The Optogenetic Catechism

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REVIEW

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An emerging set of methods enables an experimental dialogue with biological systems composed of many interacting cell types—in particular, with neural circuits in the brain. These methods are sometimes called "optogenetic" because they use light-responsive proteins ("opto-") encoded in DNA ("-genetic"). Optogenetic devices can be introduced into tissues or whole organisms by genetic manipulation and be expressed in anatomically or functionally defined groups of cells. Two kinds of devices perform complementary functions: Light-driven actuators control electrochemical signals, while light-emitting sensors report them. Actuators pose questions by delivering targeted perturbations; sensors (and other measurements) signal answers. These catechisms are beginning to yield previously unattainable insight into the organization of neural circuits, the regulation of their collective dynamics, and the causal relationships between cellular activity patterns and behavior.

What Is Optogenetics?

The 2019 revision of the Oxford English Dictionary, which may be the first to recognize the new word, will define optogenetics as "the branch of biotechnology which combines genetic engineering with optics to observe and control the function of genetically targeted groups of cells with light, often in the intact animal." Although the foundations of the field were laid in the late nineties and early naughts (*1*–*12*), the term appeared in the literature only in 2006 (*13*). Purists have remarked that "optogenetics" is a misnomer: similar coinages, such as optoacoustics or optoelectronics, refer, respectively, to interactions of light with sound and electrons. Optogenetics, by contrast, has nothing to do with interactions between light and genes; what matters is the effects of light on the protein products of genes.

What Kinds of Light-Sensitive Proteins Are Used in Optogenetics?

There are two classes of optogenetic devices (Fig. 1): sensors and actuators (*1*). Sensors translate cell physiological signals into optical signals; they make cellular function visible. Actuators transduce optical signals into physiological signals; they make cellular function controllable. The pleasing symmetry between sensing and actuation is practically important because sensors and actuators together make up a complete experimental package: Actuators deliver controlled perturbations, and sensors report system responses back.

Experimentation on Which Systems?

Optogenetics was developed to study information processing in the brain (*1*, *2*, *13*), but it is certain to find many applications outside neuroscience. One vast, still entirely unexplored territory is information processing in the immune system.

What Is Special About Constructing Light-Emitting Sensors and Light-Driven Actuators from Proteins?

Proteins can be encoded in DNA. DNA molecules are stable, portable pieces of code that can be packaged into many different kinds of delivery vehicles and integrated into the genome of nearly any organism. Once a piece of DNA has been introduced into a cell, endogenous machinery is directed to produce the required protein. This solves the problem of delivering experimental agents deep into the tissues of intact organisms: After genetic modification, the organism itself generates the tools necessary for investigating its function; biology is revealed through biology.

However, not all optogenetic devices are wholly encodable. Some sensors (*3*, *14*) and actuators (*8*–*10*, *15*, *16*) depend, in addition to a genetically encoded component, on small molecules that must be fed or injected. Others (*11*, *12*, *17*–*19*) require protein expression levels so high that they cannot routinely be achieved by

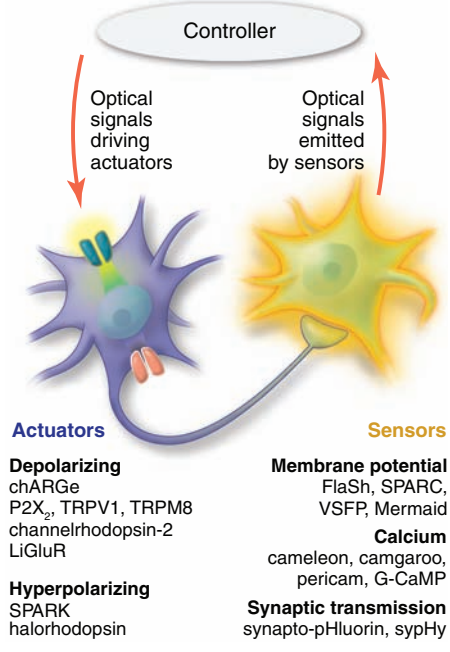

Fig. 1. Sensors and actuators. Light-driven actuator proteins are used to control genetically targeted cells in a circuit. The actuators transduce optical commands into de- or hyperpolarizing currents. Light-emitting sensor proteins report changes in membrane potential, intracellular calcium concentration, or synaptic transmission.

