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What Is the State-of-the-Art Toolbox for How Circuits Mediate Behavior?

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Abstract

A critical challenge in the field of neuroscience as well as research into the neurobiological basis of behavior has been to establish links between the cellular and biochemical processes within the brain and nervous system that occur during the mediation of behavioral events. Developments over the last 10–15 years have provided several new means to accelerate, advance, and dissect the specific mechanisms for brain function. Developments across two realms of neuroscience and engineering have afforded researchers, clinicians, and biologists advanced abilities to facilitate the dissection, observation, control, and perturbation of neural systems within intact, behaving animals. These advances include electrical, optical, pharmacological, and specialized hardware which allow for closed-loop interfaces to monitor and manipulate neural function. This chapter explores how these recent developments have become integrated into our neurobiological tool chest. It describes current advanced approaches, and the limitations of each, and explores future pathways toward even better technologies needed to dissect the molecular, cellular, and circuit basis of behavior.

Introduction

The mammalian nervous system evolved over millions of years and contains a heterogenous composition of networks and cells, which send messages to one another to communicate information. This information is communicated by a variety of signals: electrical, chemical, and anatomical (i.e., architectural). These signals converge, amplify, or inhibit the flow of information to influence ultimately behavior in the organism. In many cases, specific central and peripheral nervous system diseases are caused by dysfunctions in these brain processes at molecular, cellular, and circuit-based levels. Some of these neuropsychiatric disorders include, but are not limited to, addiction, pain, and

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emotional disorders, such as anxiety, depression, or schizophrenia. For many generations, neuroscientists have sought to understand the neurobiological basis of behavior with the specific intent of resolving and better treating these types of disorders. Most current treatments rely on pharmacological or behavioral modifications from trained clinical practitioners. In many cases, treatments do not exist or individuals are resilient to any form of intervention.

A better understanding of the mechanistic underpinnings of behavioral processes is thus needed to develop new methods to engage and hopefully adjust the brain and spinal cord's function toward a more typical homeostatic state. The complexity of the brain and spinal cord's inner workings has limited the development of new therapies, but the technologies developed over the last 10–15 years offer promise in terms of uncovering these details, which in turn could identify new targets or methods.

Furthermore, there is a growing interest in the neurobiological basis of behavior as it relates to developing artificial intelligence, brain–machine interfaces, and alternative methods for generating complex processor-based systems and/or methods for adjusting human behavior in pathological states. New probes are being developed which allow for the delivery and recording of neuronal signatures in behaving animals. These technologies will provide enhanced functionality for researchers, but they also open up new avenues in clinical realms.

For neuroscience research to examine naturalistic and pathological behavioral states, it must address a key challenge: the full integration of minimally invasive, biological sensors, actuators, and pharmacological interventions. This technology is advancing at a rapid, exciting pace, and many new approaches have started to become available. Here, I focus on these advances (i.e., where we are in the field as well as the future pipeline of neurotechnologies) and discuss limits, ideas, and concepts for both biological and hardware-based neurotechnology.

Observing, Recording, and Manipulation of Neuronal Function *in Vivo*

The architecture of the central and peripheral nervous system is composed of a series of integrated modules that span the molecular, cellular, circuit, and system's levels. At the basic molecular level, neurons and glia in the brain express a selected series of proteins, which include ion channels (including iontropic receptors), peptides, pumps, and G-protein-coupled receptors (GPCRs). In the context of cellular homogeneity and heterogeneity, the brain is composed of millions of neurons, each of which tends to be enriched with various receptors, transmitters, or proteins. Over the last several decades, neuroscientists have generally worked to classify these types of cells and have begun to understand their diversity in response, release properties, and connectivity. The diversity

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of cell types in the brain and spinal cord, along with the various scales at which the nervous system operates, have made the design and implementation of various neurotechnologies and interfaces challenging. Here I will describe several technologies currently employed to observe and record neuronal activity in awake or head-fixed animals: how the technology is currently being used, as well as future iterations of related methodologies. Devices that interface with the nervous system must have the ability to decipher signals at multiple scales as well as sufficiently interface with biological tissue in a flexible, noninvasive manner.

