards Open Access publishing of science has forced publishers, universities, libraries and even the U.S.Congress to adapt to this new landscape. First studies indicate that Open Access publication results in greater visibility and subsequent citation of scientific papers. Yet, many research scientists are still unsure about their own actions in regard to publication. This poster will explain what Open Access is, why scientists should publish in Open Access journals, the role of Public Library of Science in the new publishing environment, and the position of scientific journals within a broader online communication of science (Science 2.0).

Human

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DEC2, a New Regulator of Sleep

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Sleep is an integral part of everybody's life and when disrupted will cause cognitive impairment, psychological disorders, etc. Understanding of sleep control and how to exercise it more effectively will have significant benefit to our life. We have identified a human family with short sleep syndrome and pinpointed the cause to a single amino acid change of a transcription repressor---DEC2. A transgenic mouse model

with a same point mutation was established and shown to have similar phenotype. One of the serotonin reuptake inhibitors (SSIRs) was proved to rescue the short sleep phenotype in mutant transgenic mice and one of the patients.

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Brain Responses Following Sleep Loss Are Predicted by a Polymorphism in PERIOD3 as Assessed in Humans Using fMRI

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A variable number tandem repeat polymorphism in PERIOD3 is a genetic marker for inter-individual differences in sleep homeostasis and the effects of sleep loss on cognitive performance, in particular during the circadian alertness nadir. Individuals homozygous for the longer repeat (PER35/5) are more susceptible than individuals homozygous for the shorter allele (PER34/4). However, the brain bases of the effects of the polymorphism on cognitive performance are unknown. Brain responses to an auditory 3-back working memory task were recorded in 15 PER34/4 and 13 PER35/5 individuals during 4 fMRI sessions separated in 2 visits. In each visit, subjects were recorded in the evening, close to the circadian alertness crest, and the following morning, close to the circadian alertness trough. In one visit, they slept in the laboratory between both sessions, in the other, they remained awake (25h SD). Sleep and SD were counterbalanced. Performance and fMRI results showed that subjects could perform the task in all sessions and were affected by SD. FMRI data revealed striking differences between genotypes in the changes in brain responses observed after 25h of SD. In PER34/4, activity increased in frontal and temporal cortices, thalamus, cerebellum, and parahippocampus. By contrast, PER35/5 exhibited marked deactivations in frontal, temporal, parietal and occipital cortices.

The ability to recruit higher cognitive prefrontal areas after SD is maintained in PER34/4 but not in PER35/5. These data provide a brain basis for genetically determined interindividual differences in susceptibility to the effects of SD during the circadian alertness nadir. Support: NRS, FMRE, ULg, Wellcome Trust.

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Identifying Components of the Human Circadian Clock through Worldwide Population Genetics Studies

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Individual circadian clocks entrain differently to environmental cycles (zeitgebers, e.g., light and darkness), earlier or later within the day, leading to different chronotypes. In human populations, the distribution of chronotypes forms a bell-shaped curve, with the extreme early and late types—larks and owls, respectively—at its ends. Human chronotype, which can be assessed by the timing of an individual's sleep-wake cycle, is partly influenced by genetic factors—known from animal experimentation. To further understand this genetic influence in humans, we are conducting association studies combining chronotype data, assessed quantitatively through the Munich Chronotype Questionnaire (MCTQ), with genotype data from clock candidate genes or genome-wide tagSNPs studies. To understand how chronotype has evolved

as a complex trait, we have conducted and/or plan genome-wide analysis of populations from Europe, Asia and South America, which will allow global analysis of chronotype distribution versus genetic variability of populations living at different latitudes. Our research will in future contribute to personalize individual shift work scheduling, to improve sleep quality and the diagnosis of physiological disturbances influenced by the circadian metabolism.

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Assessment of Human CLOCK Haplotype Association with Diurnal Preference

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A polymorphism in the gene CLOCK has been associated with diurnal preference and DSPS in some populations, though not in others. Possible explanations for these differences include age, population differences in linkage disequilibrium (LD) between CLOCK 3111 (rs1801260) and a causative factor, and latitudinal effects. This study examines some of these possibilities using HapMap data from populations with European (CEU), East Asian (JPT+CHB), and Yoruban (YRI) ancestry alongside a gene-wide association study of diurnal preference. We show that CLOCK has robust LD within the HapMap data set across the entire gene (162 SNPs). Haplotypes with rs1801260 minor alleles have similar LD in all HapMap populations. This high LD was leveraged in the association study using 19 SNPs in a largely Caucasian UK-based sample of extreme eveningness and morningness (80 individuals per group). QTL analysis indicates an eveningness association with rs12648271 (Beta=-8.281, p=0.0414 after multiple-test correction). The most common haplotype in the eveningness group (17.0%) was significant (Beta = -10.750, p=0.007). This haplotype is independent of the rs1801260 minor allele and has more robust results in younger participants (<40 yo Beta=-2.240, p=0.028 vs 40+ yo Beta=-0.423, p=0.675). This haplotype is consistent with a single HapMap CEU haplotype (13% of CEU), which in turn has one difference to the most common JPT+CHB haplotype (49%), across 162 SNPs. CLOCK is a possible candidate for balancing selection based on high Tajimas D values and supported by a genome-wide HapMap screening.

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The Human Circadian Clocks Seasonal Adjustment is Disrupted by Daylight Saving Time

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About 1.6 billion people are subjected to daylight saving time (DST), a change in social, but not environmental clocks. We analyzed the changes in Mid-Sleep on Free Days (MSF) and Center of Activity (CoAct) for 8 weeks around both DST transitions in 50 subjects. MSF and CoAct adjust to the transition from DST in autumn, but CoAct does not adjust to the transition to DST in spring, especially in the late chronotypes.

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Morningness-Eveningness Preference in 237 Couples

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Physiological and behavioral processes exhibit 24-hour (h) rhythms in most organisms including humans. These rhythms are driven by a system of self-sustained clocks and are entrained by environmental cues such

as cycles of light and dark and food intake. In mammals, the master clock in the suprachiasmatic nuclei (SCN) of the hypothalamus integrates environmental information and regulates overt rhythms. Circadian clock system also influences sleep timing and diurnal preference. In this study, we assessed diurnal preference in 1814 Japanese people by morningness-eveningness questionnaire (MEQ) and found that MEQ score followed a normal distribution and diurnal preference varied widely among individuals. The survey showed that diurnal preference was associated with a number of factors, sleep onset, sleep duration, waketime, Pittsburgh Sleep Quality Index (PSQI) score, Center for Epidemiological Studies Depression Scale (CES-D) score, sex and age. Furthermore, we examined the effect of chronotype and lifestyle on sleep timing in 237 couples in this population. The current data showed that individual's seep onset was influenced mainly by his/her chronotype and sleep quality and slightly by his/her spouse's sleep onset, while individual's waketime was influenced mainly by his/her chronotype and slightly by his/her sex and his/her spouse's waketime. Our study demonstrates that individual's sleep timing depends on his/her chronotype but not on his/her lifestyle or his/her spouse's chronotype and suggests that chronotype dominantly determines sleep timing.

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Light at the Wrong Time: Short-Term Bathroom Light Influences Physiology and Behavior

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Although industrial progress and life in a 24-hour society are based on the use of artificial light at night, the endogenous circadian timing system (CTS) in humans is synchronized to the solar day by means of the environmental light-dark cycle. The proper functioning of the CTS is important to health and well-being. Under controlled laboratory conditions, however, the long-term application of bright-white, blue-enriched or blue light suppresses melatonin excretion which, for the human body, is the signal of darkness. The aim of the present study was to demonstrate that light emitted by everyday lamps in a naturalistic setting influences physiology in healthy human subjects. A total of 9 healthy subjects (6 male, 3 female, aged 22-33 yrs.) kept their average bedtimes (+/-1h) during a 7-day entrainment period (monitored by actigraphy). During the following 6-day experimental period, subjects maintained their habitual daytime schedules, attending the laboratory only in the evening hours from 7:00 PM until midnight. During lab hours, subjects were exposed to constant dim light (<10lx), with the exception of evenings 2 through 6, during which subjects were also exposed for a total of 30 minutes to light by everyday lamps (office, bathroom, industry) of different intensity (130-500 lx) and spectral distribution (4 with and 1 without blue portions) 1 hour before habitual bedtime. Melatonin suppression was measured by saliva samples taken every 30 minutes during dim-light exposure, as well as 10 minutes before, every 10 minutes during, and 10 minutes after exposure to light from the everyday lamps. Exposure to yellow light (without a blue portion) had no effect on melatonin concentrations (13pg/ml vs. 13 pg/ml). In contrast, 30 minutes of exposure to light from all four of the lamps that included a blue portion significantly reduced melatonin concentrations (13pg/ml vs. 9.9 pg/ml, 10.4 pg/ml, 8.1 pg/ml, 7.5 pg/ml). Subjective alertness was significantly increased at the end of three lighting conditions (all of which had a blue portion). Short-term exposure to everyday lamps is sufficient to influence physiology and behavior. It needs to be determined whether everyday lighting conditions during environmental night are involved in disorders that are otherwise known to occur in shift workers, such as cancer, diabetes, obesity, and depression.