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modifications of the host genome, forcing once again a resort to invasive procedures with variable penetrance: the injection of plasmids (*20*–*22*) or viruses (*23*–*25*).

Although localized agent delivery can enhance the region selectivity of expression in some circumstances (*20*, *22*, *26*), these characteristics generally undermine the elegant economy of a stably heritable, fully biosynthetic system. The need to manipulate each experimental subject individually reduces throughput, which makes optogenetic screens, if not impossible, certainly laborious. It also introduces a new source of variability, which must be controlled.

However, the delivery problem is not the only, and not the most important, impediment that a optogenetics was developed to overcome. By encoding sensors and actuators in DNA, their distribution can be restricted to particular cell types, tapping the same mechanisms that localize the expression of endogenous genes. Gene expression patterns thus provide a natural framework for exploring biological function.

Do Gene Expression Patterns Meaningfully Reflect the Functional Organization of Cells into Tissues?

Yes, but single genes are often either too loquacious or too reserved in their expression. Most isolated enhancers yield expression patterns that match behaviorally or physiologically relevant subsets of neurons only approximately; coverage is often insufficiently inclusive, inadequately exclusive, or both (Fig. 2). This is not surprising: Cell identities are thought to be specified combinatorially, by using a regulatory syntax understood only incompletely. We know, for instance, a genetic label for "dopaminergic" in invertebrates; it is "a cell in which the tyrosine hydroxylase gene is turned on." Exploiting this knowledge (and randomly some of the influence exerted on transgene expression by genomic context) made it possible to control remotely different subsets of dopaminergic neurons in the behaving fly (*10*, *27*). The origin of reinforcement signals driving adaptive behavior could thereby be mapped to a cluster of 12 dopaminergic cells—one of two existing examples of small-scale optogenetic screens (*27*, *28*). But attempts to target these cells selectively among the 200 or so other dopaminergic neurons in the fly brain failed because of an inability to translate attributes like "source of reinforcement signals," "located in the PPL1 cluster of dopaminergic neurons," or "projecting to the mushroom body" into the language of gene expression.

To take full advantage of optogenetic tools, it is essential that they can be targeted selectively and comprehensively to the cells of interest. The capacity to do so remains limited, as discriminating audiences appreciate all too well. Improving access to identified cells is as much a matter of expanding the genetic lexicon—that is, of associating as many molecular descriptors as possible with each identified cell—as it is of designing boolean operators (*1*, *29*) that can decode sets of such descriptors as unique cellular addresses.

Which Cell Physiological Signals Are Sensed?

An engineer thinking about sensor placement in a living cell might despair after a casual look at a chart of signaling pathways. Closer scrutiny, however, will reveal structural simplicity within the apparent complexity. Many signaling molecules are wired together in modules with identifiable functions, and many of these modules operate in distinct temporal frequency bands. The low-frequency band, for example, contains slow developmental, growth control, and differentiation programs that regulate, by and large, gene expression. Distinct from these are the high-frequency signals that flow through neural, immunological, and endocrine circuits and convey information about rapidly varying internal and external states.

