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Designing Tools for Assumption-Proof Brain Mapping

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Can we develop technologies to systematically map classical mechanisms throughout the brain, while retaining the flexibility to investigate new mechanisms as they are discovered? We discuss principles of scalable, flexible technologies that could yield comprehensive maps of brain function.

There has been much recent excitement about the potential for tools that might enable scalable mapping of brain circuits at the anatomical level (i.e., connectomics), the molecular level (e.g., transcriptomics, proteomics), and the activity level (i.e., dynomics). However, new fundamental mechanisms of neural function are being discovered all the time. This raises a question that sits at the junction between "big neuroscience" projects and discovery-oriented research: how should one design brain mapping technologies that can scalably acquire knowledge about classical mechanisms that we know are important, while taking in stride the continual uncovering of new mechanisms?

New Mechanisms and the Need for Mapping Them

There is no universal agreement as to what data sets are needed for a full understanding of the brain. Currently, there are efforts to understand the brain as a network made of neurons (e.g., in systems neuroscience), as well as efforts to understand neurons as networks of molecules (e.g., in molecular neuroscience). Efforts to build bridges between these levels of abstraction in the brain are much desired.

Dynomics and connectomics focus largely on mapping the spiking activity of neural populations and the synaptic connectivity of neural networks, respectively. Yet, many other mechanisms of electrical and chemical computation and communication are routinely being discovered. For dynomics, mapping the timing of discrete action potentials may reflect only part of the neural code, and full maps that reflect the analog electrical signals being discovered in many cell types may require new recording or imaging technologies. For connectomics, similar questions are being directed at the synapse. Direct electrical connections (mediated by proteins that make up gap junctions) can form local networks among interneurons with similar gene expression profiles (Brown and Hestrin, 2009), among other kinds of circuits. Direct electrical interactions between adjacent neurons—so-called "ephaptic coupling"—has been suggested to entrain the spiking of cortical neurons to extracellular electric fields (Buzsáki et al., 2012) and may play other roles in exciting or inhibiting neurons of specific geometry.

Classical neurotransmitters are of course of great importance in neural communication. But new kinds of transmitters, such as peptides, are routinely being discovered. Retrograde signaling by diffusible messengers—from postsynaptic to presynaptic neurons—is now well established, such as for the case of cannabinoids (Younts and Castillo, 2014). Nitric oxide (NO) functions as a diffusible gaseous messenger that can pass through cell membranes and can induce, in a temporally precise fashion, synaptic plasticity (Hardingham et al., 2013). Indicators for gases and other hard-to-tag molecules might be needed to understand how these nonclassical transmitters contribute to neural circuit functions.

Another mapping effort is the quest to enumerate the kinds of building blocks of the brain. One of the early flagship projects of the BRAIN initiative is to assemble a list of neuron types. Tools for mapping glial circuits, of course, might easily complement those for mapping circuits of neurons. For neurons, mapping transcriptomes has been proposed to provide a basis for classifying cell types. But whether genes are "on" or "off" is perhaps not enough: alternative splicing of genes can have profound effects on neural function. Beyond static transcriptomic snapshots, some evidence suggests that cells can change their type over time, perhaps calling into question the notion of cell type itself. In addition to dynamic changes in gene splicing as a mechanism for transcriptomic variation, neurons in adult animals can alter which neurotransmitters they use for signaling in response to environmental cues (Birren and Marder, 2013). Beyond even cells and their interconnections, it has been suggested that new tools to probe the extracellular matrix, which is implicated in the formation and preservation of memories, may be important for a full understanding of synaptic plasticity (Tsien, 2013). Tools that cannot take into account new mechanisms are essentially making the assumption that those new mechanisms are not contributing to a significant degree. Certainly, this may be the case for many well-defined problems, e.g., understanding a few seconds of neural dynamics might not require detailed understandings of how that neural activity regulates downstream gene expression over timescales of hours to days. But, when developing new mechanism mapping tools, it is useful to at least consider whether they can easily be extended to include new mechanisms.

Tools for Assumption-free Brain Mapping

The timing is right to elucidate design principles for neurotechnologies that work backward from the fundamental properties of the brain and are equal to the challenge of mapping their mechanisms, rather than working forward from known technology building blocks. In particular, we want to design technologies so that they can take new

mechanisms in stride, minimizing the reliance on assumptions that may later be shown to be false.

One key difficulty with brain mapping is that vastly different spatial and temporal scales often have to be simultaneously considered. The brain is organized with nanoscale precision, yet neural circuits can span vast regions, even tens of centimeters or larger. Individual signaling events can last milliseconds, yet learning or development or disease progression can take years. Thus, neuroscience is a kind of ''mesoscale biology,'' to borrow a term from physics.