P267

Effects of Glaucoma on the Circadian Timing System in Mice and Men

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Glaucoma is characterized by progressive, irreversible damage of the optic nerve and degenerative loss of retinal ganglion cells (RGC). However, the impact on melanopsin expressing RGCs (mRGCs) that regulate circadian responses to light remains controversial. We explored the anatomical, molecular and functional consequences of glaucoma on the circadian timing system in a rodent model of chronic ocular hypertension and in human patients with severe bilateral glaucoma. In rodents, the extent of degeneration of RGC and mRGC fiber projections was quantified using sensitive anterograde tracing and image-density analysis of axon terminals. Alteration of retinal opsins and ganglion cell markers was assayed using quantitative PCR. The capacity of animals to entrain locomotor activity to light was challenged using successive shifts of the light dark (LD) cycle associated with decreases in light intensity. The quantity of RGC axons is reduced by 40-70% in all central visual structures, and notably in the SCN. Glaucomatous rats are able to entrain to the LD cycle at all light levels, but required more time to re-adjust activity to a shifted LD cycle and showed significantly greater variability in the timing of activity onsets. Q-PCR shows that melanopsin as well as rod and cone opsin mRNAs are significantly reduced in glaucomatous retinas. Human patients with severe bilateral glaucoma showed an attenuated suppression of melatonin by light and a high variability in the time of melatonin secretion onset (DLMO). In conclusion, glaucoma affects central projections of all RGCs including mRGCs. In light of these results, the classical view of glaucoma as pathology unique to the visual system should be extended to include anatomical and functional alterations of the circadian timing system. Support: Emergence-Rhône-Alpes, ACT Vieillissement, Gis-Longévité, Allergan, Inc, Fondation Recherche Médicale stipend to ED, FP6-EUCLOCK.

P268

Long-term Effects of Artificial Dawn on Sleep Inertia: Does Melatonin Play a Role?

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Late chronotypes suffer from sleep inertia if they have to wake up early, e.g. on working days.

Objectives: to reduce sleep inertia complaints in people with difficulties in waking up early by means of artificial dawn and test whether this improvement is related to a shift in the melatonin rhythm.

Methods: An artificial dawn waking up alarm (Philips DAP B.V., Drachten, The Netherlands) was used by 51 subjects for 42 days (study 1) and by 41 subjects for 28 days (study 2) in their home environment. Study 1 consisted of 3 conditions of 2 weeks each; 0 lux (control), 50 lux and 250 lux. Study 2 consisted of 2 conditions; 0 lux (control) and self-selected maximal intensity. At the end of each condition, subjects collected saliva samples for melatonin assessment (study 1). Subjective ratings of sleep inertia were collected and sleep diaries completed. Results: No significant differences were found in dim light melatonin onset (DLMO) with increasing artificial dawn intensity (21:16 ± 1:04 h control, 21:25 h ± 51 min. 50lux,

21:17 h ± 54 min, 250lux). A significant reduction in sleep inertia duration (study 1, 16 min between 250 lux and control condition; study 2, 26 min) was found as well as significant improvements in several ratings of well being and sleep quality. Conclusion: The use of artificial dawn wake-up-light® has beneficial effects according to subjective ratings on sleep inertia, well being and sleep quality. However a shift of the biological clock, measured by melatonin onset, cannot explain these improvements.

P269

Effects of Diurnal and Nocturnal Bright Light Exposure on Human Performance and Wake EEG

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Sleep deprivation in humans results in sleepiness, decrements in performance, and changes in spectral parameters of electrical brain activity (EEG). Bright light at night counteracts sleepiness and influences spectral parameters of brain activity. The effects of bright light on performance and brain activity are less well studied during daytime than at night. Therefore we compared time-of-day-dependent effects of bright light exposure on sleepiness, performance, and spectral changes in electrical brain activity (EEG) during wakefulness. In both experiments, each including 12 young male subjects, subjects were exposed to either bright light (5000 lux) or dim light (< 10 lux, control condition); between noon and 4:00 P.M. (experiment A) or between 12:00 A.M. and 4 A.M (experiment B). Hourly measurements included a neurobehavioral test battery and 3-min recording of wake-EEG (C3-A2) with eyes closed. Irrespective of the time of day, bright light increased performance and reduced sleepiness and power densities in the slow waves (3-4.5 Hz) of the wake EEGs. Despite the increase in performance, test accuracy did not benefit from the light. Time-of-day-dependent effects of bright light included a significant reduction in simple reaction times and an increase in power in the alpha band during the night. During the day there was a non-significant decrease in power in the alpha frequency. Based on the wake EEG, bright light seems to prohibit subjects from falling asleep during the night, but not during the daytime. Apparently the relationships between wake-EEG alpha activity and sleepiness and performance are more complex than often presumed.

P270

The Effect of Incremental Changes in Light Onset on Reentrainment Rate of Octodon degus following A 12-Hour Phase Advance

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We investigated the effects of incremental light cycle changes prior to a 12-hour shift on reentrainment rate using the diurnal Chilean rodent, Octodon degus. Because a 12-hour shift occurs midway through a circadian cycle, there can be a period of both advancing and delaying of the circadian system, resulting in a reentrainment rate that is slower than expected, given the size of the shift. We tested the hypothesis that the internal clock can be primed for a larger shift by using smaller incremental shifts similar to seasonal changes in light. We predicted that the animals that experienced daily 15-minute advances of the light:dark cycle over a period of 5 days prior to a 12-hour advance would reentrain faster than animals that experienced the 12-hour shift with no priming. Preliminary data suggest that incremental advances of the LD cycle prior to a 12-hour advance reduce the length of time required for reentrainment. Final data will be discussed with implications for research and application to human jet lag recovery.

P271

Non-visual Sensitivity to Light in Humans

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Although melanopsin-expressing ganglion cells are undoubtedly the major photoreceptive system in the mammalian retina, it is becoming increasingly clear that classical photoreceptors (rods and cones) are functionally involved in circadian photoreception. The relative contribution of classical and non-classical photoreceptors is unknown, and may depend on spectral composition of light, irradiance, and light history. In order to clarify the mechanisms involved in non-visual response to light in humans, we investigated 1) the acute effects of light exposure (spectral sensitivity), and 2) the immediate after-effects of light exposure in healthy young males and females, and 3) the effects of combinations of monochromatic lights. The sensitivity of light-induced melatonin suppression was assessed, for each subject, with monochromatic lights of equal photon density (3.16x10e12 photons/cm2/sec) at 9 different wavelengths spread over the visual spectrum (420-620 nm). Blood samples were collected every 15-60 min before, during, and after a 60-min nocturnal light exposure session in subjects with fully dilated pupils. Our results show a peak sensitivity of melatonin suppression at approximately 480 nm. As we recently found in mice (Benyahya et al. Neuron 2007), our results suggest that L/M cones play a role in circadian photoreception. Finally, in accordance with our recent work in rodents (Mure et al. 2007), the temporal combination of monochromatic lights suggest that non-visual sensitivity can be modulated by prior light exposure in humans. Taken together, our results provide new insight into the mechanisms involved in circadian photoreception. Our results can be of clinical significance as they could be used to refine the current treatments (light therapy) of depression and circadian sleep disorders. Support: Emergence Rhône-Alpes, GIS Longévité, INSERM ACT Vieillissement, ACI MRT, FP6-EUCLOCK.

P272

Differences in Breast Cancer Risk in Blind Women with and without Light Perception

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Blind women are reported to have a lower risk of breast cancer compared to sighted women. Differences in cancer risk are often attributed to differences in light exposure between the sighted and the blind. Reported history of breast cancer was compared in blind women with some degree of light perception (LP) to those with no perception of light (NPL), to determine whether differences in breast cancer risk are associated with light perception. Participants included 958 LP and 396 NPL women, aged 19-98, living in North America. Data were collected via a multi-format, self-administered survey. Odds ratios and 95% confidence intervals were calculated using logistic regression models. We found a lower proportion of NPL women with a past history of breast cancer compared to LP women (OR = 0.45, CI: 0.25-0.80). Furthermore, we found ORs for breast cancer increased with increasing visual acuity as compared to NPL women. When adjusted for current age, history of full term pregnancy, and smoking, the effect was attenuated (OR = 0.56, CI: 0.30-1.02). When stratified by age 50, however, we found that NPL women over age 50 had a lower breast cancer risk compared to LP women over age 50 in the adjusted analyses (OR = 0.48, CI: 0.25,

0.91). These results are based on survivors since those women with aggressive disease did not survive to be included in this study.

Our findings are consistent with the reported reduced risk of breast cancer among the blind and suggest that NPL blind women have a reduced risk of breast cancer compared to LP blind women.

