Integration of light-controlled neuronal firing and fast circuit imaging

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For understanding normal and pathological circuit function, capitalizing on the full potential of recent advances in fast optical neural circuit control will depend crucially on fast, intact-circuit readout technology. First, millisecond-scale optical control will be best leveraged with simultaneous millisecond-scale optical imaging. Second, both fast circuit control and imaging should be adaptable to intact-circuit preparations from normal and diseased subjects. Here we illustrate integration of fast optical circuit control and fast circuit imaging, review recent work demonstrating utility of applying fast imaging to quantifying activity flow in disease models, and discuss integration of diverse optogenetic and chemical genetic tools that have been developed to precisely control the activity of genetically specified neural populations. Together these neuroengineering advances raise the exciting prospect of determining the role-specific cell types play in modulating neural activity flow in neuropsychiatric disease.

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Genetically targeted circuit control technologies

Several technologies recently have been developed to control the activity of genetically specified neural populations. Chemically triggered genetic silencing technologies include MISTs [1••], AlstR [2••], GluClαβ [3••], and modified GABA-A receptors [4•]; all of these allow for inducible and reversible 'knock out' of genetically specified cell populations from neural circuits after application or washout of appropriate small molecule drugs. This chemical-genetic strategy (reviewed in this issue by Tervo and Karpova) already has shown feasibility in awake, behaving animals performing amphetamine-induced rotational behavior, a classic behavioral assay of Parkinson's disease models [3••].

Complementing these elegant chemical-genetic neural control methods are recently developed optogenetic tools that allow for fast stimulation and inhibition of genetically targeted neural populations on the millisecond timescale. Indeed these optical control tools work well in intact mammalian brain tissue, as is needed for probing neuropsychiatric disease models. An optical neural interface based on channelrhodopsin-2 (ChR2) can be used to drive behavioral output in mammals *in vivo* [5••], and the halorhodopsin from *N. pharaonis* (NpHR) can knock out single spikes or long spike trains in intact mammalian brain tissue [6••]. Importantly for neuropsychiatric disease studies, these microbial opsins do not require addition of chemical cofactors in mammalian brain tissue [7], relying on endogenous retinoids as chromophores. Indeed, the causal role of specific disease-relevant cell types even deep in the brain can be probed with these high temporal-precision optogenetic methods in freely moving mammals [8••]. Other relevant technological advances include light-activated ionotropic glutamate receptors (LiGluR) [9] modified by an exogenous chemical photoswitch; vertebrate rhodopsin, which can reduce neuronal activity via second messengers [10]; transmembrane receptors that can activate neurons in response to flash uncaging of exogenous chemical ligands [11]; and technology for precise stimulation in prescribed spatial patterns [12••,13•], that will allow fast circuit mapping with optogenetic technologies.

Integrating fast optical control with fast optical imaging of activity flow

The chemical and optogenetic tools described above in principle allow characterization of the contribution of genetically defined cell types to neural activity flow through intact circuits. Complementing these control strategies, recent advances in high-speed optical imaging now allow for quantification of activity flow through intact neural circuits with high spatiotemporal resolution. Activity imaging presents clear advantages over alternative techniques in quantifying whole-circuit activity flow. For example, traditional electrode-based techniques provide relatively limited information on the spatial distribution of evoked activity or the type of active cells. Conversely, fMRI and PET can determine the spatial distribution of activity across the entire brain, albeit with coarse spatial and temporal resolution. However, circuit imaging with fast optical activity reporters could in

principle permit quantification of activity of intact neural circuits on the millisecond timescale and with near-micron spatial resolution [14,15•]; and when combined with chemical or optogenetic control tools could illuminate how specific cell types control neural activity flow.

Imaging of neural activity typically involves either calcium or voltage-sensitive reporters. Calcium dyes (e.g. Fura-2) report activity through binding to calcium and have higher signal-to-noise ratios (SNR) than voltage-sensitive dyes, but also much slower overall kinetics, affecting their use in quantifying millisecond-scale kinetics of activity propagation [16]. Voltage-sensitive dyes (e.g. di-4-ANEPPS, RH1691) report activity changes on much faster timescales (<1 ms), though correspondingly reduced SNRs have limited their application relative to calcium dyes [17,18•]. Both modalities have been applied to imaging neural activity in culture, slice, and *in vivo* preparations [16,19] and choice of reporter is heavily dependent on the experiment in question (see [16] for a review of these considerations). Emerging genetically encodeable reporters of both calcium (e.g. GCaMP2, Camgaroo) and membrane voltage (e.g. hVOS, VSFP1) will allow a further level of specificity in quantifying activity flow of genetically defined neural populations [16].