Electrophysiological Methods

For nearly a century, the electrical properties of the brain and nervous system have been empirically measured and recorded. Investigators have used electrical probes to stimulate and mimic neuronal activity patterns in investigations of neural circuit functions during behavior. For many years, researchers were severely limited by the "channel count" of the electrophysiological probes for *in vivo* measures. This meant that we could not sufficiently sample large populations of neurons in behaving animals. More recently advanced materials engineering and manufacturing approaches have, however, brought forth new technologies, including large Utah arrays and Neuropixels (Jun et al. 2017). This latter technique integrates 960 recording locations within a 70×20 μm^2 area and allows for single unit action potentials to be isolated at very high resolution across multiple brain regions. In addition, it reportedly allows for stable tracking of single neuron activity over multiple days, thus allowing investigators to measure dynamic changes within neural circuits that change over time. This method permits significantly advanced throughput in neuronal recordings and provides the ability, given their small size, for simultaneous recordings of activity to be made over a wide range of brain regions.

Recent advances have also worked toward developing neural probes that are softer and more flexible in their ability to interface with the brain. Most neural probes are composed of hard surfaces, including metal, glass, and silicon semiconductors; these materials can activate the brain's immune response and lead to severe lesion, inflammation, and cell death. Recently, an injectable platform composed of mesh-based electronics, including 16 channels of platinum recording and stimulating electrodes, has become available (Zhou et al. 2017). This device was designed in a flexible, open manner to facilitate better integration with surrounding brain tissue and has the ability to isolate local field potentials as well as single units. It can also be operated chronically in the rodent brain for many months. This method provides additional surface and material structure that can interact with the brain in a noninvasive manner, while providing high-resolution actuation and observation data sets.

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Current Limitations

Limitations of these new technological recording methods include the following:

- Neuropixel devices collect a large volume of data. Thus, due to the channel count and multiple-site recordings, data processing can be slow, time-consuming, and difficult.
- Integrating multiple streams of data over time while assuring for quality and meaningful data remains a significant challenge.
- Large teams of computational neuroscientists are required to ensure that the data is sufficiently and carefully managed, processed, and fit into a larger picture.
- These devices also do not include the means for optical coupling and isolating of selected optical approaches, which might help to identify the neuronal type being recorded.

Future iterations of the technique will need to employ streamlined data processing pipelines (now underway, as I understand it) as well as full integration with optical methods for applications (discussed below). In the case of flexible probes and the architecture therein, there are some limits to the technique in terms of channel count and integration with related optical approaches. Future iterations, however, may be able to incorporate some modifications to include these features.

Neuronal Actuators and Related Devices

Electrical recording offers precise, high-resolution information related to specific activity of regions, networks, and single cells in the behaving animal. However, one key limitation of the method is that investigators cannot simply rely on a unit's electrical signature waveform to classify the neuron as a certain type. Although some investigators do use this method, it is not considered definitive in most subdisciplines of the field. For over a decade, neuroscience has employed the use of genetics to specify particular cells and to manipulate these cells by incorporating light-sensitive proteins in the continually developing and evolving field of optogenetics (Lerner et al. 2016). The "workhorse" in the field has been channelrhodopsin-2 (ChR2), which acts to depolarize neurons upon activation. This tool is expressed in a genetically defined manner to allow for selected excitation of a given neuronal population, alongside experiments related to spatiotemporal sufficiency of a given neuronal subgroup in a particular behavioral output. In the field of neural circuits and behavior, along with *in vivo* neurophysiology, ChR2 has been used in numerous experiments to dissect the specific properties of various behaviors, including reward, decision making, addiction, pain, anxiety, depression, social interaction, feeding, and other homeostatic processes. In recent years, variations in ChR2 have been

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generated to provide powerful new abilities that further extend the function and utility of the optical approach. These include channels with faster kinetics, step-function properties, altered activation spectra (redshifted), and cellular localization (Yizhar et al. 2011; Klapoetke et al. 2014).

As these optogenetic stimulation tools became available, additional approaches were being developed to allow investigators to "silence neuronal activity." Here, the principal strategy was to develop photosensitive cation channels which could act to hyperpolarize a neuron and thus significantly minimize and prevent that neuron from generating an action potential and "firing." These inhibitory opsins include pumps for protons hydrogen (H^+) (called archaerhodopsin or bacteriopsin), sodium (Na^+), and chloride (Cl^-) (called halorhodopsin) (Yizhar et al. 2011). Like ChR2, these proteins have been modified to enhance function, expression, sensitivity, and wavelength, thus affording investigators more advanced methods to manipulate neural circuits. The key advantage to these inhibitory opsins is their ability to be harnessed for the determination of how a genetically defined neuronal population is necessary for a given behavioral event. The investigators can time lock activation of the optical silencing method within a given group of neurons and observe the behavioral consequences of that particular manipulation in real time.

