Title

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Journal

ACS Nano, 18(44)

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Publication Date

2024-11-05

DOI

10.1021/acsnano.4c07256

Peer reviewed

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PERSPECTIVE

Integrated Bioelectronic and Optogenetic Methods to Study Brain–Body Circuits

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Cite This: *ACS Nano* 2024, 18, 30117–30122

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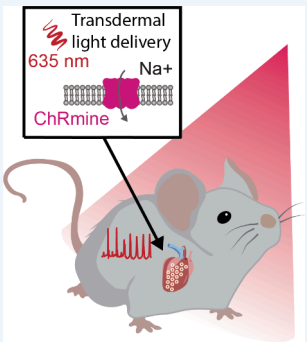
ABSTRACT: **The peripheral nervous system, consisting of somatic sensory circuits and autonomic effector circuits, enables communication between the body's organs and the brain. Dysregulation in these circuits is implicated in an array of disorders and represents a potential target for neuromodulation therapies. In this Perspective, we discuss recent advances in the neurobiological understanding of these brain–body pathways and the expansion of neurotechnologies beyond the brain to the viscera. We focus primarily on the development of integrated technologies that leverage bioelectronic devices with optogenetic tools. We highlight the discovery and application of ultrapotent and red-shifted channelrhodopsins for minimally invasive optogenetics and as tools to study brain–body circuits. These innovations enable studies of freely behaving animals and have enhanced our understanding of the role physiological signals play in brain states and behavior.**



KEYWORDS: *optogenetics, interoception, bioelectronics, neuromodulation, neuroscience*

INTRODUCTION

The central and peripheral nervous systems work in coordination to monitor and respond to the changing physiological states of our internal organs.[1−3] For example, sensory neurons in the body use chemical and mechanical sensory signals to relay information about visceral organs (e.g., breathing, heart rate, bladder extension) to the brain.[1−3] Interoception, which is defined as the representation of the body's internal state, enables organisms to sense and regulate their homeostasis, which is essential for survival.[1−3] Dysregulation of interoceptive signaling is linked to a wide range of disorders, from psychiatric disorders like anxiety and depression to physiological disorders like irritable bowel syndrome and cardiovascular diseases.[3−5]

Determining the circuit basis of interoceptive processing will not only enhance our understanding of physiology and cognition but also inform new treatment approaches for bioelectronic medicines.[5,6] Bioelectronic medicines aim to treat chronic diseases by harnessing neural activity of specific circuits to exert a therapeutic effect.[5] However, recording and stimulating cells within dynamically moving tissue in the body are significant technical challenges.

By engineering less-invasive, flexible systems to record and stimulate cells, tissues, and organs across the brain and body, conformable bioelectronic devices could be used to treat diseases ranging from autoimmune diseases to mental health disorders through direct control of the body's natural regulatory signals.[5−8] This Perspective discusses recent progress in neuroscience, bioengineering, and bioelectronics, which has advanced our understanding of interoception and bioelectronic medicines. We highlight integrated technologies that utilize both bioelectronic devices and genetically encoded proteins to enable functional studies of cellular physiology within freely moving animals. Our goal is to demonstrate how the combination of miniaturized bioelectronic design with improved channelrhodopsins (light-sensitive microbial ion channels and pumps) have enabled a suite of tools to accelerate our neurobiological understanding of interoception, with a strong focus on highlighting the potential of recently discovered ultrasensitive and red-light-responsive opsins (Figure 1).

UNDERSTANDING THE NEUROBIOLOGY OF INTEROCEPTION

The body's interoceptive nervous system collects sensory data from nearly every organ system and engages both autonomic and central effector pathways to maintain organ health (Figure 1a).[1−3] While sensory signals are relayed to the brain through many pathways, the vagus nerve is a major conduit of visceral

Received: June 2, 2024
Revised: October 8, 2024
Accepted: October 11, 2024
Published: October 23, 2024

