SYNTHETIC BIOLOGY

Neuron-targeted electrical modulation

Engineering neurons to make conductive polymers enables cell type–specific behaviors

By **Kevin J. Otto** *and* **Christine E. Schmidt**

Conductive polymers have been widely studied and used for biomedical applications—including as biosensors, neural prostheses, and bioactuators—and for drug delivery and tissue engineering (*1*). Conductive polymers are organic chains of alternating single and double bonds, which endow the polymers with metal-like semiconductive properties. Exogenous application of electrical stimulation to these polymers can promote cellular activities such as proliferation, adhesion, migration, differentiation, and protein secretion. Because many cells and tissues, particularly neurons, are responsive to electrical fields, conductive polymers are attractive for biological and medical applications. On page 1372 of this issue, Liu *et al.* (*2*) report a genetically targeted approach to assemble conductive polymers in neurons. This in turn remodels membrane electrical properties and enables cell type–specific cellular and behavioral modulation, such as control of neuronal firing, as demonstrated in cultures of rat hippocampal neurons, mouse brain slices, human cortical spheroids, and in living *Caenorhabditis elegans* worms.

Commonly studied conductive polymers include poly(3,4-ethylenedioxythiophene (PEDOT), polyaniline (PANI), and polypyrrole. Conductive polymers have traditionally been synthesized as standalone biomaterials that are used in cultured cells or for implantation in vivo (*1*). Integrating conductive polymers into tissues is critical for localized delivery of electric fields. There have been attempts to polymerize electroactive materials directly into tissues to provide a more seamless interface between the conductive substrate and cells. The first reports of successful in vivo polymerization of PEDOT in the brains of rats demonstrated that it did not negatively affect behavior (*3*, *4*). Although these studies show some local specificity, the polymers are ubiquitous throughout the neural space and thus do not provide cell-type specificity. Alternative injectable neural interfaces are being developed (*5*); however, these are also not targeted to specific cells.

J. Crayton Pruitt Family Department of Biomedical Engineering, Herbert Wertheim College of Engineering, University of Florida, Gainesville, FL, USA. Email: kevin.otto@bme.ufl.edu; schmidt@bme.ufl.edu

Liu *et al.* demonstrate in vivo polymerization of PANI conductive polymers that are manufactured according to specific cell types and modify the electrical properties of the cell membrane. They genetically engineered neurons, using adeno-associated virus (AAV) vectors, to express peroxidase enzyme on the outside of cell membranes. Peroxidases catalyze polymerization of aniline monomer and dimer precursors when infused into cells or tissues. This approach could provide more cell-specific targeting of electric fields (see the figure).

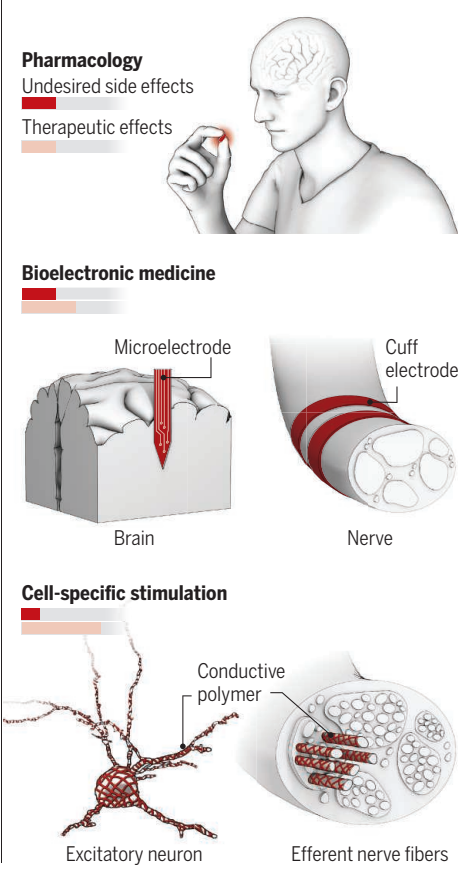
Bioelectronic medicine, which aims to electrically modulate neural elements for target- and organ-specific effects, promises improved specificity and efficacy over traditional pharmaceutical medicine (*6*). The approach has been heralded as the frontier of medicine (*7*), and a roadmap has guided numerous funding opportunities (*8*). Intended therapeutic benefits of bioelectronic medicines are often contingent on activating a predominant effect (such as excitatory or inhibitory) and/or conveying unidirectional information (efferent or afferent) in nerves. Side effects arise from the activation of off-target cells or tissues and can result in undesired outcomes, such as stimulation of the cough reflex in vagal nerve stimulation and seizure episodes in deep brain stimulation. Thus, targeting a subpopulation of neurons is attractive. This is challenging in current systems because of spatial proximity of different neural elements, such as excitatory neurons located near inhibitory elements or efferent axons close to afferent axons. Genetically targeting cell-specific expression of conductive polymers could overcome this challenge.