While the optical tools described above offer spatiotemporal and optical control of neuronal activity through excitation or inhibition of given subsets of neurons, due to the constraints of their binary impact and the fact that their activation does not necessarily mimic naturalistic neuronal activity, additional optogenetic approaches have been developed. These include, for instance, methods for specifically manipulating cellular signaling, neuromodulation, and gene expression. In particular, a newer approach was developed whereby GPCR signaling (the primary mediator of neuromodulatory function in the nervous system) can be mimicked in a cell type- and neural circuit-specific manner. These chimeric optogenetic tools have been engineered using seven transmembrane-spanning opsins that contain the intracellular loops and C-terminal tail of GPCRs, which are typically expressed within mammalian neurons or glial cells. One of the first families of opto-XR receptors was the adrenergic receptor system, whereby optically active beta-2 and alpha-1 adrenergic receptors were generated (Airan et al. 2009; Siuda et al. 2015). Subsequently, a series of new opto-XRs, developed over the years, can activate a whole host of G-protein signaling pathways and neurons, including Gi, Gq, and Gs among others (Spangler and Bruchas 2017).

Finally, several other photoactivatable proteins have recently been employed in cellular studies and are beginning to be used *in vivo* in brain tissue. These optogenetic tools target the inhibition or activation of second messenger cascades (for a review, see Wiegert et al. 2017). These tools regulate downstream signaling using allosteric or proximity-based effects. They incorporate the use of flavoprotein domains, such as the light-oxygen-voltage domain and cryptochromes. These flavoproteins generally fit into one of

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three categories and act to initiate enzymatic activity, dimerize, or change conformation, all in response to light (Spangler and Bruchas 2017). The advantage of these newer optogenetic systems is that they can selectively and discretely target, in a spatiotemporally precise manner, specific intracellular signaling components within intact neurons or at the systems level; this allows investigators to probe how a specific cellular signaling, trafficking, or physiological event can be potentially directly linked to a behavioral outcome in real time.

Additional approaches, now widely used in behavioral neuroscience research, include "chemogenetic actuator" tools. These biological tools allow for selective modulation of GPCR signaling in specific tissues or cell types. Like their complimentary optogenetic partners, chemogenetic tools are very easily adapted to behavioral contexts. The most widely used of the chemogenetic tools are GPCRs, which have been designed to respond to specific ligands and couple to specific excitatory (Gq, Gs) and inhibitory G-protein linked neuronal pathways. In most cases, as with opsins, investigators use these DREADD (i.e., designer receptors exclusively activated by designer drugs) proteins by combining a particular genetic method (animal, viral, or both) to introduce the DREADD into a particular cell type. Through molecular evolution of the human muscarinic or kappa-opioid receptor, Roth and his group engineered a family of mutated receptors that are only activated by clozapine-N-oxide (CNO) or other ligands such as salvinorin B and compound 21, among others (e.g., Roth 2016). Currently, most investigators use the DREADD proteins hM3Gq and hM4Gi to excite (enhance probability) or silence neuronal or gliotransmission, respectively. These two proteins are typically introduced via a specific viral method (e.g., an adeno-associated virus, lentivirus, or herpes simplex virus) into particular cells in the nervous system, after which investigators inject a systemic ligand to activate each receptor protein for the desired effect. The activity of these receptors and the drug CNO typically peaks at 90 minutes. This approach, while providing cellular and network-level access, lacks spatiotemporal precision compared to optical methods. Thus, it is somewhat more challenging to incorporate into particular systems-level models of a given circuit's role in behavior.

Additional methods are now in the process of being engineered and tested in a variety of systems. These include the development of magnetically sensitive proteins as well as proteins that are sensitive to high-frequency vibrations, such as ultrasonic actuation (Stanley et al. 2015; Wheeler et al. 2016). These developments remain controversial (Meister 2016) yet could offer, if successful, a completely new noninvasive means to perturb and probe neural circuit function.

The use of various optogenetic and chemogenetic tools within neuroscience is continuing to expand on an almost daily basis. The key advances in protein engineering, crystal structure, cryogenic electron microscopy, and biochemistry are allowing for continued progress in this type of toolbox. The

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development of various sensitivities, color activation spectra, and functions coupled with novel hardware approaches will continue to advance the field and allow for more nuanced physiological neuronal activity patterns to be utilized. Furthermore, with the advances in biological substrates in optogenetics, additional hardware-based neuronal probes have been developed which offer advanced optogenetic targeting and function to decrease neuronal tissue damage or allow for tether-free animal movement in more naturalistic settings. These devices include new Michigan- and Utah-based optoelectronic probes (Deisseroth and Schnitzer 2013), along with printed flexible microLEDs and multifunctional polymer-based fibers. In some cases, devices can be powered using near-field or radio frequency communication parameters for completely untethered control of LED function, and these devices can also be used in most common behavioral setups in a closed-loop manner (Shin et al. 2017).