The acute slice preparation provides an excellent experimental system for determining the changes in neural activity flow that underlie behavior, with both optical and behavioral assays conducted in the same animal. While Ca^{2+}-Fura-2 imaging (excitation wavelength 340 nm) can be integrated with ChR2/NpHR optical control in acute slices [6••], fast VSDI technology also can be integrated with fast optical control (Figures 1 and 2). We have described experimental and analytical methods for quantitative high-speed voltage-sensitive dye imaging in acute hippocampal slices [20••]; moreover, circuit activity controlled by ChR2 (excited at ∼470 nm) can be imaged with the voltage-sensitive dye RH-155, which has a voltage-sensitive absorption band at ∼700 nm that is sufficiently spectrally separated from the excitation peaks of ChR2 and NpHR to permit smoothly integrated imaging of activity (Figure 1) [21,22•]. Indeed, integrated millisecond-scale optical control and imaging can be achieved, here using acute brain slices from Thy1::ChR2-YFP transgenic mice [12••,23••]. These mice express ChR2 in excitatory projection neurons, and in the hippocampus ChR2 is chiefly expressed in the CA1 subfield (Figure 2a). Imaging optically evoked neural activity flow on the millisecond-timescale in this preparation can be conducted, and data can be collected and quantified from the entire imaged circuit (Figure 2), in principle allowing

Figure 1
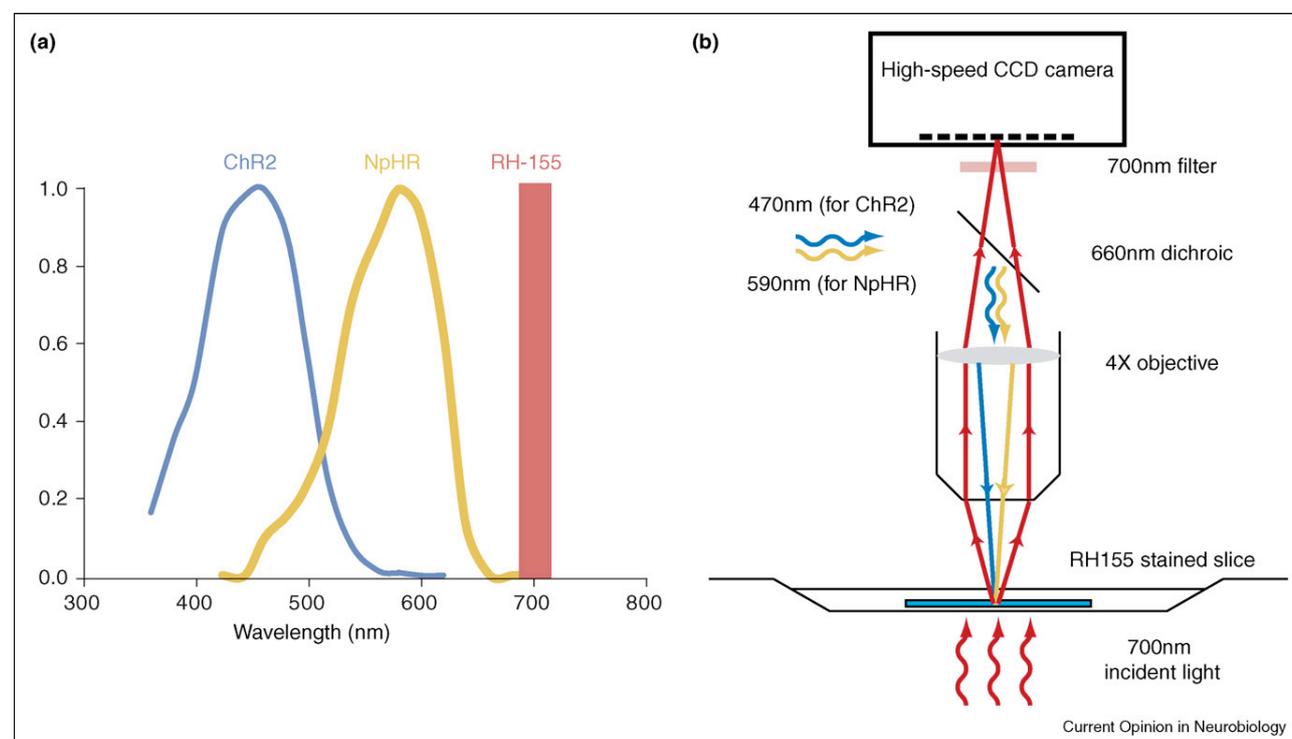
Design for integrating fast optical control and recording technologies. **(a)** Voltage-sensitive absorption band of RH-155 overlaid on action spectra of ChR2, NpHR; spectral separation suggests that high-speed all-optical control and recording of neural activity flow can be conducted with this combination of optical tools. **(b)** Schematic of microscopy apparatus for high-speed all-optical control and recording of circuit activity.