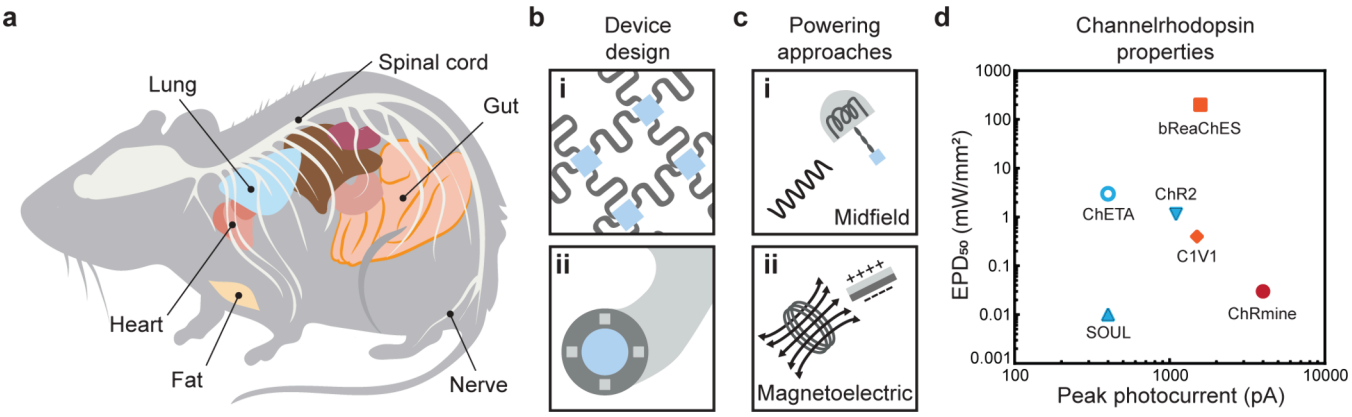


Figure 1. Design considerations for optogenetic targeting of peripheral circuits and organs. (a) Examples of circuits and organs outside of the brain that have been targeted for optogenetic control. (b) Example design of bioelectronic devices for studies of brain−body pathways: (i) serpentine geometry of lithographically defined interconnects enables stretchable electronics, including deformable μLED arrays and antennas[6] and (ii) thermal drawing of multifunctional neural probes for optogenetics, electrophysiology, and microfluidic delivery.[23] (c) Examples of wireless powering approaches for tetherless stimulation and/or recording in freely moving animals, including (i) inductive powering by midfield energy transfer of a μLED in a resonant cavity[43] and (ii) magnetoelectric implants for electrical stimulation.[26] (d) Plot of effective power density for 50% activation (EPD50 (mW/mm^2)), a measure of channelrhodopsin light sensitivity, and peak photocurrent (pA) for cationic channelrhodopsins color coded by response to blue (~488 nm), orange (~594 nm), or red (~635 nm) wavelengths of light. Ultrasensitive opsins like ChRmine exhibit both low EPD$_{50}$ and large photocurrents. Values are estimated from refs 34, 36, 44, and 45.

information. The vagus nerve is composed of both afferent sensory and efferent parasympathetic pathways.[9] Afferent vagal neurons in the nodose and jugular ganglia convey visceral information—including blood pressure,[10] inflammatory state,[11] and gastrointestinal stretch and nutrients[12]—primarily to the nucleus of the solitary tract in the brain stem. Input sensory information is relayed to higher brain centers that influence emotional and behavioral states as well as efferent cholinergic pathways that can control organ function. While extensive reviews exist on interoceptive pathways,[1−3] this section will highlight some recent findings in order to discuss how bioelectronic tools can be applied to advance our understanding of brain−body circuits and their effects on animal physiology and behavior.

Neural Control of Autonomic Bodily Functions. Breathing, both involuntary and volitional, is precisely controlled by specific neural circuits. The pre-Bötzinger complex, the brain's breathing control center, is composed of a heterogeneous population of neurons, whose roles are only recently being identified through molecular, anatomical, and optogenetic methods. For example, somatostatin neurons in the pre-Bötzinger complex can cause unusual or stronger breaths depending on brief or continuous optogenetic activation.[13] Sensory feedback from the lungs by distinct vagal sensory neurons can also halt breathing (*P2ry1*$^+$ neurons) or induce rapid, shallow breaths (*Npy2r*$^+$ neurons).[14]

Optogenetic methods have also helped to map neural circuit control of cardiovascular function. The baroreflex consists of sensory feedback loops that adjust the heart rate to maintain blood pressure. The sensory inputs to the brain were found to be transmitted by *Piezo1/2* expressing mechanosensitive neurons in the vagal nodose ganglion to the nucleus of the solitary tract.[10] Activation of *Piezo2*$^+$ neurons strongly reduced blood pressure and heart rate through a negative feedback loop. Further molecular dissection of the nodose ganglion revealed a *Npy2r*$^+$ population that relays ventricular chemical information to the area postrema in the brainstem, triggering the Bezold−Jarish reflex—a response that leads to syncope.[15] These findings show how vagal sensory signals are relayed to different brainstem nuclei for bidirectional modulation of breathing, heart rate, and blood pressure.