P273

Room Light Alters the Timing of Melatonin Synthesis

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Although it was once thought that the human circadian system was relatively insensitive to light, we have shown that exposure to room light is sufficient to elicit near-maximal phase shifts and suppression of the melatonin rhythm, if preceded by exposure to dim light (<15 lux). Under normal entrained conditions, however, the importance of room light in determining the timing of melatonin synthesis is not as well established. To test the effects of room light on melatonin phase, we examined the plasma melatonin rhythm in 100 subjects (18-40 yrs) entrained to a 16:8 light-dark cycle during an inpatient protocol. On days 1-2, subjects were exposed to <200 lux during the wake episode, and on day 3 subjects were exposed to <200 lux for the first 8 hours, followed by exposure to dim light (<3 lux) in the 8 hours before bedtime. Exposure to room light prior to bedtime resulted in a later melatonin onset time in 99.0% of subjects, with the onset occurring on average 1.53 hours ± 0.07 (SEM) later, as compared to exposure to dim light for 8 hours prior to bedtime. In contrast, the timing of melatonin offset did not differ in room light versus dim light. Our results suggest that melatonin onset is significantly suppressed by exposure to ordinary room light. As the melatonin onset may appear artificially to be delayed under such conditions, the circadian phase of melatonin onset cannot be determined accurately in subjects exposed to ambient room light. Support: NCCAM (AT002129, SWL); NIMH (MH045130, CAC); NIH/NHLBI (T32 HL07901, CAC).

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Subjective Tiredness, Wrist Activity, and Sleep Fragmentation Score under the Influence of Daytime Low UV Light Radiation in Humans

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Effects of daytime VITA-LITE (full-spectrum natural light) with / without a ultraviolet (UV) cut filter were examined on sleep related functions; rectal temperature, wrist activity, subjective fatigue, self sleep assessment and urinary melatonin, in young healthy male subjects. During four-day-long experiment, seven subjects stayed in the isolated chambers, obeying the strict experimental schedule, such as sleep-wake / light-dark cycle, and meal and shower time. And they were exposed to the VITA-LITE at their back skin for 8 hours from noon on the third day ((+)UV, 25 kJ/m2 for 8 hour's UV radiation), and to the VITA-LITE covered with the UV cut filter for the same period on the other days ((-)UV). Compared with the (-)UV light condition , the subjective tiredness during the (+)UV light condition and the amount of wrist activity from the end of the (+)UV light condition into the bed-time were increased, and at the following night, the elevated sleep fragmentation score was observed. The rectal temperature rhythm and amount of urinary melatonin excreted during the sleep period were not different between the (+)UV and (-)UV light

conditions. Sunbath is known as an easy way to get sufficient light intensity to reset our circadian clock, so we have to immediately confirm the safety timing, duration and intensity of UV radiation to protect our sleep and wake quality against UV radiation. This study was financially supported by the Grant-in-Aid for Scientific Research (A) from the Ministry of Education, Culture, Sports, Science and Technology (no. 17207020).

P275

Correlations among Inter-Individual Differences in Non-image Forming Effects of Light at Night

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There are inter-individual differences in non-image-forming effects of light. We examined the correlations among inter-individual differences in light-induced melatonin suppression, phase shift of internal circadian rhythm, changes in rectal temperature, alertness and behavioral functions. Fourteen healthy male adults participated in the study. Salivary melatonin concentration, rectal temperature, Karolinska sleepiness scale (KSS) and psychomotor vigilance task (PVT) were measured under dim light (15 lux) every hour from 18:00 pm to 8:00 am on three consecutive nights. On the second night, the subjects were exposed to white fluorescent light (1000 lux) for four hours. Starting time of exposure to light was 5.5 hours before the time of nadir of rectal temperature of each subject. The dim light melatonin onset (DLMO) was measured as a marker of internal circadian phase. The inter-individual difference in light-induced melatonin suppression was positively correlated with phase delay of DLMO. However, the inter-individual difference in light-induced melatonin suppression was not correlated with changes in rectal temperature, subjective sleepiness and reaction time of PVT. The inter-individual difference in light-induced change in reaction time of PVT was negatively correlated with change in rectal temperature, and positively correlated with change in subjective sleepiness. The results suggest that correlations among non-image-forming effects of light depend on the measurements, and that light-induced melatonin suppression was significantly correlated with phase delay of internal circadian rhythm.

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Acute Effects of a One-Hour 10,000 Lux Light Exposure on Subjective Sleepiness Ratings

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Prolonged exposure to bright light (4-6.5 h) is able to decrease subjective sleepiness ratings during both day-time and night-time exposures. In the current study, we aimed to measure the alerting effects of short-duration (1 h) light exposure on subjective sleepiness. Thirty-three healthy male and female subjects (18-30 years) participated in a 9-10 day study in a time-free environment. Following three baseline days, subjects underwent a 30-52 hour Constant Routine (CR) in <3 lux and, after an 8-hour sleep, were randomized for exposure to one hour of either 10,000 lux white light (4100K) (n = 17) or background light (<3 lux) (n = 16) exposure. The light exposure was centered during a 16-h wake period and scheduled at one of 18 circadian phases ~20 degrees apart. Subjects completed the Karolinska Sleepiness Scale every 30 minutes while awake. Changes in sleepiness were expressed as deviations from the mean value for the light exposure day for both measurements. Exposure to one hour of 10,000 lux white light reduced subjective sleepiness significantly as compared to continuous dim light exposure ($p<0.05$), regardless of circadian phase when

the bright light pulse was given. These findings confirm that bright white light is able to improve subjective alertness and demonstrate that short-duration bright light in particular is effective. Short-duration bright light exposure may have the potential to be developed as a non-pharmacological sleepiness countermeasure.

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Total Daily Excretion of Urinary 6-Sulphatoxymelatonin During 3 Consecutive Days of Simulated Night Work

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Epidemiological studies have revealed an increased risk of cancer in long-term night shift workers. It has been suggested that decreased melatonin production, due to acute suppression of pineal melatonin secretion by light exposure during night work, could be a significant risk factor for breast and prostate cancers. However, some circadian adjustment to the night work schedule can shift the episode of melatonin production and/or extend its duration. Therefore, total daily melatonin production may not necessarily decrease. In this study, 38 subjects (15 M/ 23 W; 20-35 y.) were studied in a laboratory simulation of night work (00:00 h–08:00 h). Light intensity was 50 lux during day and night shifts. Daytime light intensity after night shifts was designed to mimic the profiles of light exposure previously recorded by ambulatory monitoring in night nurses. The entire urine production of each subject was collected, every 2 h during wake time, and after each sleep episode. Concentration of 6-sulphatoxymelatonin was determined by ELISA, and total excretion (in ng) was then computed from midnight to midnight for the day shift and for the 3 consecutive night shifts. Data were log-transformed to normalize the data prior to statistical analyses. Complete data were available for 29 subjects. Total daily 6-sulphatoxymelatonin excretion decreased during night work ($p = 0.02$). When each day was considered separately, a significant decrease was found only on the last day of night work ($p = 0.01$). These results suggest that consecutive days of night work can decrease total daily melatonin production.

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The Association between Sleep and Rate of Change in Body Temperature across Circadian Phase and the Menstrual Cycle

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Decreased core-body temperature (CBT) and increased heat loss at the extremities contribute to rapid sleep-onset, and are influenced by a circadian/menstrual interaction. We investigated the rate of change (ROC) in temperature during naps across the circadian and menstrual cycles to explore the relationship between thermoregulation and sleep. Eight women were studied during their mid-follicular and mid-luteal phases using an ultra-rapid sleep/wake cycle (36 cycles of 60-min wake/60-min nap episodes). CBT and distal temperature (DT) were recorded and used to calculate the distal-core temperature gradient (TG) and ROC (°C/hour) of CBT, DT and TG throughout naps. Subjective sleep-onset latency (SSOL) and quality (SSQ) were assessed following each nap. A significant circadian variation in ROC was observed for CBT, DT and TG, though no menstrual effects were detected. CBT generally decreased (ROC < 0) during naps, with maximal rates of decrease near habitual bedtime and early night. DT and TG increased (ROC > 0) during naps, with maximal rates of increase at late afternoon/early evening and an early morning trough.

CBT ROC was positively correlated with SSQ and negatively correlated with SSOL, whereas the opposite occurred for DT and TG ROC. These results establish a variation in the ROC of body temperature during naps throughout the day, which may influence sleep propensity and quality. The inverse relationship between DT and TG ROC and improved sleep may arise because maximal DT and TG ROC were observed near the time of the circadian CBT crest, which corresponds to the evening forbidden zone for sleep.