Autonomic neural modulation (such as vagus nerve stimulation) is a subset of bioelectronic medicine in which cell-type specificity is particularly desirable. Autonomic neural modulation involves electrical stimulation of the autonomic nervous system—for example, to decrease sympathetic activity ("fight or flight") and increase parasympathetic activity ("calm and composed") as a therapeutic strategy for the treatment of diseases such as epilepsy and depression. The first in-human evidence of autonomic neural modulation—vagal stimulation for epilepsy (*9*)—paved the path for other clinical uses. Yet, there is room for clinical improvement. Autonomic nerves often contain motor and sensory axons as well as sympathetic and parasympathetic information. Parasympathetic motor axons can drive hormonal release in off-target organs; sensory axons could miscommunicate organ state information to the brain. As greater understanding of the innervation of end organs is discovered, it is likely that cell type–specific activation will result in desirable organ effects without the undesirable side effects.

Another approach of bioelectronic medicine uses neuromodulation for prostheses. Stimulation of peripheral nerves has been enabled by advances in materials,

Modes of neural modulation

Altering nerve activity is an important therapeutic strategy. Pharmacological modulation results in systemic and unwanted side effects. Bioelectronic approaches increase targeting of defective neural pathways. However, ensuring that specific cells are modulated could allow behavioral modification with minimal side effects.



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electronics, and devices (*10*, *11*) and has emerged as a successful approach for amputees by conveying sensory information from limb prostheses directly to the users' nervous system (*12*–*14*). Cell-type specific modulation could improve these applications; for example, sensory fibers related to a prosthesis's physical interaction with the environment (somatosensation and proprioception) need to be excited while avoiding undesirable side effects if motor axons were simultaneously excited.

Integrating cell type–specific conducting polymers into tissues could also enhance the regeneration of tissues after injury or disease. The body contains electric fields that play roles during embryogenesis and wound healing by guiding cell migration and eliciting protein secretion. Exogenous application of electrical fields affects similar processes. Conductive polymers have been explored as electroactive substrates for regenerative medicine to stimulate tissue repair (*15*).

There remain considerable hurdles to the translation of cell type–specific neural modulation approaches. Liu *et al.* use AAV transfection, which is still being developed for applications in humans because of the regulatory challenges of gene therapy. It also remains to be elucidated whether this approach will be viable in higher-order species, especially vertebrates. Last, it is unclear whether the biologically assembled conductive polymers are transmembrane or extracellular, which could affect cellular modulation. ■

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ACKNOWLEDGMENTS

The authors are supported by National Institutes of Health, National Science Foundation, and Defense Advanced Research Projects Agency. We thank M. Urdaneta, B. Ibarra, E. W. Dirr, and J. Murbach for helpful discussions.

10.1126/science.abb0216

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EVOLUTIONARY BIOLOGY

Shallow ponds prompt fitness-favorable species interbreeding

Dry conditions liberate female toads to drive sexual selection of male mates

By **Marlene Zuk**

If there is one thing that biologists used to agree on, it was that mating between two animals of different species yields unfavorable results. (Plants have a dodgy reputation for insouciant polyploidy and hence have often been ignored.) Basic biology classes teach that different species usually cannot interbreed successfully and that rarely produced crossbred offspring (hybrids) are often infertile or of lower fitness. Now, on page 1377 of this issue, Chen and Pfennig (*1*) contradict conventional wisdom about the disadvantages of hybridization and provide a connection between species diversification, sexual selection, and, ultimately, the context dependence of behavioral evolution.

In general, scientists expect mate selection to favor precise species-recognition mechanisms, and indeed, many organisms display such finely tuned abilities (*2*, *3*). Architects of the Modern Evolutionary Synthesis (which merges Mendelian genetics with Darwinian evolution) were so convinced of the importance of species recognition that they virtually dismissed sexual selection—that is, selection due to reproductive competition—as a force in evolution. Indeed, in 1942, evolutionary biologist Ernst Mayr proclaimed (*4*), "it is now recognized that many phenomena previously thought to promote intraspecific sexual selection are actually specific recognition marks."

Hybrids now have a more respectable reputation. Spurred partly by advancements made in genomics over the past few decades, biologists have come to acknowledge more and more the importance of hybrids in generating biodiversity at a macroevolutionary scale (*5*). For example, whole-genome analysis facilitated the assessment of introgression (gene flow from one species into the gene pool of another) and revealed a history of hybridization in numerous taxa (including humans) without having to rely on observable morphological differences between groups (*5*). Scientists also now understand that sexual selection in the form of mate choice and mate competition account for differences in male and female behavior, appearance, and life history that go far beyond recognition of conspecifics (*6*).

Chen and Pfennig studied hybridization between two species of spadefoot toads that live in ephemeral ponds of varying

Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, MN, USA. Email: mzuk@umn.edu

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Science **367** (6484), 1303-1304.
DOI: 10.1126/science.abb0216