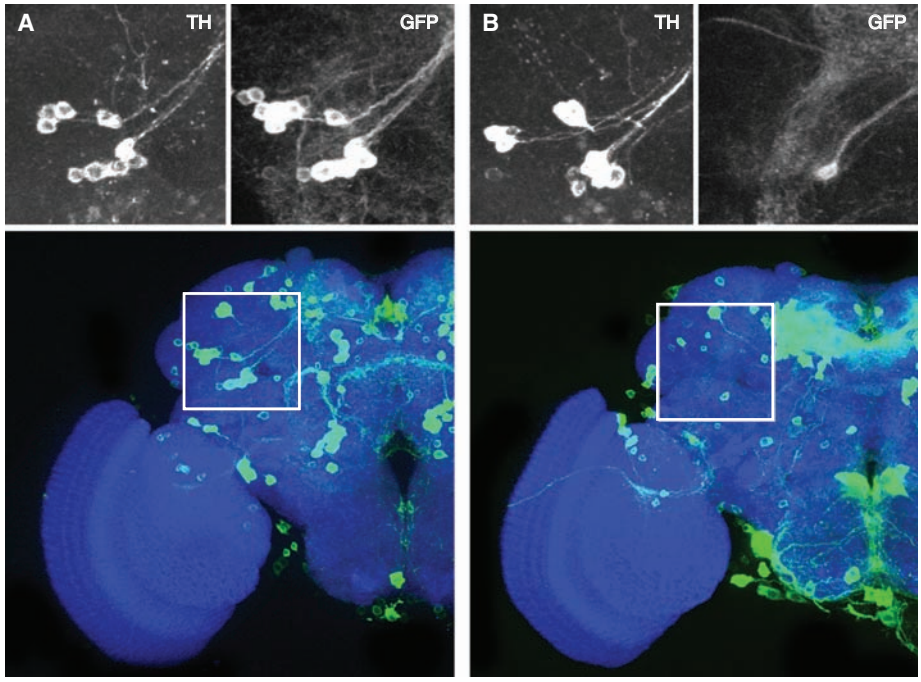

Fig. 2. Genetic access to neurons in the fly brain. Two different enhancer elements are used to drive the expression of GFP. The two enhancers each label neurons in the same dopaminergic cluster, which is visible in the left top images after staining with an antibody against tyrosine hydroxylase (TH). The enhancer in (**A**) labels the cluster exhaustively, whereas the enhancer in (**B**) targets a single identified neuron in it. Views of the whole brain (bottom images) reveal that neither enhancer expression pattern exclusively captures the dopaminergic neurons of interest.

Although there is much diversity in the molecular carriers of these messages along a communication chain, many of them are eventually translated into a cellular Esperanto of membrane potential changes, calcium admission from extra- and intracellular reservoirs, and secretory events triggered by calcium. The most versatile sensors are those that detect and report these changes.

Protein-based optical sensors for the three key variables of voltage, calcium, and secretion now exist (*4*–*6*) (Fig. 1). In addition to these generalists, there is also an ever-expanding range of specialist probes for analytes such as adenosine 3′,5′-monophosphate (cAMP) and for processes such as cell cycle progression (*30*).

How Do Sensors Work?

Virtually all optogenetic sensors are derivatives of the green fluorescent protein (GFP), engineered to modulate their light output in response to a physiological variable. One of the two common mechanisms of modulation is chromophore protonation-deprotonation cycles, which are typically driven by conformational changes that allow or restrict solvent access to the chromophore. The second mechanism is variations in the strength of electrodynamic interactions between nearby chromophores, caused by changes in proximity and/or dipole orientation. The calcium sensor G-CaMP illustrates the first mechanism, the calcium sensor cameleon the second (*1*, *30*, *31*).

How Well Do Sensors Perform?

A biophysicist's answer will be framed in terms of the sensor's response kinetics, dynamic range, absolute brightness, photostability, specificity, the potential for perturbation of cellular function, and so forth. Because many of these parameters are in conflict with each other, sensor optimization tends

to force substantial trade-offs; these and the signal detection theory behind them are discussed elsewhere (*1*, *29*–*32*). As a simple illustration, consider the problem of optically detecting a neuronal action potential (*5*, *32*). Naïvely, one might suppose the best approach to consist of packing the neuron's cell membrane densely with the fastest possible voltage sensor. However, resolving the light emissions of individual neurons in thick, scattering tissue generally requires some form of scanning microscopy, which samples each spatial location only intermittently. This has two important consequences.

First, to avoid detection failures while looking elsewhere, each location must be revisited at intervals shorter than the typical duration of an optical event. To detect an action potential of 1-ms duration by using a membrane potential sensor with a fast impulse response, the sampling rate must exceed 1 kHz. This limits the number of cells that can be surveyed.

Second, short events have few photons associated with them, making detection statistics unfavorable. The problem could, in principle, be ameliorated by expressing more sensor molecules, but this increases the risk of sometimes violent interference with the variable of interest. Such an observer effect plagues the expression of genetically encoded voltage sensors: When these probes are present at the levels required for action potential detection, they suppress synaptically evoked electrical activity (*14*). The culprit is the capacitive load added by the mobile probe charges sensing the cellular membrane potential (*14*, *32*).