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Circadian Rhythms in Behavioral Inhibition and Flexibility

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Circadian rhythms in cognitive performance can be due to variations in executive functions, which are responsible for problem solving and decision making. Two components of these functions are behavioral inhibition and flexibility. The objective of this study was to identify possible circadian rhythms in inhibition and flexibility. Participants were eight female undergraduate students, aged 17.5±0.93 yr (16-19 yr). Rectal temperature was recorded every minute; subjective sleepiness and tiredness, as well as a Stroop task were assessed each hour in a constant routine protocol for 30 h. The Stroop task required the participants: to read 48 words written in an incongruent color ink; to name the color of the ink, and to shift from one criterion to the other, reading half of the words and naming the color of the others. The index of inhibition was the time to name the colors; and the index of flexibility was the time to complete the shifting criteria section. Data were detrended to remove a possible fatigue effect. There were circadian variations in the time to name colors (Friedman $X2=50.84$, $p<0.001$) and the time to complete the shifting criteria section (Friedman $X2=37.04$, $p<0.05$); performance in both indices decreased at 05:00-08:00h. Cross-correlation analysis showed a 2 h phase delay of inhibition and a 3 h phase delay of flexibility with respect to rectal temperature. In conclusion, there were circadian variations in behavioral inhibition and flexibility (components of executive functions). These results may explain circadian variations in the performance of many complex tasks.

P280

The Effects of Sleep Deprivation on Pheromone Attraction

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Pheromones are olfactory chemosensory molecules that are secreted by animals, including humans, that can have a large impact on communication between individuals. Previous research has shown that pheromones can play a role in attraction, but little research has been completed on what can affect pheromone production in humans. This study examines the effect of sleep deprivation on the attractiveness of pheromones of individuals who were well-rested and then sleep-deprived during consecutive evenings. We predicted that sleep deprivation would negatively affect the attractiveness of individuals' odors, as ranked by participants. After each night of scheduled sleep (8 hours or 4 hours of sleep), donors showered and wore a clean t-shirt for 2 hours while relaxing. College-recruited participants later rated the attractiveness of each pair of donor samples. Data will be discussed in terms of the effects of sleep deprivation's effects on pheromone production.

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Circadian Rhythms of Functional Brain Lateralization in Humans

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Hemispheric asymmetries appear to be a ubiquitous feature among species, occurring in invertebrates, for instance fruit flies, and in vertebrates, such as fish, amphibians, reptiles, birds, and mammals, including humans. Hemispheric asymmetries also exist on various levels from neuroanatomy and neurochemistry to cognitive behaviour. One source of dynamic variations in transcommissural interactions and changes in functional hemispheric asymmetry may happen during the course of the 24h day, when significant circadian changes occur at all levels in the brain, including but not limited to variations in hormones affecting transcallosal communication. Many human physiological and cognitive functions are known to be regulated by circadian rhythms. This study investigates the role of circadian rhythms as a contributing factor to the plasticity of brain asymmetry. We have tested 54 extreme chronotypes (Evening and Morning) in a counterbalanced repeated measures design both in the morning and in the afternoon. We assessed both auditory and visual lateralization in computerized testing, took cortisol as well as oral temperature measures, and administered mood and sleep questionnaires (e.g., PSQI, Epworth). Behavioral effects of Time of testing, time of day preference, and gender will be reported. Investigating the impact of circadian arousal on functional hemispheric lateralization will not only shed light on the neurobiological mechanisms underlying the plasticity of brain function, but it may also elucidate practical implications as to when cognitive tasks requiring left- or right- hemispheric strategies should be scheduled during the day taking into account individual differences in time of day preference.

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A Circadian Phase-Dependent Acute Effect of Moderate-Intensity Exercise on Melatonin Levels in Humans

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Exercise has been reported to have no effect, to increase, and to decrease melatonin levels. We investigated whether the acute effects of moderate-intensity exercise on melatonin levels in humans were dependent on the circadian time of exercise. We examined the effect of exercise on plasma melatonin levels in seven subjects using a within-subjects 44-day, 20-hour forced desynchrony protocol. Subjects were exposed to exercise or a control posture across circadian phases under dim light conditions (<15lux). Exercise consisted of 45-min long bouts on an ergometer at an intensity of ~65% of maximal heart rate. Melatonin was significantly higher during exercising than during the control posture ($p<0.005$). There was a significant exercise group by circadian time interaction ($p<0.01$). Post hoc analysis revealed that exercise increased melatonin levels at CT20 ($p<0.005$) and at CT0 ($p<0.005$). We found that melatonin concentrations were increased up to 25% by exercise, but only at circadian phases when melatonin was elevated. This suggests that exercise increases plasma melatonin concentrations during the nocturnal melatonin secretory episode but does not elicit melatonin secretion during the subjective day, when the pineal does not otherwise secrete the hormone. This is consistent with the finding of no exercise-induced increase in melatonin levels when exercise occurs during the day and an increase in levels during the night. As melatonin can entrain the circadian system, our results suggest that exercise may act as a nonphotic Zeitgeber via increased melatonin concentrations during the subjective night, but not during the day.

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At-Sea Testing of Alternate Watchstanding Schedules on U.S. Navy Submarines

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U.S. Submarines typically employ a 6 hrs-on /12 hrs-off (6/12) watchstanding schedule at sea. This schedule was adapted from a 4/8 schedule in the mid 1960's to provide a longer consolidated sleep opportunity, offer more time on- and off-watch for duties, and reduce the number of watch turnovers per day. Years of operational rigor and disciplined training of Submariners has engrained much confidence in this schedule. However, the 6/12 produces circadian desynchrony, chronic sleep restriction and often includes periods of total sleep deprivation. Modeling alternative watchstanding schedules (based on theoretical work/rest hours) using the Sleep, Activity, Fatigue, and Task Effectiveness (SAFTE) model supports the idea that 24-hr based schedules could improve individual performance underway. In this study, authors were granted access to a 688-class submarine. The boat followed the 6/12 watchstanding schedule for 18 days and an 8/16 schedule for 11 days. Data were collected from 28 Submariners including wrist-actigraphy, PDA-administered cognitive tests and subjective ratings of crewmembers. Saliva was collected every 2-3 hours from subjects while awake for seven days, stored and assayed subsequently for cortisol, melatonin and alpha-amylase. Total sleep time (per 24hr) was similar between the two schedules although sleep was more consolidated on the 8/16 schedule. SAFTE modeling of actigraphy data predicts increased cognitive performance on 8/16 and our data support this hypothesis with higher throughput on a 'matching to sample' test. Crewmembers' subjective ratings supported the 8/16 watchstanding schedule and our study demonstrates that an 8/16 schedule can improve cognitive performance underway.

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Sleep-Activity Circadian Rhythm Abnormalities Reflect Self-Assessed Depression and Anxiety in Patients with Advanced Non-Small Cell Lung Cancer

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Advanced NSCLC patients have a high incidence of depression and anxiety and significantly disrupted circadian rhythms of sleep-activity. This study demonstrates that the presence and severity of objectively measured disruption in the normal circadian sleep-activity pattern is positively correlated to the presence and severity of self-assessed depression and anxiety among advanced NSCLC patients. Eighty-four advanced NSCLC patients (42 inpatients; 42 outpatients) completed a Hospital Anxiety and Depression Scale (HADS) questionnaire, a commonly used screening tool to evaluate the incidence of anxiety and depression, prior to their first chemotherapy cycle. A 3-day wrist actigraphy, an objective measurement of circadian function, was performed at home prior to and in the hospital during the first chemotherapy cycle for the outpatient and inpatient groups, respectively. Anxiety was reported in 40% and depression in 25% of all subjects at baseline. All patients demonstrated extremely disturbed sleep-wake cycles compared to healthy individuals (AMI database), but outpatients had more robust daily activity patterns and better nighttime sleep than inpatients. Spearman rank correlation at 0.05 level revealed that a more disrupted circadian sleep-activity rhythm (e.g. lower daytime amplitude and peak activity, less nighttime sleep and more daytime sleep) is associated with a higher depression and/or anxiety score in outpatients. No such associations were found in the inpatient group. Our data suggest that cancer-related depression and/or anxiety may be alleviated by circadian organization enhancement or repair strategies such as good sleep hygiene, optimally-timed daily exercise, meals, bright light or melatonin.

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Sleep Quality and Circadian Function in Patients with Advanced Lung Cancer

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Many patients with cancer report poor sleep quality, despite having adequate time and opportunity for sleep. Satisfying sleep is dependent on a healthy circadian time structure and the circadian patterns among cancer patients are quite abnormal. Cancer-related sleep disturbances can continue years after successful anti-cancer treatment have concluded. Wrist actigraphy has been validated with concurrent polysomnography to objectively measure many standard sleep parameters, and daily activity. It was found that actigraphic and subjective sleep data are in agreement when determining activity/sleep patterns and sleep quality/quantity, each of which are severely affected in cancer patients. In this study, the actigraphic pattern of an individual advanced lung cancer patient's circadian organization is compared with the patient's report of sleep quality. Each patient's rest-activity cycle was measured over a 4-7 day period and sleep was assessed using the Pittsburgh Sleep Quality Index. Prior to treatment, 84 patients were studied between two centers and actigraphy was obtained in the inpatient setting at the MRMC and in the home for the VAMC patients. Actigraphy shows that sleep quality of lung cancer patients differs significantly from the normal population. The duration of nighttime sleep for lung cancer patients is 35% less than the control subjects and the longest sleep episode was 40% shorter for these patients. The correlation of circadian activity/sleep time with PSQI documented sleep indicates that actigraphy can objectify/complement subjective assessments of sleep quality in patients with advanced lung cancer. These results suggest that improvements to circadian function may also improve sleep quality.