Numerous cell types have also been identified to convey gastrointestinal information—such as ingested nutrients and stomach stretch—to the brain. For example, vagal *GLP1R*$^+$ neurons are mechanosensitive and optogenetic activation can increase gastric pressure.[12] By contrast, vagal *GPR65*$^+$ neurons respond to intestinal nutrients, and photoactivation can block gastric contractions. Additional sensory pathways conveyed through dorsal root ganglia have also been studied. For example, while mechanosensitive *Piezo2*$^+$ neurons from both vagal and dorsal root ganglia pathways innervate the gastrointestinal tract, only somatosensory neurons in dorsal root ganglia are required for gastrointestinal transit.[16] Together, these anatomical and functional studies have revealed several "labeled lines" pathways that have distinct functions to both monitor and control particular organ systems. While these studies involve optogenetic stimulation of peripheral nerves and organs, invasive procedures were required in order to expose and deliver light to target cell populations deep in the body. As a result, these experiments were conducted primarily in anesthetized animals. Although it remains poorly understood how the brain coordinates signals from these interoceptive pathways with exteroceptive inputs (external sensory information), these sensory pathways are known to engage distinct brain regions that contribute to adaptive behavior.[17,18] In the next section, we discuss emerging bioelectronic devices and optogenetic tools that can enable independent recording and control of cellular activity throughout the brain and body during active behavior. These technologies can be applied to elucidate how changes in bodily signals can affect internal states and behaviors to deepen our understanding of the neurobiology of interoception.

INTEGRATED BIOELECTRONIC TECHNOLOGIES FOR BRAIN−BODY NEUROSCIENCE

Advances in materials science and microfabrication techniques have enabled the development of miniaturized and flexible bioelectronic devices that integrate seamlessly into tissue.[6−8]

https://doi.org/10.1021/acsnano.4c07256
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Unlike traditional rigid neuromodulation and recording devices, these soft bioelectronics can record and stimulate electrical and chemical signals while conforming to the biomechanical properties of the tissue. When combined with molecular tools like optogenetics, precise cell types can be studied *in vivo* while minimizing tissue damage. These technologies have been detailed in various reviews,[6−8] and some are highlighted here to illustrate their utility in studying peripheral signals in animal behavior (Figure 1b,c).

Miniaturized Microelectronic Devices. Applications of integrated circuit and microelectromechanical systems microfabrication strategies have enabled the creation of soft, multifunctional bioelectronic implants.[6,7] For example, devices made from patterned serpentine metal interconnects encased in polyimide and silicone elastomers are capable of powering μLEDs and wirelessly recording physiological signals in untethered mice. The miniaturization and flexible nature of these devices have enabled the study of the spinal cord and other dynamically moving systems and have highlighted the important role nociceptive sensory neurons play in the activation of inflammation[19] and the function of a subclass of spinal cord interneurons in restoring walking after paralysis.[20] These advanced optoelectronic systems have enabled chronic studies of targeted neural pathways across the brain and body. However, depending on the choice of soft dielectric materials, many of these devices face challenges in long-term and reliable operation,[21] which can be improved with new material selection.[22]

Fiber-Based Devices. To sidestep the spatial limitations of flat, layer-by-layer fabrication, another method to synthesize devices utilizes fiber-based form factors. Using thermal drawing techniques, waveguides for optical stimulation and recording, electrodes for electrical recording, and channels for microfluidic delivery were integrated into multifunctional fibers with both small footprints and isotropic deformability.[23] Similarly, a hybrid approach to turn two-dimensional lithographically defined electronics into a one-dimensional fiber by physically rolling the device enabled recording of neural activity and gut motility by incorporating pressure sensors and active electrodes.[24] Hydrogel materials with high refractive index, stretchability, and fatigue resistance have also been molded into peripheral nerve cuffs for optogenetic stimulation of the sciatic nerve without impeding movement in mice.[25] Continued enhancements to increase electrode density, simplify the integration of multifeature backend connections, and incorporate novel material properties will broaden the utility of one-dimensional neural probes in studying brain−body physiology and in other applications that require devices to operate within dynamically moving tissue.