Can Optogenetic Sensors Detect Naturalistic Activity Patterns in Brain Circuits?

No. All optical sensors are fundamentally limited by their photon statistics. The circle of collecting sufficient photons from many optically resolved cells at high sampling rates, so that activity can be detected reliably, has not been squared, not with synthetic and not with optogenetic probes.

Existing encodable voltage probes (*5*, *14*) interfere with electrical excitability (*14*, *32*). Calcium (*4*) and exocytosis sensors (*6*) are better tolerated than voltage probes and able to resolve single isolated action potentials and synaptic impulses under favorable conditions. However, they still lack the temporal resolution to distinguish such events in naturalistic settings (*1*, *29*, *30*).

Even though functional optical imaging is so far unable to keep pace with dynamics on electrophysiological time scales, it offers insights into the organization of cells and systems that would be difficult to obtain by other means. Maps of orientation or direction selectivity in visual cortex could, in principle, be pieced together from hundreds of single-unit recordings, but only functional images reveal at once, in one individual, the geometry and self-similarity of these maps across macroscopic and microscopic scales (*33*). The genetic resolution of encodable sensors makes them ideal for eavesdropping at the mezzanine level: They can discriminate how macroscopic order emerges from layering and interactions of distinct groups of microscopic elements (*34*–*36*).

Are Optogenetic Actuators Able to Control the Key Cellular Information Carriers: Voltage, Calcium, and Secretion of Signaling Molecules?

In broad outline, yes. Because the majority of actuators (Fig. 1) are ion channels (*8*–*10*, *15*), ion pumps (*17*–*19*), or proteins that regulate ion channels (*7*), they are able to influence transmembrane voltage. Many of these channels and pumps—TRPV1 (*8*), TRPM8 (*8*), $P2X_2$ (*8*), channelrhodopsin-2 (ChR2) (*11*, *12*, *17*), and the light-gated glutamate receptor (LiGluR) (*15*)—conduct calcium ions (*37*), so they can also control calcium-dependent processes, including regulated secretion. Because calcium influx and exocytosis are tied to voltage changes, it is not usually feasible to regulate these processes independently: to elevate intracellular calcium levels without altering membrane potential or to trigger secretion directly.

As in the sensors' case, there are also specialized actuators, such as light-activated adenylyl cyclases, transcription factors, kinases, and heterotrimeric guanine nucleotide–binding protein (G protein)–coupled receptors. Biodiversity prospecting and bioengineering will undoubtedly extend this range further.

How Is Light Sensitivity Built into Actuators?

In actuators regulating membrane potential, photons exert force by driving chemical changes in either retinal or exogeneous light-sensitive small molecules (*1*, *37*). Retinal, an endogenous vitamin A derivative, functions as the chromophore in two large families of optically responsive proteins, known as opsins in free form and as rhodopsins when in complex with retinal. The visual rhodopsins of vertebrate and invertebrate eyes are G protein–coupled receptors; when illuminated, they engage intracellular signaling cascades that control the opening or closing of ion channels in the plasma membrane or in intracellular compartments (*7*, *12*).

Microbial rhodopsins, in contrast, are light-driven proton or chloride pumps, which couple the photocycle of retinal to the translocation of ions (*37*). When coupling is leaky, as in ChR2 (*38*), the pump cycle includes passively conducting intermediates: The pump functions (intermittently) as a nonselective channel (*17*).

The alternative to retinal as a light-operated lever is irreversible photolysis (so-called uncaging) or reversible photoisomerization of synthetic photochemicals. Specificity of action is ensured in two ways. One is orthogonality: The photolytically liberated compound lacks activity against endogenous targets and thus requires the expression of an exogenous receptor (*8*, *10*). The other mechanism is recruitment: The activity of a broadly active photoswitchable ligand is localized (and potentiated) by docking the molecule to an adhesive patch on the actuator protein (*9*, *15*, *26*).

How Well Do Actuators Work?