For the case of neural activity, it will likely be important to map neural activity not only at the single-neuron level, but potentially with neural subcompartment resolution. Observing the propagation of neural activity through parts of neurons, e.g., in the dendritic tree, may be required to understand how neurons integrate inputs toward their neural code outputs. Despite the need for such spatial resolution, however, it is also clear that neurons in widely distributed circuits are operating in close coordination, and thus technologies for brain activity mapping must span these large spatial scales. The temporal precision required is also demanding, one millisecond or even better, which makes the recording of behaviorally relevant neural activity patterns (that might take hours to days or longer) daunting from a data analysis and perhaps even data storage standpoint. The joint criteria of spatial precision at circuit-wide scale, and temporal precision at behavioral scale, makes this problem all the more challenging.

In the connectomic and molecular mapping space, there is similarly a problem of achieving fine spatial discrimination, while scaling to the spatial extent of behaviorally or disease-relevant circuits. The requisite spatial resolution for assumption-free structural brain mapping is probably in the tens of nanometers or even better (if the goal is to resolve individual proteins, important to understand synaptic strength and dynamics, for example). Thus, nanoscale imaging systems that can scan quickly will be required; rather than just going for precision, or speed, of an imaging system, the ideal systems will need to do well along both performance axes. The ability to systematically map molecular mechanisms will probably require new kinds of observable tags and imaging systems.

Integrated Tools

An ideal technology would be able to map many kinds of variables (anatomical, molecular, physiological) in the same brain. Surprising organizational features of the connectivity of circuits are often apparent only after looking at many neurons within a single instantiation of a circuit and their topology of connectivity. Within a circuit, self-organization via plasticity mechanisms occurs to ensure network operation within the evolutionarily selected bounds of behavior, but two neurons in two different brains would not experience any such interaction. Such mechanisms of homeostasis could prove to yield important organizing principles of neural circuitry.

Correlations between multiple variables can of course be seen even at the population level. Gene expression and projection patterns are linked variables for neurons in the cerebral cortex (for example, Sorensen et al., 2013). Technologies that only reflect connectomic or only gene expression patterns, and not both, would miss such linkages. Studies linking cell shape and gene expression pattern have also revealed rich interdependencies, although the mapping is not one-to-one between single markers and overt morphologies (Markram et al., 2004), raising the question of how best to represent the geometry of a cell for informatic analysis, and the converse question of how many genetic markers it takes to define a cell type. More complete descriptions of cell shape and gene expression, as well as mechanistic links between the two, would be valuable to map in intact circuits, as well as tools that enable surveillance of cell type changes over time. Ideally, of course, we could map molecular, connectomic, and activity patterns—including new mechanisms governing or contributing to each—throughout circuits. Integrative mapping technologies must be compatible with each other—e.g., if you want to acquire an activity map from a brain, and then obtain its molecular and anatomical maps, you ideally would not alter the molecular or anatomical maps in the initial experiments on activity mapping.

Tightening the Loop between Discovery and Mapping

How can one design assumption-resistant, scalable, brain mapping technologies that can be extended to new mechanisms as they are found? It is important to work backward from the properties of the brain that need to be mapped and then to design the technology to meet that need. But this approach can be limiting if it cannot take into stride undiscovered mechanisms. One strategy is to bring forth new models of collaboration that connect people from different backgrounds so that technologies are designed ideally without excluding potential mechanisms that might to be considered in the future. It also requires systematic thinking in design. For example, attempting to make roadmaps of all possible directions before picking a path has in our experience helped narrow focus on paths that obey physical laws and can, potentially, match the complexity of the brain. In this ''architecting'' strategy, we actively recruit experts on different potential technology building blocks, bringing them together to consider not just the quantitative evaluation of potential paths, but new creative ideas or intuitions that might help generate an integrative technology.

For example, we recently completed a study of how different modalities—optical, radiofrequency, ultrasonic, molecular, and so forth—might contribute to brain activity mapping (Marblestone et al., 2013). Working across 14 different departments and organizations, we collectively mapped out a variety of paths. We aimed collaboratively to achieve some of the milestones thus outlined, e.g., pursuing the adaptation of lightfield microscopy to neural activity imaging, yielding whole-organism dynamics for *C. elegans* (Prevedel et al., 2014). Another collaboration has been pursuing algorithms and robots for automated intracellular neural recording in live mammalian brain (Kodandaramaiah et al., 2012). Thus, although neurotechnology may seem omnidisciplinary, and thus daunting, bringing together the right teams has already proven itself to yield impactful technologies. ''Architecting'' works best often when people from solution-providing engineering fields and problem-driven scientific fields are brought together in the right combinations, as all the incentives

are naturally in place to encourage people to work together (e.g., engineers want more impact; scientists want more solutions).