Wireless Communication and Powering. The integration of wireless power transfer and data streaming has enhanced animal behavioral studies by allowing observations in more naturalistic environments.[26,27] These systems use various wireless power solutions, including coupled resonances and uncoupled power transfer. For instance, one type of coupled wireless device features a miniaturized near field communication antenna and a temporary capacitive power bank, enabling on-demand, closed-loop optogenetic stimulation in freely behaving animals.[27] Magnetoelectric composite films that convert magnetic fields into electrical fields can be used to power electrical stimulation devices.[28] Acoustic powering is also an alternative wireless energy transfer modality. For example, a millimeter-scale implant with a piezoceramic transducer or soft poly(vinylidene fluoride) ferroelectric thin films can be mounted on sciatic nerves to perform repeatable electrical stimulation cycles, expanding the methods available for wireless neuromodulation.[29,30]

Flexible bioelectronics represent a significant advancement in studying brain and body pathways in animals under natural conditions. However, maintaining intimate contact with target cells in dynamically moving tissue remains a technical barrier, particularly in recording electrophysiological and chemical signals from populations embedded deep within visceral organs. Enhancing wireless communication bandwidths and onboard computational power for data processing represent other areas for improvement. These challenges highlight exciting interdisciplinary pathways for future bioelectronic research and development. Nevertheless, these technologies have matured over the past decade and are increasingly being used by researchers to dissect complex interactions between the brain and body in freely moving animals.

INTEGRATED BIOELECTRONIC AND OPTOGENETIC METHODS TO MAP BRAIN−BODY CIRCUITS

To study the effects of body-to-brain signals on influencing behavioral states, methods integrating flexible devices with channelrhodopsins restricted to specific cell types in transgenic animals have been developed. For example, optogenetic photoactivation of *Calca*$^+$ vagal fibers in the stomach with a wireless, implantable μLED revealed the negative valence associated with these sensory afferents to suppress appetite.[31] Multifunctional fibers with wireless optogenetic stimulation of *Phox2b*$^+$ vagal sensory afferents in the gut initiated appetitive behaviors such as place preference,[32] further confirming the role gut signals play in the rapid engagement of reward pathways.[18] These interdisciplinary studies highlight the significant role physiological signals have on behavior and may potentiate future studies to control bodily states to influence other cognitive functions, such as attention, learning, and emotional processing.[3,4]

Ultrasensitive Opsins for Brain−Body Optogenetics. Potent optogenetic tools can also minimize tissue damage and inspire new device design to study brain−body pathways (Figure 1d). While most of the interoceptive studies described in this Perspective have relied on Channelrhodopsin 2—one of the earliest opsins used for optical control of neural activity that responds to blue light[33]—opsins with higher photosensitivity, larger photocurrents, and a red-shifted absorption spectrum are better suited for minimally invasive optogenetic approaches. For example, we recently discovered that the ultrapotent and red-shifted opsin ChRmine enabled photoactivation of genetically defined circuits using a distal light source placed up to ∼7 mm away from the target population.[34] ChRmine exhibits several ideal photophysical properties, including absorption of red light ($\lambda \approx 600-650$ nm), large photocurrents (∼4000 pA), and, with structure-guided engineering, can be mutated to have high-speed kinetics or greater red-shifted action spectra.[35,36] When compared with other excitatory opsins, the biophysical properties of ChRmine allow cells to be photoactivated with some of the lowest effective power density for 50% activation (EPD50) (Figure 1d). By coupling evolved viral serotypes and cell-type-specific gene regulatory elements, targeted expression of ChRmine to serotonergic neurons in the raphe nucleus enabled brain-noninvasive optogenetic control of prosocial behavior in

https://doi.org/10.1021/acsnano.4c07256
ACS Nano 2024, 18, 30117−30122

mice—which remains the least invasive cell-type-specific control of a deep brain structure reported to date.[34]

Red-light-responsive inhibitory channels may also be suitable for potent optogenetic inhibition of neural activity with minimal tissue damage. A red light-responsive chloride pump variant called Jaws enabled suppression of neural activity with transcranial light delivery up to 1−3 mm away from the light source.[37] The recently discovered kalium channelrhodopsin KCR, which has even greater photocurrents, avoids the unintended axonal neural excitation effects of proton or chloride pumps while affording red light photoinhibition.[38,39] However, the suitability of KCRs for transcranial photoinhibition is only starting to be investigated.[40]

ChRmine as a Tool for Cardiac Interoception. By taking advantage of the exquisite properties of ChRmine, we have recently demonstrated that specific photoactivated ventricular rhythms can accentuate anxiety-like behavior in mice (Figure 2).[41] ChRmine was targeted to cardiomyocytes using a mouse troponin promoter and systemic viral delivery of AAV9, which has natural tropism in the heart (Figure 2a,b). Unlike prior demonstrations of optogenetic cardiac pacing, which required exposure of the heart or suturing of a μLED device on the heart's epicardium,[42] we found that a μLED placed on skin overlying the heart was sufficient to photoactivate ventricular contractions (Figure 2c,d). By introducing brief increases in heart rhythms, we found that mice exhibited increased anxiety-like behavior in several behavioral assays, including decreased open arm exploration in an elevated maze assay (Figure 2e).