A biophysicist will look to several performance indices: the actuator's single-channel conductance, its response kinetics, the open probability in the dark, cofactor requirements, the capacity to regenerate the optically responsive state, the potential for controlling on and off transitions independently, and the precision with which the optical signal driving the actuator can be localized in scattering tissue. As in the sensors' case, there are substantial trade-offs to consider and a growing literature to help weigh them (*1*, *26*, *29*, *37*).

Perhaps the most important balance to strike is between actuator force and speed. Unsurprisingly, the tighter the mechanistic link between photochemical process and ion flux, the faster the actuator's kinetics. Actuators that are gated by diffusible intermediates, whether products of signal transduction pathways (*7*, *12*) or photolysis (*8*, *10*), respond with temporal precision ranging from ~10 ms to seconds. Actuators with built-in chromophores, such as ChR2 (*11*, *12*, *17*), LiGluR (*15*, *16*), and halorhodopsin (*18*, *19*), can be controlled with higher millisecond or submillisecond accuracy. Unfortunately, however, the fastest actuators are also the weakest: The unitary conductance of ChR2 is only 40 to 50 fS (*17*, *38*) and that of LiGluR, 250 fS (*16*, *26*); TRPV1 and $P2X_2$, by comparison, have conductances of 30 to 35 pS at resting potential (*8*, *37*). This nearly 1000-fold difference in the force exerted by a single actuator molecule explains the ineffectiveness of microbial opsins in some settings (for example, the intact adult fly central nervous system) and the requirement for massive overexpression in many others.

What Have Actuators Been Used for?

The earliest applications of encodable actuators were naturally proof-of-principle experiments: demonstrations that remote activation of neurons known to control particular functions produced the predicted outcomes (*10*, *18*, *23*). Many recent studies have continued in this vein, voyaged into the known, and discovered the expected. Where truly novel insights have emerged, they tend to fall into three areas: tracing of functional connections (*20*, *22*), analysis of mechanisms by which neural circuits (self-) regulate their activity (*24*, *25*, *28*, *39*), and searches for the neural underpinnings of cognition and behavior (*21*, *24*, *27*, *28*, *39*).

An example from the first category is the demonstration that inputs from ascending, local, and descending axons are segregated onto discrete dendritic domains of neocortical pyramidal cells (*22*). Transmitting information through synaptic clusters rather than distributed connections may result in more efficient coupling between pools of synaptic partners. One (perhaps rather

too high) estimate suggests that at most 300 action potentials in layer 2/3 pyramidal cortical neurons represent a basic parcel of propagating activity (*21*).

An advance in the second category, insight into mechanisms of neural control, is the discovery that animals harbor functional circuits for behaviors they never express. The smoking gun is a cryptic capacity of female fruit flies for displaying male-specific courtship (*39*). This hidden talent was uncovered after optical activation of neurons expressing a principal sex determination factor. Latent bisexuality may be a vestige of shared brain development in males and females, on which sex-specific action selection mechanisms are later superimposed.

In the third category, even the neuronal substrates of some higher-order cognitive functions, such as the construction of valuations (*27*) or the gating of sensory responses (*24*), have been delineated. They include not only narrowly circumscribed spatial (*27*) but also distinct temporal domains (*24*) of activity.

Is It Important for Artificial Actuation to Mimic Naturalistic Activity?

It is unlikely that it can, and it is not clear that it needs to or should. The reasons are manifold.

In practical terms, we do not know what a naturalistic neuronal activity pattern looks like because no one has been able to record one. We have many examples of isolated spike trains from different types of neurons and in different conditions. But what is generally lacking (with a few notable exceptions) is knowledge of the temporal relationships between the spike patterns of many cells during simultaneous activity. Probing the importance of these phase relationships in information processing is a key future application of optogenetic technology, one whose outlines are just beginning to appear (*24*, *25*). But these experiments require bold systematic variation, not slavish imitation, of naturalistic activity.