Figure 2
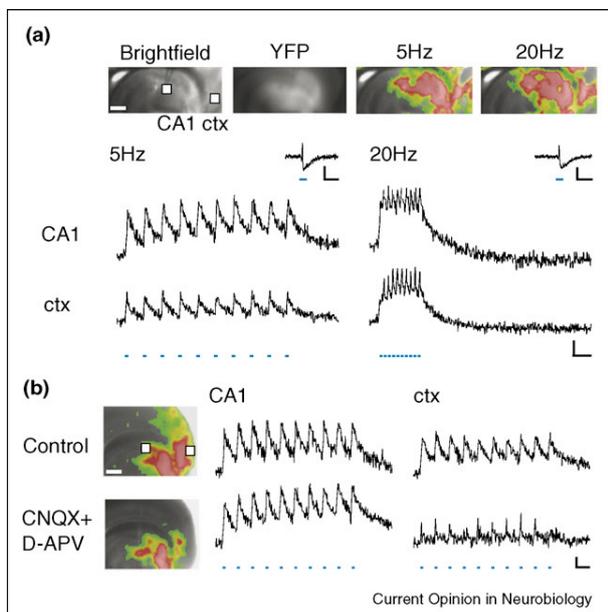
Experimental integration of high-speed all-optical circuit control and imaging. **(a)** Top (left to right) *Brightfield*: image showing a 350-μm horizontal hippocampal slice prepared from a Thy1::ChR2-YFP transgenic mouse (six weeks) stained with RH-155 (0.1 mg/mL for 3 h). White boxes indicate sources of VSDI traces in CA1 and cortex (ctx); field recording electrode can be seen in upper middle region of image. Scale bar: 350 μm. *YFP:* image showing labeling of neurons with ChR2-YFP primarily in CA1 of hippocampus; same field as brightfield image. 5 Hz, 20 Hz: sample VSDI images of neural activity flow in CA1 and cortex following ChR2-mediated optical stimulation (10 ms pulses of 470 nm light delivered by the Sutter DG-4 high-speed optical switch); same field as brightfield and YFP images. Note the lack of optical signal in non-YFP expressing CA3; the optical signal seen in adjacent cortex is synaptically induced as shown in **(b)**. Bottom: Single-pixel VSDI traces corresponding to white boxes for 5 Hz (left) and 20 Hz (right) optical stimulation. Blue dashes indicate timing of 10 ms blue light pulses used to stimulate ChR2. Scale bars: 0.1% dF/F and 200 ms. *Insets*: sample field recording traces for one optically stimulated event delivered in trains of 10 pulses at 5 Hz (left) and 20 Hz (right). Scale bars: 0.5 mV and 20 ms. **(b)** Optical recording of optically evoked neural activity flow following pharmacological modulation. Blue light directly stimulates CA1 neurons and triggers stimulation of adjacent cortex via excitatory synapses as CNQX (10 μM) and D-APV (25 μM) block cortical responses to optical stimulation determined by VSDI. Image scale bar: 350 μm. VSDI trace scale bars: 0.1% dF/F and 200 ms.

determination of the real-time impact of genetically defined cells on intact neural circuit dynamics.

Preclinical disease models

Could this kind of method also be applied to quantifying-altered circuit dynamics in neuropsychiatric disease models? For the slice preparation method to be effective, stable changes in the circuit of interest would have to be involved in the pathophysiology of the neuropsychiatric phenotype. A strategy for quantitative delineation of the

changes to neural circuitry underlying neuropsychiatric disease would entail (Figure 3): first, identification of a validated animal model and target brain regions; second, measurement of the disease-relevant behavior using a validated behavioral assay; third, preparation of acute slices from the hypothesized brain regions labeled with the activity reporter; fourth, fast imaging of the spatio-temporal dynamics of optically, chemically, or electrically evoked activity within the brain area of interest; and fifth, analysis of the imaging data to identify features of evoked activity linked to the behavioral state of the individual animal. This experimental paradigm has already been applied to determining the role of key hippocampal pathways in an animal model of depression [20**]. In this study, animal models of depression and its treatment were induced using combinations of chronic mild stress (CMS), an ethologically relevant well-validated model of depression [24], and widely used antidepressants (fluoxetine and imipramine). The modified Porsolt forced swim test (FST) served as a behavioral endpoint, given its predictive validity in assaying models of depression and antidepressant activity [25]; and high-speed voltage-sensitive dye imaging (VSDI) was employed to quantify alterations in hippocampal activity flow associated with this model of neuropsychiatric disease.