We further performed neural activity recording and optogenetic inhibition in the posterior insula, a brain region known for both interoceptive signal processing and regulation of emotions, during optogenetic pacing of the heart.[4] We found that optical pacing of the heart increased neural activity in the insula and that inhibition of its activity was sufficient to reverse the cardiogenic anxiety-like effects. Importantly, our set of studies may be applied to other organ systems, including the gut, bladder, and skeletal muscle, to determine how different visceral signals can influence emotions and other cognitive behavior.

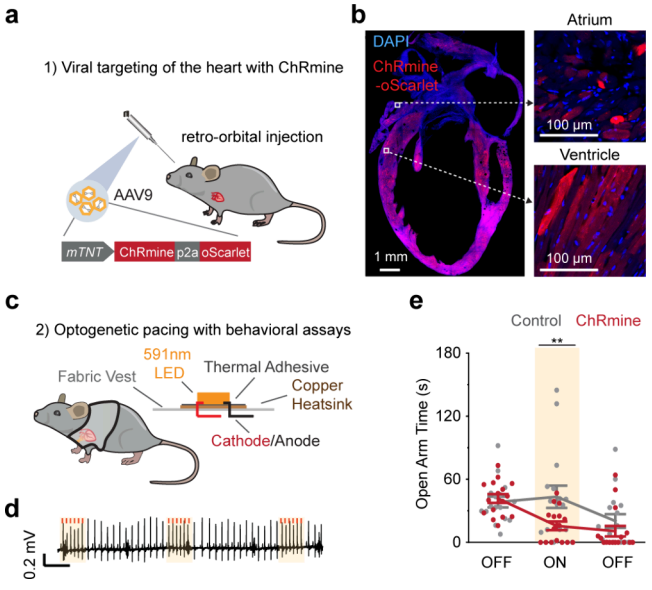

Figure 2. Cardiac optogenetics accentuates an anxiety-like state in mice. (a) Schematic for noninvasive targeting of ChRmine to heart cardiomyocytes via retro-orbital injection of AAV9-mTNT:ChRmine-p2A-oScarlet. (b) Confocal image of a mouse's heart transfected with AAV9-mTNT:ChRmine-p2A-oScarlet. (c) Schematic of a custom wearable μLED vest for optical pacing of the heart without invasive device implantation used for behavioral assays. (d) Representative electrocardiogram trace showing noninvasive optical pacing at 15 Hz for 500 ms every 2 s. (d) Example mouse path trace of a control (gray) and optically paced (red) mouse during an elevated plus maze assay (EPM). Note, mice with anxiety-like behavior will spend less time in open arms. (e) Quantification of the time mice spent in the open arm during an elevated plus maze behavioral assay. Optically paced mice (red) showed increased anxiety-like phenotypes by showing decreased open arm exploration time upon stimulation (ON) compared to control mice (gray). Adapted with permission under a Creative Commons CC BY License from ref 41. Copyright 2023 Springer Nature.

CONCLUSION AND OUTLOOK

The integration of optogenetics with bioelectronic devices reveals how visceral signals influence behaviors in freely moving animals. Rapid maturation of multifunctional bioelectronic technologies for simultaneous recording and stimulation of organ physiology also enables scalable and user-friendly applications in large-cohort animal studies. The continued discovery and evolution of channelrhodopsins—with different action spectra, improved light sensitivity, and selective ion conductance—will further guide the design of bioelectronic devices with longer working distances and multiplexed capabilities to target different populations of cells or bidirectional excitation and inhibition of cell activity. As new interoceptive pathways are continuously discovered that influence immune, metabolic, hormonal, and other physiological states, the application of these technologies may reveal new therapeutic strategies for bioelectronic medicines.

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https://doi.org/10.1021/acsnano.4c07256
ACS Nano 2024, 18, 30117−30122

Author Contributions

R.C. conceptualized and wrote the Perspective with contributions from all authors. All authors have given approval to the final version of the manuscript. Q.R.Z. and S.X.W. contributed equally.

Funding

This work was supported by a NIH 4R00NS119784 grant, the UCSF Program for Breakthrough Biomedical Research (funded in part by the Sandler Foundation), the Klingenstein-Simons Fellowship in Neuroscience, the David and Lucile Packard Foundation, and the Shurl and Kay Curci Foundation.

Notes

The authors declare no competing financial interest.

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https://doi.org/10.1021/acsnano.4c07256
ACS Nano 2024, 18, 30117−30122