Even if we knew the timing of every spike in every cell of a circuit, playing back such an activity pattern to a volume of tissue would be no simple technical feat. Setting aside questions about the efficiency and precise spatial addressability of optogenetic actuators, patterned illumination of hundreds to thousands of locations simultaneously but independently poses enormous challenges. These challenges are magnified because positive actuation alone—translating the desired spike pattern into a pattern of light pulses and delivering these pulses—will generate uncontrolled runaway behavior. To ensure stability and constrain the system to a prescribed trajectory, negative control signals will have to be applied in addition to positive actuation, just as de- and hyperpolarizing currents are both necessary for keeping a neuron under voltage clamp. Akin to a voltage-clamp amplifier, these control signals can only be determined if the system's behavior is sensed in real time and fed back to the controller. The unsolved problem of densely sampling neuronal spike patterns at high temporal resolution again rears its head.

Empirically, even rather crude and highly artificial optogenetic actuation has proved remarkably effective. In many instances, firing a sizeable number of neurons at once or forcing a population of cells periodically produces, instead of chaos, coordinated behavior: flies fly (*10*) and court (*39*); fish swim (*28*); memories can be written (*27*); mice are nudged from sleep to wakefulness (*23*); decisions can be biased (*21*); cortical neurons synchronize (*24*, *25*). This suggests that neural systems do not easily adopt any of the astronomical number of dynamical states that are theoretically open to them; rather, they appear to switch between a more limited repertoire of preferred activity patterns. After a perturbation, be it sensory input, motor command, update of the contents of working memory, or optogenetic intervention, the circuit settles into one of these so-called attractor states. Attractors can be thought of as minima in the free energy landscape characterizing the circuit's dynamics, with a surrounding basin of initial states that feed into them. The hallmark of attractor dynamics is that, irrespective of where exactly in the basin the circuit lands after a perturbation, its trajectory will evolve toward a common dynamical state. The unreasonable effectiveness of optogenetics in eliciting physiological responses hints that attractor dynamics is a common feature of brain circuits. Of course, testing this interpretation rigorously will require that the dynamic endpoints reached from many different initial states are mapped systematically.

This leads to the statistical argument that complex systems are best probed, not by replicating any one particular activity pattern, but by applying whole families of perturbations which may be unapologetically artificial. The core of this argument was articulated by R. A. Fisher (*40*): "No aphorism is more frequently repeated […] than that we must ask Nature few questions, or, ideally, one question, at a time. The writer is convinced that this view is wholly mistaken. Nature, he suggests, will best respond to a logical and carefully thought out questionnaire […]." Statisticians recognize Fisher's questionnaires, also known as randomized multifactorial perturbations, as the most powerful experimental design for uncovering causal relationships in networks of interacting components, and biologists are taking note. Geneticists, for example, increasingly seize the advantages of analyzing the simultaneous phenotypic effects of thousands to millions of allelic differences instead of looking, one at a time, at a collection of single-gene mutants. Neuroscientists have long embraced randomized multifactorial perturbations under the rubric of white noise analysis and related methods of systems identification in sensory physiology (*41*). The advent of optogenetic control is an incentive to revive this tradition but also to step beyond single neurons and sensory systems and insert synthetic signals directly into defined neural elements deep in the brain. The development of stimulation protocols and technology that address many such elements with intelligently constructed test signals, such as spatiotemporal m sequences, can provide a global characterization of responses over many possible inputs and thus expose a circuit's dynamic modes and their functional importance.

Why Not Just Watch?

Mechanistic understanding requires intervention. This is as true for a geneticist wishing to link a phenotype to a gene as it is for a biochemist seeking to purify an enzyme, and it is of course also true for a neuroscientist attempting to isolate the neural signals that drive behavior. The information-carrying features of these signals rarely pop out from recorded neuronal chatter. Rather, they are found by systematic deletion, variation, and reconstitution of activity. Even if observation suggests that information is represented in a particular aspect of a neuronal activity pattern, the leap from correlation to causality still demands experimental intervention (*10*).

It is difficult to imagine how the genetic code could have been broken by observation alone. The decisive advance occurred when the genetic codebreakers instructed ribosomes with simple synthetic messages (*42*). Optogenetic instruction of the nervous system can play a similar role in the quest to decipher neural codes.