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Circadian Rhythm Analysis: A Pilot Study of Single vs. Serial Section Cosinor Analysis

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Disrupted circadian rhythm profiles have been reported in mechanically ventilated ICU patients. Data sets, however, typically are 48 hours long and sampling intervals vary from 2 to 12 hours. The data set is usually analyzed as a single unit, which can bias against detecting changes between cycle periods. To better describe core body temperature circadian rhythm in an 87-year-old mechanically ventilated patient, rectal temperature sensor data were captured every 5 seconds for 6 days and subsequently averaged into 1-minute bins. The data were then divided into 24-hour serial sections, which were analyzed individually and averaged as a population of sections within the individual. The entire time series was also analyzed as a single unit and compared to the serial section analysis. Using cosinor models with 24 hour periodicity, the overall Midline Estimating Statistic of Rhythm (MESOR) was the same for each analysis method (37.7C). The mean acrophase for the serial section was -215 degrees (06:02); the acrophase for the entire time series was -327 degrees (22:36). Amplitudes were .30 and .14, respectively. Percent rhythm values were 45% for serial section and 3% for the entire time series ($p < .0001$). Serial section analysis showed MESOR increase accompanied by amplitude reduction on days 4 and 5. In this patient, disruption of core body temperature rhythm occurred concurrently with deteriorating clinical condition. Serial section analysis offers the advantage of describing changes in rhythm parameters over time while allowing changes between cycles to be examined. Data collection from additional patients continues.

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Chronic Deterioration in Psychomotor Vigilance Performance during Resident Work Schedules

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Under laboratory conditions, chronic sleep deprivation induced by limiting time-in-bed to 4 or 6 h/night for two weeks gradually impairs average daily performance to a level approximately equal to 72 or 48 h of continuous sleep deprivation, respectively. Medical resident work schedules allowing 24-30 h shifts every third day (Q3) reduce total sleep time to 6.6 h/day and are likely to induce chronic and acute sleep deprivation. In the current study, we compared interns working both the Q3 and an intervention schedule which abolished shifts longer than 16 hours to assess the impact of chronic sleep deprivation on psychomotor performance. Nineteen medical interns were studied during two three-week rotations in intensive care units, each during both the traditional and intervention schedules. PVT (10-min) tests were scheduled 3-5 times/day during work shifts. Mean reaction time (RTms) and the transformed number of vigilance lapses (RT>500ms) were averaged by week and compared with two-tailed paired Student's t-tests. RT slowed and lapses increased significantly from the first to the third week under both schedules. There were no differences in average weekly performance between the two schedules during weeks 1-2. By week 3, however, interns working the Q3 schedule performed significantly worse (RT+/-SD = 343+/-69 ms; 4.3+/-1.8 lapses) than when the same interns worked the intervention schedule (313+/-65 ms and 3.6+/-2.1 lapses; $p<0.05$). In addition to imposing acute sleep deprivation, traditional schedules employing extended shifts also exacerbate performance impairments due to chronic sleep deprivation.

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Predicting Risk of Cognitive Performance Decrements during Resident Work Schedules

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Circadian Performance Simulation Software (CPSS) permits the prediction of cognitive performance levels according to sleep-wake behavior and circadian phase. Under normal sleep-wake conditions, CPSS predicts that performance will drop below 80% of maximum after 16 hours of continuous wakefulness. Recent studies highlight risks posed to medical interns and their patients due to acute sleep deprivation as a consequence of extended (>24-30 hr) work shifts every third day (Q3). The risk ratio (RR) of the predicted duration spent below the 80% threshold was compared in medical interns' schedules with and without extended shifts using CPSS. Self-reported sleep-wake and work data from 20 interns were used to create the three-person team Q3 schedule and a four-person team intervention schedule (which abolished scheduled shifts longer than 16 hours) necessary to provide continual coverage for three weeks. CPSS predictions were binned by minute and RR values (95% confidence intervals) were calculated for each team for the number of minutes exposure below 80% performance for all work hours, overnight duty (23:00-7:00h), and the hour after the end of each shift. The RR of sub-optimal performance on the traditional schedule as compared to the intervention schedule was 1.67(1.63,1.70) for all work hours, 1.79 for overnight duty (no CI due to no time above 80% performance during the Q3 overnight) and 1.64(1.55,1.74) during the commute home. The CPSS-predicted risk of sub-optimal performance paralleled closely the increase in medical errors, attentional failures overnight and motor vehicle crashes on the commute home observed in interns while working on these schedules.

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Efficacy of Blue-Enriched Fluorescent Light for Melatonin Suppression and Circadian Phase Resetting

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Separate analytic action spectra show peak wavelength sensitivity for the circadian system in the blue portion of the spectrum, which differs from the classical visual system. The aim of the study was to compare the efficacy of standard white (4,100 K) to blue-enriched (17,000 K) fluorescent lamps for their capacity to suppress and to phase-shift melatonin rhythms in healthy humans. Two separate groups (N=8/group) were exposed to nine irradiances of white or blue-enriched light (full visual field exposure) and a dark control condition from 02:00–03:30 without mydriasis to assess melatonin suppression. Both groups showed a significant intensity-related suppression of control-adjusted percent plasma melatonin change scores ($p<0.001$) determined by one-way ANOVA. The coefficients of correlation for the fitted sigmoidal fluence-response curves were high ($R2>0.90$). Using the same lamps, a second study was initiated to compare an equal photon, 6.5-hour exposure during the biological night for their capacity to suppress and phase-shift melatonin rhythms in a seven-day protocol. Target intensities for this study were derived from the melatonin suppression fluence-response curves above. These studies are ongoing. These findings confirm that both the standard white and blue-enriched fluorescent lights suppressed plasma melatonin in healthy young subjects in a clear dose-response pattern with higher irradiances eliciting progressively stronger hormone suppressions. These data will help to characterize the photoreceptor system(s) mediating the circadian, neuroendocrine and neurobehavioral responses to complex polychromatic light stimuli.

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Interaction over Endogenous Circadian System and Behavioral Stressors on Cardiovascular Risk Factors in Humans

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The risk of adverse cardiovascular events peaks in the morning (~9AM). We tested the effect of the circadian system, behavioral stressors, and their interaction on markers of cardiovascular risk. 12 healthy adults (6 female) underwent a Forced Desynchrony protocol (12 recurring 20-h 'days' in dim light). Subjects performed mental (serial addition), postural (head-up tilt), and exercise (cycling) stress tests evenly distributed across the circadian cycle (assessed by body temperature; fitted minimum=0°). There was a large circadian rhythm in plasma epinephrine (peak to trough: 60-110% of mean for each stress test), peaking during the biological day (~180°=~16:30). The circadian effect was comparable to the independent effect of tilt (+93%) and exercise (+87%). There was an endogenous circadian rhythm in systolic blood pressure peaking at ~240°, but no rhythm in diastolic blood pressure or platelet aggregation. Cardiac vagal markers peaked during the biological night (~0°). There were no significant interactions between circadian and stressor effects except for vagal markers where the circadian rhythm disappeared during tilt and exercise. These data demonstrate significant circadian effects on hemodynamic and autonomic function, but not on

hemostatic function. These potential risk factors did not peak at the vulnerable circadian phase equivalent to ~9 AM, suggesting that circadian rhythms in cardiovascular function in young healthy subjects may actually be cardioprotective. Alternatively, the rate of change of cardiovascular risk markers may be more clinically important. Finally, the day/night distribution of potential behavioral triggers also likely affects the pattern in overall risk. Support: R01-HL76409, K24-HL76446, NCRR-GCRC-MO1-RR02635, and R21-AT002713.

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Using Circadian Time To Optimize Hematopoietic Stem Cell Harvest for Clinical Transplantation

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Hematopoietic stem cells (HSCs) can be released from the bone marrow (BM) into the circulation following stimulation with various compounds. These mobilized HSC can be used for life-saving transplantation procedures for the treatment of cancers and other hematologic diseases. However, between 5-20% of healthy donors and up to 60% of cancer patients fail to mobilize enough HSCs for transplantation. Under steady-state, HSCs circulate in blood at low levels. Our recent studies have revealed that under homeostasis HSCs exit the BM following a robust circadian rhythm that peaks at zeitgeber time 5 (ZT5) and reach a trough at ZT13 (Mendez-Ferrer Nature, 2008). Here, we have evaluated the circadian release of HSCs in humans and whether normal rhythms could be exploited to enhance HSC harvest. In mice treated with granulocyte-colony-stimulating factor (G-CSF), HSC yields were increased by 2-fold at ZT5 compared to ZT13 ($p<0.05$). In addition, G-CSF-induced mobilization was reduced when mice were housed in a constant-light environment or after light shifts (jet lag), suggesting that this pharmacological enforced mobilization use the endogenous physiological machinery. In healthy humans, HSC numbers were significantly higher at night (8:00PM) compared to the morning (8:00AM), suggesting that the rhythms are reversed in humans compared to mice. This finding is consistent with the activity levels of diurnal humans and nocturnal mice, and indicates that the maximal release of HSCs in blood occurs in the resting period of both mammals. To address whether HSC rhythms influenced G-CSF-induced mobilization in humans, we carried out a retrospective analysis 81 healthy mobilized donors. The mobilization yield was 0.35±0.02 CD34+ cells/blood volume (ml)/donor weight (kg) for subjects collected around 11:30AM and 0.55±0.05 CD34+ cells/ml/kg for donors collected around 1:30PM; 1.5-fold increase, $p<0.0002$). Although a prospective clinical study is needed to provide definitive results, these data suggest that circadian rhythms can be exploited to increase HSC mobilization efficiency for clinical stem cell transplantation.