Key Limitations

Although optogenetic and chemogenetic approaches have provided unprecedented new knowledge about neural circuit function as it relates to behavior, they are constrained by several limiting functions. Of primary importance is the fact that sufficiency experiments that rely on using DREADD or ChR2 (and related opsins) utilize broad neuronal activation via light or chemical entity to activate a genetically defined neuronal population. In the case of chemogenetic hM3Gq-DREADD activation, neurons that express the receptor will respond in unison to the drug application; this increases their firing all at once, in a similar manner, as the drug is exposed to and binds the DREADD receptor onto a given neuron. In a similar manner, ChR2-based (or comparable) optical excitation results in photostimulation across the entire field of cells, whereby light can reach, with sufficient power, through the tissue to depolarize the population of neurons. The problem with this approach is that we know from *in vivo* electrophysiological studies and optical imaging approaches that neurons do not typically respond in a monolithic synchronous fashion. Indeed, neurons fire in patterned, stochastic ways to encode various behavioral responses. This is a severe limitation of current DREADD-, fiber- and LED-based optogenetic strategies utilized in behavioral neuroscience. The assumption is that by stimulating or inhibiting all the neurons in a given region at once, we can mimic the activity of the neural circuit as it relates to a particular behavior. This "binarization" of neuronal activation in circuit neuroscience should be questioned and resolved, so that we can better understand the discrete, heterogenous nature of circuit encoding and behavior in the nervous system. There is, however, some promise with respect to this limitation, and a few papers have recently utilized and highlighted this new method (Packer et al. 2015; Jennings et al. 2009). By using spatial light modulator-based two-photon microscopy, investigators have been able to image particular neuronal activity patterns and then "play back," in a finite manner, those patterns using optical stimulation across

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a single planer view. This approach is limited to a fixed neuronal number due to laser power and coupling restraints; however, the approach suggests that advanced optical holographic methods can be utilized to overcome the limits of monolithic, synchronous optical manipulations.

Detection and Visualization of Neural Circuit Function and Transmission

The complexity of neural circuits in the mammalian nervous system has been a daunting task to dissect. Canonical approaches have utilized *in vivo* electrophysiological methods to record activity of neurons and ensembles within discrete brain regions during behavioral tasks in awake, freely moving, or head-fixed animals. This method, used for many decades, has provided neuroscientists with a rich framework to understand how various networks in the brain respond under various behavioral states. While extracellular and multidimensional (high channel count) recordings have been instrumental to our understanding of neural circuits, they have been limited, for instance, by the following factors:

- They lack genetic or cell-type identification.
- They are unable to track neurons across days and trials with confidence, due to limitations of maintaining a single neuron during recording over multiple sessions.
- Channel and cell count are limited by region and array size, and for deep brain, significant issues arise due to lesioning of more dorsal structures in an attempt to reach limbic structures.

Fortunately, several modern approaches have advanced our ability to detect and visualize discrete neural circuits as well as to make claims about causality of various cell types, circuits, and networks in mediating a particular behavior. Although some of these approaches have only begun to be utilized widely in the community, they are at the forefront of neural technology and are likely to lead the field's efforts in the coming years.

In vivo Calcium and Voltage Imaging

Advances in genetically encoded calcium indicators (GECIs) represent the most recent avenue by which some of the limitations listed above are beginning to be resolved. Older versions of GECIs were limited in their capacity to resolve deep brain structures due to low signal, noise, and poor dynamic range. Newer variants have helped to resolve many of these limitations (Chen et al. 2013b; Odaka et al. 2014). The general principle of these GECIs is that calcium ions enter the cell following an action potential, and these sensors are then used to detect subtle changes in neuronal calcium by converting that

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signal into a fluorescent signal that can be measured using advanced microscopy approaches. This allows for a reliable proxy measure of action potential firing patterns, along with synaptic calcium dynamics in real time.