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REVIEW

Modalities, Modes, and Models in Functional Neuroimaging

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In this, the 21st century, human-brain mapping celebrates 21 years of cognitive activation studies. This review looks at imaging neuroscience and key ideas it has pursued; some ideas portend exciting developments, and others have failed gloriously. In terms of achievements, there is much to celebrate, in the sense that it is difficult to imagine modern neuroscience without brain imaging. I will look at recent advances from the perspectives of functional segregation and integration in the brain, paying special attention to approaches that deal with the distributed and integrated nature of neuronal processing and the questions they address.

Neuroimaging is now the predominant technique in behavioral and cognitive neuroscience. The volume of papers and number of fields it pervades are unrivaled (Fig. 1). Despite this, it is curiously difficult to summarize its achievements. The simplest summary falls back on the two guiding principles that shaped brain mapping at its inception: namely, functional segregation and integration. Neuroimaging has established functional segregation (the segregated or modular deployment of functional specialization within brain regions) as a fundament of brain organization. Furthermore, we can now characterize the integration of different brain areas in terms of functional and effective connectivity (Fig. 2). But beyond this, what have we learned? If you ask any imaging neuroscientist, they will recount exciting developments in their own field, ranging from the detailed functional architecture of retinotopically mapped visual cortex to the role of the ventral striatum in emotional learning. However, the question is more difficult to answer in terms of generic principles that underlie the brain's function and its relationship to anatomy. To see how people have tried to access these broader principles, I will look at recent trends in functional magnetic resonance imaging (fMRI), with a special focus on the questions that have been addressed. This focus is particularly important for functional neuroimaging, whose contributions will be measured by the depth of the questions asked, not the elegance of the method or, perhaps, the answers.

I first consider four themes that have caught people's imaginations recently and examine their underlying motivations, noting that there are many other exciting developments I could have addressed [such as genetics in neuroimaging, psychopharmacological fMRI, invasive and non-invasive electrophysiology, retinotopic mapping, computational anatomy, tractography with diffusion weighted imaging, lesion-deficit mapping, magnetic resonance spectroscopy, optical imaging, and technical advances such as polarization transfer (*1*)]. I then consider a couple of failures and conclude with a discussion of the implications for future directions; this discussion is illustrated with a few questions or model-led examples.

Multimodal Fusion

For years, we have heard about the promise of multimodal fusion, in which the spatial precision of fMRI will be complemented with the temporal precision of electroencephalography (EEG) to provide unprecedented spatiotemporal accuracy. However, this has not happened, despite the fact that we have the technology to acquire both modalities simultaneously (*2*) and have sophisticated biophysical models mapping from neuronal activity to both hemodynamic and electromagnetic measurements (*3*). So why is multimodal imaging not commonplace? Perhaps because there are many questions about functional anatomy that do not need bilateral spatial and temporal precision. Most questions about structure-function mapping and neuronal processing come in two flavors: where is it? or when is it? Functional magnetic resonance imaging is quite sufficient for questions of where and electromagnetic measurements [EEG and magnetoencephalography (MEG)] are the modalities of choice for questions of when; however, there are also questions about how imaging signals are generated that rest on fusion.

Multimodal fusion refers to the use of a common forward model of neuronal activity that explains different sorts of data. Several years ago, this was thought to be the best way to integrate fMRI and EEG because model parameters that are under-constrained by one modality might be informed by the other. In retrospect, this may have been a little misguided because the added value afforded by fusion requires unknown quantities generating data to express themselves in both modalities. Ironically, it may be that the complementary aspects of fMRI and EEG subvert the benefits of fusion. This may explain the success of simpler approaches to multimodal integration, in which the results from one modality constrain models of the other. These approaches use fMRI to provide precise spatial constraints (priors) on the source reconstruction of electromagnetic signals (*4*). Conversely, the temporal precision of EEG has been exploited in epilepsy research, in which explanatory variables based upon EEG features provide temporal constraints (in the form of explanatory variables or regressors) to model fMRI data (*5*).

So what questions call for fusion? A nice example is fusion of EEG and MEG data to measure evoked or induced responses, in which each modality alone is blind to certain sources (*6*). However, multimodal fusion really comes into its own when trying to understand the neurophysiology of brain-imaging signals and how they reflect underlying computations: Questions about func-

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