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Does the Timing of "Morning" Alcohol Administration Differentially Affect Sleepiness?

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Introduction: Roehrs and colleagues show that alcohol administered in the morning increases sleepiness compared to placebo, but not alcohol administered in the afternoon. Is time of day, circadian phase, or length of prior wake the critical factor in these disparate outcomes? This preliminary analysis examined effects of alcohol administered in the "morning" but at two distinct circadian phases. Methods: Alcohol or placebo (Conditions randomized and counterbalanced) was administered in the "morning" to two groups of participants undergoing 20-h forced desynchrony: Group 1 = 1000; Group 2 =2200 administration.

Twelve healthy young adults ages 21-26 y (M=23, SD=1 for both groups) were studied. Beverage was given about 2hrs after waking. The Multiple Sleep Latency Test (MSLT), including 6 naps spaced 2 hours apart, was performed following drinking. A moderate dose of alcohol (0.49 g/kg women, .54 g/kg men) was used; placebo beverage was the same quantity of fluid. Results: MSLTs were scored for sleep latency (SL; time to first sleep epoch), and data were analyzed using MANOVA. A main effect of Condition (p=.039) distinguished longer SL overall with alcohol (M=12.1, SD=6.1 mins) compared to placebo (M=9.5, SD=4.8). Main effects of Group (longer SL in 1000 Group) and SLT (shorter SL in later tests), and an interaction of Group by SLT (longer SL for later tests in 1000 Group) were also observed. Discussion: These preliminary data indicate that a moderate alcohol dose in the "morning" affects sleep latency differently depending on circadian phase. Additional analyses will examine other circadian phases and times of "day."

Sleep

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Basal Forebrain Activation in Day-Active and Night-Active Grass Rats

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In the diurnal grass rat, access to a running wheel switches some individuals to a predominantly nocturnal activity pattern (night-active individuals, NA), while others keep their diurnal activity pattern (day-active individuals, DA). In the present study we determined whether Fos expression in the basal forebrain (BF) is related to wheel-running rhythms. We assessed this question in DA, NA (both with access to wheels), and control (with no wheels) male grass rats kept on a 12L:12D cycle, by examining the expression of Fos and/or choline acetyltransferase in the BF at two time points (ZT 4 and 16). Preliminary results indicate that all the groups had a similar expression of Fos at ZT 4. In contrast, at ZT 16 NA individuals had significantly more Fos expression than controls, whereas DA showed a trend toward significantly higher levels. A similar trend was observed in cholinergic cells expressing Fos. We are currently evaluating the differences in activation among different BF nuclei. Overall, the results suggest that access to a wheel, regardless of the displayed activity pattern, modifies the profile of BF Fos expression. Given that in grass rats access to a wheel has no effect on the SCN, and that cholinergic projections to this site are reduced in comparison to nocturnal lab rats, the BF activation seen in DA and NA individuals with access to wheels is likely to produce its effects on arousal downstream from the SCN, perhaps through projections to other arousal systems. This research was supported by the National Institute of Mental Health grant MH 53433.

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Sleep Responses to 6-H Deprivation in Male Juvenile Octodon degus

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There has been very little work establishing a developmental and diurnal rodent model of sleep. Previous work has established degus display more sleep during the night versus the day, as well as increased bouts of activity around the light transitions in adult males, yet sleep patterns in juvenile males has yet to be explored. The aim of this study was to examine electrophysiological sleep-wake patterns of young animals under baseline conditions and following sleep deprivation. Males (3 mo. old) were housed on a 12:12 Light/Dark cycle at 18°C. Degus were implanted with EEG electrodes and thermistors to record brain temperature. Infrared devices were used to detect activity. Baseline sleep recordings were collected

for 72 h then animals were sleep-deprived by gentle handling for 6 h in the middle of the dark phase (ZT15-ZT21). Following deprivation, animals were allowed 48 hours recovery sleep. EEG was assigned in 12 s epochs. In juveniles, preliminary data suggest the amount of NREM and REM sleep in day versus night did not differ. These data differ from adult males, which do demonstrate significantly more NREM sleep at night versus day. Surprisingly, juvenile degus also did not demonstrate significant differences in sleep during recovery following 6 h deprivation, which is contrary to our data for adults. Juvenile and adult degus demonstrate increased activity around the light transitions. Further work will elucidate circadian and homeostatic components of sleep in young degus in response to photic phase advances and fully characterize developmental changes in sleep architecture.

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Subregional Suprachiasmatic Control of Circadian Outputs

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The ventrolateral (vl) and dorsomedial (dm) SCN can be desynchronized in rats by exposure to a 22-hr light-dark (LD) cycle. This protocol results in desynchronized circadian rhythms that are presumably sustained independently by these two regions. For example, locomotor activity and non-rapid eye movement sleep (NREMS) show a light-entrained 22-hr rhythm as well as a 24.9-hr free-running rhythm. However, the rhythms of activity-independent core body temperature (i.e. changes in body temperature that occur independently of variation in locomotor activity; CBT) and REMS exhibit only a free-running 24.9-hr period. Here, we show that the circadian expression of REMS in desynchronized rats was associated with per1 gene expression in the dmSCN, suggesting that this region directly regulates this sleep state and supporting our hypothesis of SCN region-specific control of circadian outputs. The vlSCN and dmSCN can also be transiently desynchronized by an abrupt phase shift of the LD cycle in rats housed under a 24-h LD cycle, providing an alternative paradigm with which to test our hypothesis. Here, we also show that, as predicted by our hypothesis, when rats were exposed to a 6-hr phase delay, the phase of NREMS and locomotor activity adjusted immediately to the new LD phase. However, the rhythms of REMS and activity-independent CBT lagged behind, requiring several cycles to align with the new LD schedule. These data support the hypothesis that circadian behavioral and physiological outputs rely on the activity of different SCN subregions. Furthermore, they suggest this region-specific output control may underlie some of the circadian dysregulation symptoms associated with transmeridional flights.

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Does Disruption of Circadian Rhythms Mediate the Inhibitory Effects of Sleep Deprivation on Hippocampal Neurogenesis in the Adult Rat?

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We have recently shown that 96-h of REM sleep deprivation inhibits cell proliferation in the hippocampus of adult male rats, independent of adrenal stress hormones (Mueller et al, 2008). During this procedure, daily rhythms of sleep-wake, activity and drinking are also attenuated, raising the possibility that inhibition of cell proliferation is a consequence of disrupted circadian organization rather than sleep loss. Consistent with this idea, constant light (LL) suppresses circadian rhythms in rats and impairs hippocampus-dependent spatial learning and synaptic plasticity (Ma et al, 2007), processes that have been linked to hippocampal neurogenesis. A daily rhythm of hippocampal cell proliferation in rats has also been reported, suggesting circadian gating of proliferation (Guzman-Marin et al., 2007). The present study examined whether loss of circadian behavioral rhythms by LL alters basal levels of hippocampal neurogenesis. Young adult male Sprague Dawley rats exposed to LL for 10 weeks exhibited strong suppression of locomotor

activity circadian rhythms. Age-matched rats in LD 12:12 exhibited normal circadian rhythmicity. To label new cells, all rats received a single IP injection of 5-bromo-2-deoxyuridine (BrdU, 200mg/kg) and were sacrificed 2-h later. BrdU-immunoreactive cells in the granule cell layer/subgranular zone and hilus were counted separately. No significant difference between LL and LD housed rats in the number of BrdU+ cells was detected in either region. These results indicate that normal circadian organization is not necessary for regulation of cell proliferation in the hippocampus, and appear to rule out disruption of behavioral rhythms as the cause of reduced cell proliferation in REM sleep deprived rats. Supported by NSERC, Canada.