VSDI of acute hippocampal formation slices demonstrated that activity percolation was decreased in the dentate gyrus and increased in CA1 following CMS; a pattern reversed by antidepressant treatment [20**], defining a neurophysiological endophenotype of depression-relevant behavior. This metric correlated strongly with FST scores on an individual animal basis ($r^2 > 0.55$), across all treatment groups. Interestingly, amplitude of neural circuit activity at any one point was not consistently altered; instead, affective state modulation controlled the areal spread of activity, a result that would have been very difficult to identify without an imaging approach. This identification of a neurophysiological endophenotype may be combined with chemical and optogenetic control tools to allow for cell-type-based dissection. For example, hippocampal neurogenesis has been suggested to have a pivotal role in depression pathophysiology and treatment [26]. By selectively expressing ChR2 and NpHR in targeted cell populations, it may be possible to quantitatively determine the causal influence of newborn neurons or other cell types in mediating the antidepressant-induced changes in circuit dynamics that have been observed [20**].

Many other animal models of disease symptomatology could be studied with these methods. Although no animal model completely simulates all the signs, symptoms, and etiologies of any human disease (e.g. environmental and genetic influences on diabetes, suicidality in depression), neuropsychiatric disease models do simulate core symptoms (e.g. anhedonia in depression). These models are

Figure 3
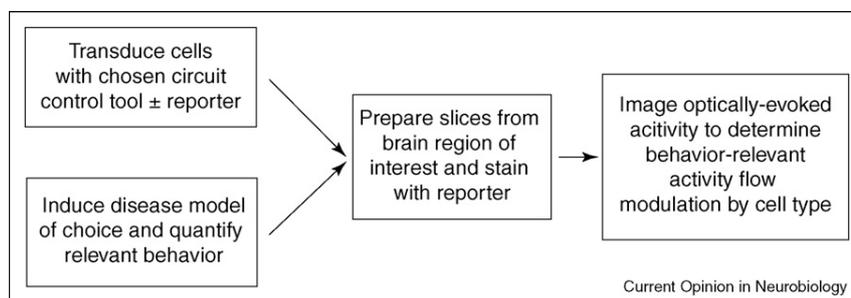
Experimental design for using high-speed all-optical control and recording to probe neuropsychiatric disease.

validated based on several sources: face validity — the animal behavior models the human symptoms well; construct validity — the model construction parallels known etiology of the disease; and predictive validity — the reaction of the model to a given treatment predicts reaction of the human disease to analogous treatment (e.g. drug) [27]. By understanding the contribution of target cell types and brain regions to the physiology underlying the behavioral phenotype of a particular animal model, we will gain understanding of the physiology that underlies the corresponding human neuropsychiatric symptoms.

Neurological diseases may also be addressed with combined high-speed optical imaging and control. For instance, while DBS to inhibit the subthalamic nucleus (STN) is effective in treating some Parkinson's disease symptoms, it is unclear which cell populations (e.g. excitatory or inhibitory neurons) mediate the relevant changes in activity within this structure [28,29]. To determine the role of these populations in mediating successful DBS, inhibitory optogenetic probes (e.g. NpHR) or chemical genetic probes (VAMP/Syb MIST) [1••] can be expressed in excitatory neurons in the STN (e.g. by driving expression with the selective promoter CaMKIIα). Furthermore, while it is known that dopamine depletion in the basal ganglia is important in Parkinson's disease, there is still controversy regarding the exact quantitative role of different dopamine-receptor expressing populations in movement-related deficits associated with the disorder [29]. By imaging basal ganglia activity flow following millisecond-scale optogenetic control of D1-expressing or D2-expressing neural populations, a more quantitative model of this circuit may result that could guide future pharmacological or electrical stimulation-based treatments.

As a final perspective, it is important to note that imaging whole-circuit activity in the awake, behaving animal is close to realization. Activity imaging of cerebellar and cortical neural networks has already been achieved *in vivo*

using both calcium and voltage-sensitive reporters [19,22•,30,31••,32••]; indeed *in vivo* activity imaging can be carried out with two-photon microscopy developed by Denk and colleagues, even in deep-brain structures with the Schnitzer endoscopic system, in freely moving animals. Dyes and genetically encodeable reporters of both membrane potential and calcium described above can be delivered with a variety of methods including electroporation, viral transduction, or using transgenic animals [16].

Conclusions

By driving expression of optogenetic probes (or their chemical counterparts) in specific cell types, circuit elements can be controlled to determine their role in behavior. With high-speed imaging, activity propagation can be quantified both in normal circuits and in animal models of neuropsychiatric disease. When these two classes of fast optical technology are combined (Figures 1 and 2), a high-speed experimental platform results that may further our quantitative understanding of neuropsychiatric diseases (Figure 3), extend our ability to design and screen for novel treatments, and deepen our understanding of neural circuit dynamics arising from properties of individual cellular components.

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