The recent development of ultrasensitive protein calcium sensors, GCaMP6.0 series, has been transformative for the field at large, because they allow for very reliable detection of calcium transient activity and circuit activity in deeper structures when coupled with the advent and use of both cranial window-based imaging and the gradient refractive index lens (GRIN), which provides optical advantages for researchers using single-cell imaging methods within deep brain limbic circuits. Cranial window-based imaging in multiphoton applications can provide larger fields of view, so that hundreds and even thousands of neurons can be imaged in a single animal over multiple behavioral sessions. New "mesoscope" microscopes are becoming available for this very purpose and will greatly expand the field. The utilization of these new biological and hardware-based imaging tools with fiber photometry as well as single- and two-photon imaging permits reliable terminal field and single-cell detection of calcium transient activity over single and multiple trials spanning days to weeks. Various hardware has been developed and optimized for maximizing the types of behavioral experiments in which GCaMPs can be used. Fiber photometry has gained substantial popularity in recent years due to its relative ease of use. This method employs a simple fiber optic and photometer-based detection system and provides a computationally simple data pipeline (Gunaydin et al. 2014) for measuring "bulk" calcium dynamics in a given neuronal population, and ease of use in freely moving behavioral studies. Further specificity of single-cell activity has been gained through the advent of GRINs lens-containing mini-endoscopes (Ghosh et al. 2011; Barretto and Schnitzer 2012); this allows for a less than 2 g microscope to be mounted to an animal's head, and a complementary metal oxide semiconductor image sensor for high-speed detection of calcium transients in single cells. This mini-scope approach allows for deep brain imaging during freely moving behavior, together with single-cell tracking over the course of multiple behavioral sessions (Mukamel et al. 2009; Xia et al. 2017). Finally, two-photon imaging in head-fixed, awake-behaving rodents allows for high-resolution long-term imaging with either cranial windows or GRIN lens implants for deep brain applications. This head-fixed two-photon imaging can be coupled with complex behavioral tasks, including virtual reality systems and spherical treadmills with simulated environments for increased complexity in both endoscopic and cranial window-based imaging platforms (Zhang et al. 2018; Jennings et al. 2019).

Additional tools are under development and being tested. These include an expansion of the color range for calcium indicator protein sensors, such as red fluorescent calcium indicators (RCaMP) and jRGECO (Akerboom et al. 2013). The advantage of these additional sensors is that they will improve the imaging depth within intact brain tissue, since near-infrared light scatters less through tissue. The other advantage of these red indicators is the future ability to allow

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for simultaneous multicolor imaging of diverse genetically or circuit-specific neuronal populations. These newly fashioned indicators could also allow for dual imaging of presynaptic axon terminals or coupling with other optogenetic actuator-based approaches. These indicators are currently limited by their signal-to-noise and dynamic range, but multiple groups are working to resolve and optimize these issues using forward genetic screens in high throughput testing scenarios.

One disadvantage of these calcium-based sensors is that they only sense calcium transient activity, not actual action potentials. That is, they resolve activity on a scale of seconds, not milliseconds. Compared to extracellular recordings and phototagging, this has caused some in the field to remain skeptical of the advantages of genetically encoded calcium imaging methods. However, recent advances in genetically encoded voltage indicators may hold some promise in resolving this issue. The recent developments of Archon, QuasAr2, and CheRiff allow for reliable voltage detection (Adam et al. 2019), which has been validated by electrophysiological studies to match kinetics directly with neuronal action potentials.

Imaging and Detecting Neurotransmitter and Neuromodulator Activity

A very recent and exciting development in biosensors for other biomolecules and neurotransmitters has come to the forefront. For many years it has been difficult to measure the actions of both fast neurotransmitters and neuromodulators in real time, using genetically encoded tools, during freely moving behavior. Classically, these molecules have been measured using microdialysis-based methods, which afford pristine detection with mass spectrometry but are limited by spatial and temporal resolution. Dialysis is also generally unable to detect larger molecules (e.g., neuropeptides), although some recent progress has been made with opioid detection (Al-Hasani et al. 2018). These include probes for glutamate (SF-iGluSnFR), acetylcholine (GACh), glycine (GlyFS), GABA (Chamelean), dopamine (dLight and GRAB-DA) (Patriarchi et al. 2018; Sun et al. 2018), as well as norepinephrine (GRAB-NE) (Feng et al. 2019). These sensors rely on technology based on fluorescent-resonance energy transfer or they take advantage of circularly permutated green fluorescent protein (cpGFP, also used in GCaMPs) fusion proteins within the third intracellular loops of specific GPCRs or related coupling domains. This allows the sensor to detect "binding" of the transmitter or modulator, and thus reveals the presence of a substance in a given response within a region or circuit. These new sensors are promising because coupled with modern viral and genetic tools, we can selectively express the sensors in various brain regions, cell type, and circuits, and record activity in discrete behaviors in real time.