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Unihemispheric Sleep in the Bearded Dragon throughout the 24-Hour Period and under Threat Conditions

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Unihemispheric sleep is a form of behavior that allows half of the brain to sleep while the other half is awake, allowing an animal to perform important behaviors and sleep at the same time. It has been observed in dolphins, which must continually surface to breathe, even while sleeping, and in birds, possibly as a means to recover sleep while flying long distances while migrating over large bodies of water. It can be observed through the observation of unilateral eye-closure (both eyes open, one eye open, both eyes closed) as well as electroencephalographic (EEG) analysis between hemispheres. The bearded dragon, Pogona vitticeps, is a diurnal lizard from eastern and central Australia. We predicted that the bearded dragons perform unihemispheric sleep both during the day, when they are napping, and at night to enable them to watch for predators while basking and sleeping. In this study, recordings of eight bearded dragons were made using a five-video camera setup during a 24-hour period, and scored by judging eye-state at one-minute intervals. Presence of unihemispheric sleep was defined as distinct periods during which one eye is open and the other is closed. Data indicate that the breaded dragon does engage in unihemispheric sleep during the day. Data from night-time behavior and in the presence of a predator threat will also be discussed, along with the evolutionary implications of unihemispheric sleep in lizards.

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Per1 and Per2 Expression in the Forebrain of SCN Lesioned and Intact Mice in Response to Sleep Deprivation

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Sleep is regulated by two main processes, a circadian process (C) and a homeostatic process (S). Process C depends on the master pacemaker, the suprachiasmatic nucleus (SCN), and its rhythmic expression of several clock genes, including period1 (per1) and period2 (per2). However, these genes also oscillate in many extra-SCN areas. Furthermore, several clock-gene knockouts exhibit sleep alterations that suggest a role for these genes in Process S, consistent with our observations that per1,2 expression in extra-SCN brain regions increase with wake and decrease with sleep. To study the relative contributions of these two processes on clock-gene expression outside the SCN, we used real-time PCR and in situ hybridization to measure per1,2 expression in the brains of SCN lesioned (SCNx) and intact mice after 6-h sleep deprivation (SD) beginning at light onset and in respective time-matched controls. We observed that SD elevated per1,2 cortical expression in SCNx and intact mice. Higher per1,2 baseline levels displayed in

SCNx mice are also consistent with the higher day-time activity in these mice relative to controls. In intact mice, SD produced no change in SCN expression of per1,2, consistent with the primary role of these genes in regulating circadian timing in the SCN. In conclusion, our results show that sleep-wake dependent changes in clock-gene expression in extra-SCN brain regions are preserved in mice without a functional circadian pacemaker, supporting the idea that outside of the SCN, clock genes are driven by vigilance state and may not always follow a 24-hr periodicity. Supported by NIH MH067752.

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Glutamatergic Neurons in the Doromedial Hypothalamic Nucleus Regulate Rhythms of Locomotor Activity

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Previous studies have shown the dorsomedial nucleus of the hypothalamus (DMH) to be a major relay and integrator in the circuitry through which patterns in arousal state, behavior and endocrine rhythms are temporally organized. For example, cell-specific lesions of the DMH produce a significant reduction in the amplitude of circadian rhythms of locomotor activity, wakefulness, corticosteroid secretion and feeding. The DMH receives both direct projections from the suprachiasmatic nucleus (SCN), the master circadian pacemaker, and indirect (disynaptic) projections from the SCN via the ventral subparaventricular hypothalamus. Because the DMH sends excitatory (glutamate and thyrotropin releasing hormone) projections to wake-promoting neurons in the lateral hypothalamus (LH) as well as inhibitory (GABAergic) projections to sleep-promoting neurons in the ventrolateral preoptic nucleus, it has been hypothesized that the DMH influences circadian rhythms in physiology and behavior by promoting arousal. To test the importance of glutamatergic projections of the DMH to the LH in vivo, we employed a conditional knock out mouse, bearing loxP modified alleles for vesicular glutamate transporter 2 (VGlut2). VGlut2 is the only vesicular glutamate transporter isoform present in DMH neurons. By stereotaxically injecting an adeno-associated viral vector expressing Cre recombinase into the DMH, we were able to eliminate glutamatergic neurotransmission from this region. Loss of VGlut 2-expression in this nucleus correlated with a measurable decrease in the amplitude of locomotor activity rhythms in constant darkness.

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Differences in Sleep Patterns Affect Recovery in A Segregating Population of Sleep-Deprived Mice

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While a great deal is known about sleep recovery after acute sleep deprivation, little is known about how differences in phase-specific baseline sleep affect how an animal responds to this challenge. In order to better understand this relationship, we collected and analyzed 48 hours of sleep-wake data comprised of 24 hours of baseline data, 6 hours of sleep deprivation during the light phase (ZT 2-8) and a 16 hour recovery period in N=105 genetically heterogeneous mice from a (C57BL/6Jx129S1/SvImJ)F2 cross. We find that the amount of time an animal spends in NREM sleep during the dark (active) phase is significantly correlated with a decreased amount of sleep fragmentation, as represented by brief arousals, during the first 4 hours of sleep recovery ($r=-0.22$, $p<0.05$). Therefore, an increased amount of NREM sleep during the dark phase results in a more consolidated sleep recovery period compared to baseline. Conversely, the amount of NREM sleep during the light (inactive) phase has no effect on fragmentation after sleep deprivation. We also show that a high number of stage shifts, or less consolidated sleep, in the baseline

inactive phase positively correlates with the amount of NREM rebound sleep in the four hours following sleep deprivation ($r=0.20$, $p<0.05$), while a high number of stage shifts in the active period negatively correlates with NREM rebound following deprivation ($r=-0.21$, $p<0.05$). We show here that the response to a homeostatic challenge is correlated to baseline sleep and is specifically dependent upon characteristics during NREM sleep that are phase-specific under baseline conditions.

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Diurnal Differences in Correlations of Sleep-Wake Phenotypes and Depressive-Like Behaviors

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Clinical depression in humans often presents with a sleep disturbance component, with many depressed patients complaining of excessive daytime sleepiness and/or insomnia. In a study of N=105 genetically diverse male mice maintained on a LD 12:12 cycle from a (C57BL/6J x 129S1/SvImJ)F2 cross, we found correlations between depressive-like behavior and both NREM sleep and fragmentation, at phases of the activity-rest cycles that correspond with sleep-wake disruptions associated with depression in humans. Using various behavioral measurements taken during a six-minute tail suspension test that measure depressive-like behavior in mice, we found that more depressive-like behavior was associated with more NREM sleep during the normal active phase (12 hour dark phase) ($r = 0.24$, $p<0.05$). This is consistent with human data showing that depressed patients complain of excessive daytime sleepiness. We also saw positive correlations between sleep fragmentation and depressive-like behavior in the light phase, or the rest phase ($r = 0.34$, $p<0.001$), again consistent with human data showing that a large majority of depressed patients suffer from insomnia, resulting in fragmented sleep during the normal sleeping phase. These data show that behavior and sleep correlates depend highly on phase. Sleep behaviors in the rodent have a strong phasic component in how they correlate to depressive-like behavior, as do sleep-wake traits associated with clinical depression in humans. These data are expected to aid in finding endophenotypes for depression, and ultimately, the genetic components associated with depression and sleep.

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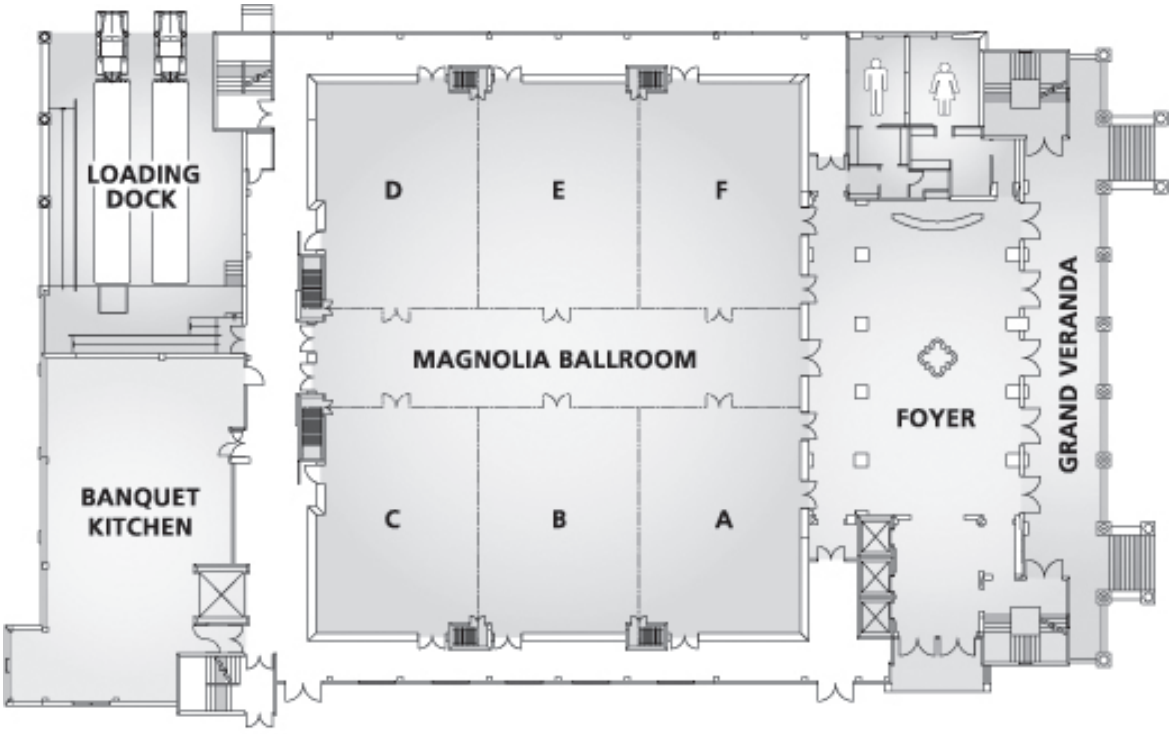
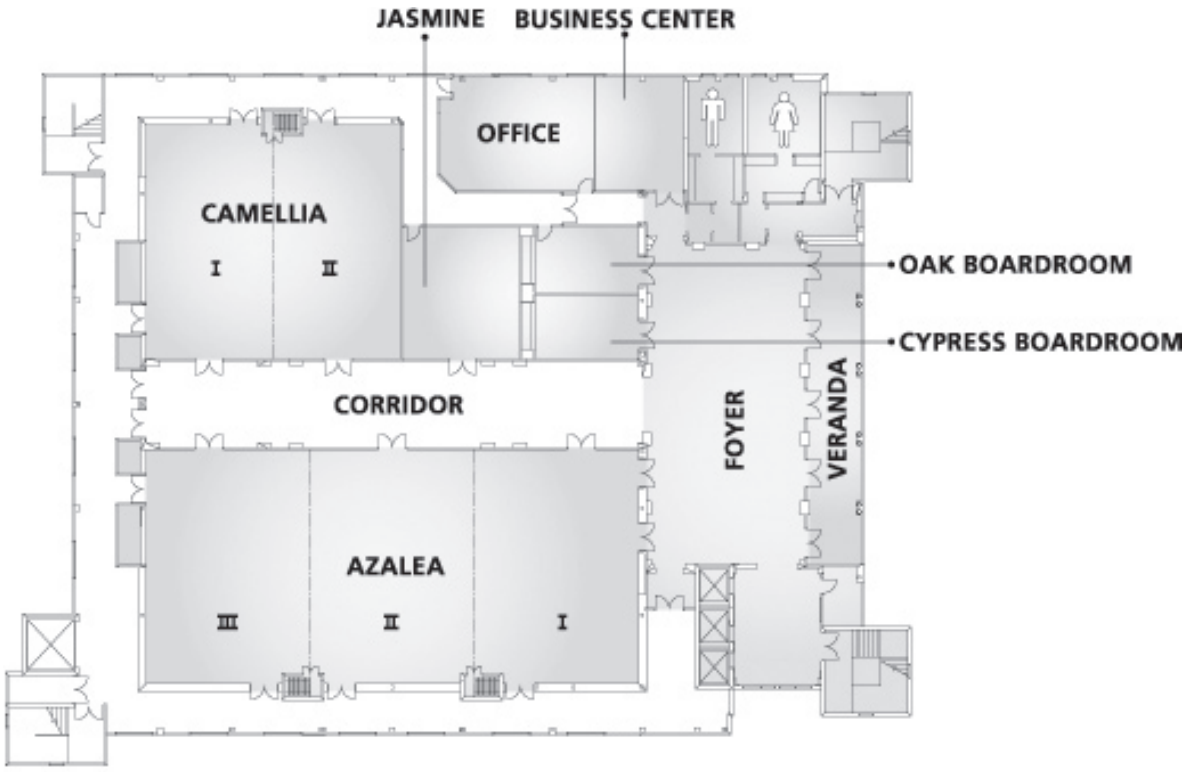
Notes

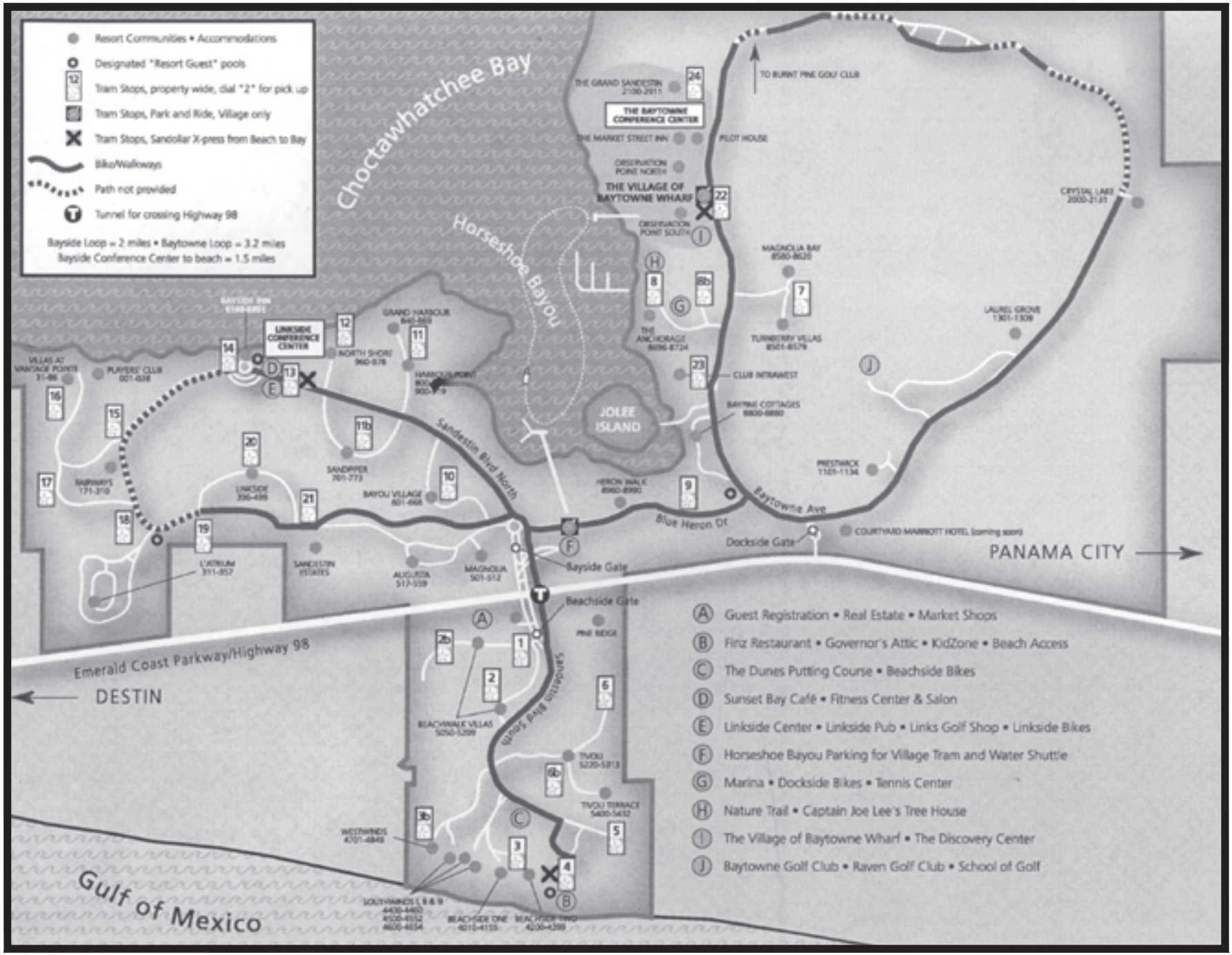
Maps



THE BAYTOWNE CONFERENCE CENTER - MAIN LEVEL



THE BAYTOWNE CONFERENCE CENTER - SECOND LEVEL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Tram Stops, Park and Ride, Village only
Tram Stops, Sandollar X-press from Beach to Bay
Bike/Walkways
Path not provided
Tunnel for crossing Highway 98
Bayside Loop = 2 miles • Baytowne Loop = 3.2 miles
Bayside Conference Center to beach = 1.5 miles
Choctawhatchee Bay
Horseshoe Bayou
JOLEE ISLAND
THE VILLAGE OF BAYTOWNE WHARF
THE BAYTOWNE CONFERENCE CENTER
LINKSIDE CONFERENCE CENTER
TO BURNT PINE GOLF CLUB
Sandestin Blvd North
Sandestin Blvd South
Baytowne Ave
Blue Heron Dr
Emerald Coast Parkway/Highway 98
DESTIN
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Dockside Gate
A Guest Registration • Real Estate • Market Shops
B Finz Restaurant • Governor's Attic • KidZone • Beach Access
C The Dunes Putting Course • Beachside Bikes
D Sunset Bay Café • Fitness Center & Salon
E Linkside Center • Linkside Pub • Links Golf Shop • Linkside Bikes
F Horseshoe Bayou Parking for Village Tram and Water Shuttle
G Marina • Dockside Bikes • Tennis Center
H Nature Trail • Captain Joe Lee's Tree House
I The Village of Baytowne Wharf • The Discovery Center
J Baytowne Golf Club • Raven Golf Club • School of Golf