While these protein-based sensor approaches offer a significant amount of promise in terms of our ability to dissect the role and function of specific

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neurotransmitters and modulators in real time during freely moving and head-fixed behavior, there are still several limitations, and advances will be forthcoming. Currently available sensors are mostly directed toward the detection of small molecules, including fast transmitters, cholines, and monoamines. While efforts are ongoing to use the same technology to detect neuropeptides, tool development has been limited due to the high-affinity nature by which neuropeptides bind to receptors and the inclusion of the cpGFP into the third intracellular loop of the GPCR. This is the same region of the receptor that dictates G-protein coupling and high affinity binding. There are some promising developing neuropeptide GPCR-based sensors on this front, none of which have yet been published or validated *in vivo*; however, if neuropeptides can be detected in a meaningful and genetically defined manner, it will open a host of very exciting possibilities in how neuropeptides coordinate neuromodulatory function within neural circuits that mediate a variety of behaviors (e.g., stress, anxiety, fear, addiction, and reward seeking).

These new transmitter sensors could open new avenues and enable us to address long-standing questions about neurons that co-release fast transmitters (e.g., GABA and glutamate) while simultaneously releasing monoamines and neuropeptides. We may be able to answer important questions about whether neuropeptides encode specific information on their own or in conjunction with specific fast transmitters under certain circumstances. Furthermore, these types of tools coupled with imaging would allow us to expand our understanding into the molecular and cellular basis of organization within neuromodulatory and neurotransmitter circuits. Are peptides active at specific locations, released in dendrodendritic form in some cases, or just released in mass in a volumetric manner? Although these types of biosensors hold great promise, further enhancement of their quantum yield (signal to noise) and sensors for other intracellular signaling molecules (including cAMP, kinases, and other cascades) will be needed for *in vivo* systems-level experiments in awake-behaving animal studies.

Conclusion

Optical tools provide unique methodologies in our quest to dissect neural circuits associated with behavior. Equipped with novel biological tools as well as new, less restrictive hardware and/or systems with higher resolution for imaging activity within neural circuits, investigators have been able to resolve specific spatiotemporal properties of discrete cell types, neurotransmitters, and neuromodulatory pathways in real time during discrete behavioral events. The challenges posed by these new methods include their invasiveness, their lack of temporal resolution (particularly with GECIs), as well as their dynamic range. Hardware limitations, in terms of the imaging window, pose limitations, for instance, on data stream management. As richer, high-resolution data becomes

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available through these new approaches, deciphering the data and utilizing new computational approaches becomes more imperative. Computational models, in turn, are needed to handle the new data, thus posing a future challenge.

The advent of these new technologies begs the question as to whether any of the tools described above might eventually pave the way to a better understanding of intrusive thoughts in animal models, and whether these could be applied in clinical translation. From the discussions at this Forum, it is clear that there are a variety of possible uses for novel tools in establishing causality and translation—provided that some of the limitations can be overcome. These include using closed-loop sensing of neuronal activity (GCaMP or other) and optogenetic (ChR2 or halorhodopsin equivalents) or pharmacological manipulations in a wireless setting. Real-time sensing during established behaviors defined to represent "intrusive thoughts" across species, alongside real-time feedback with optogenetic and pharmacological control, would establish causality and mechanisms for intrusive thoughts, at least in one sense. For example, deep brain stimulation has been widely used in clinical settings for a variety of neurological and psychiatric diseases, yet it has not been used in a closed-loop setting, whereby certain neuronal signatures, biomarkers, or other measures would be detected followed by a closed-loop infusion of a drug or optical/electrical stimulation.

The approaches outlined here, including optogenetic, chemogenetic, and electrical perturbation, could be amenable to these ideas if we could (a) measure signatures of intrusive thoughts that span particular brain regions with particular biomarkers and (b) overcome the limitations of expressing viruses in the human brain. Recent developments in retinal research and clinical trials with adeno-associated viruses, along with other viral delivery methods and many new hardware developments, could assist translational approaches in the future.

Collaboration between cross-disciplinary computational neuroscientists, biologists, psychologists, behaviorists, and clinical psychiatrists is of paramount importance and needs continual encouragement. Notwithstanding these challenges, the range of tools in the neuroscience toolbox continues to grow, offering innovative ways to resolve specific pathways, networks, and behaviors with increased granularity. Future efforts require specific focus on cross-species corroboration, computation, and analysis.

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