A curious direction for the future is whether new neurotechnologies or at the very least technology building blocks might be "hiding in plain sight" in the literature. After all, neurotechnology is not a fundamental engineering discipline like mechanical engineering or chemical engineering; rather, it ideally dips into all these other disciplines as needed in order to solve the problem. It is interesting to note that, even a decade or more before a tool comes to prominence, precursors to the tool can sometimes be found in the literature. For example, the use of light-activated ion pumps (microbial opsins) to control a eukaryotic cell was actually achieved in 1994, in a paper where yeast were genetically engineered to produce chemical energy in response to light—a primitive form of photosynthesis, if you will (Hoffmann et al., 1994). This paper preceded the publication that kicked off the use of microbial opsins for optogenetic control of neurons by a full decade (Boyden et al., 2005). Similar stories apply to other inventions of importance in biology and medicine, such as the polymerase chain reaction, which was described in outline form in a paper (Kleppe et al., 1971) a full decade before the physical implementation at Cetus (Saiki et al., 1985). New tools that allow surprises to be mined from the literature, perhaps software based, may be of use in the future for helping generate new technologies. In the meantime, teaching engineers not only about the big problems in neural circuits that we want solved now, but about the ambiguities and unknowns as well, may help them make better inventions not only now, but going forward into the future.

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NOTE: This is an extended version of the essay published in Neuron, with an assortment of references that may be of interest to the reader (although there is no pretense at being comprehensive; the goal is simply pedagogy about the logic of our approach), as well as further analyses, analogies and heuristics.

Designing tools for assumption-proof brain-mapping

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Abstract

There is much excitement in neuroscience about the potential for tools that might enable scalable mapping of brain circuits at the anatomical, molecular, and activity levels. In parallel, new fundamental mechanisms of neural function -- novel transmitters, new cell types and structures, unanticipated genetic and molecular signaling modalities -- are being discovered all the time. This raises a question: how should we design brain mapping technologies so that they can scalably acquire knowledge about mechanisms we already know we want to understand, while taking in stride the novel mechanisms as they are uncovered, so that comprehensive and integrative pictures of brain function, in the end, emerge? Here we discuss the design principles governing mapping technologies so that they can meet these somewhat contrary goals – scalability and flexibility.

Keywords: neural circuits, optogenetics, signaling, connectomics, dynomics, neural codes, cell types

Mapping mechanisms vs. discovering new ones

There has been much recent excitement in neuroscience about the potential for tools that might enable scalable mapping of brain circuits at the anatomical level (i.e., connectomics) (Morgan, 2013; Helmstaedter, 2013; Takemura, 2013), at the molecular level (e.g., transcriptomics) (Lein, 2006; Grange, 2014; Toledo-Rodriguez, 2005; Khazen, 2012), and at the activity level (i.e., dynomics) (Alivisatos, 2012; Prevedel, 2014; Vladimirov, 2014; Kopell, 2014). However, new fundamental mechanisms of neural function -- epigenetic changes that affect memory, gaseous neurotransmitters that sculpt plasticity, retrogradely diffusing cannabinoids that alter synaptic strength, ephaptic coupling that synchronizes oscillations, roles for glia in learning and sleep -- are being discovered all the time. This raises a question that sits at the junction between potential "big neuroscience" projects and discovery-oriented mechanism research: how should one design brain mapping technologies that can scalably acquire knowledge about what we already know we want to understand, while taking in stride the continual uncovering of novel mechanisms?

Imagine "sequencing the genome" in an era when only the nucleotides A, T and C had been identified, but G remained unknown. Except to the most statistically-minded of biologists, the resulting "genome sequences" would be not too interesting. For the brain, the continuous discovery of new mechanisms implies that many neurobiological analogues of G are still around the corner. In other biological fields, like molecular biology, progress is sometimes regarded as resting on the availability of "ground truth" datasets -- datasets that are unambiguous because they are at an appropriately detailed level of abstraction as well as comprehensive. "Ground truth" often took (and continues to take) the form of a reduction to a chemical structure -- complete genomic sequences, as mentioned before, but also other kinds of chemical structures, such as x-ray crystallographic structures. Applying this kind of thinking to the brain would require radically new tools, and furthermore, for some properties of the brain such as patterns of brain electrical activity, it is unclear whether chemical structures are even the proper representation. Accordingly, there are no universally agreed upon paradigms for declaring what datasets are needed to enable the brain to be understood. Currently, there are efforts to understand the brain as a network made of neurons (e.g., in systems neuroscience), as well as efforts to understand neurons as networks of

molecules (e.g., in molecular neuroscience). Much progress has arisen by studying species and circuits, or by developing technologies, that enable bridges to be built between these low- and high-level abstraction levels.

In the first century of neuroscience, well characterized mechanisms of disease were relatively scarce (a state that is now changing rapidly as a result of new tools, such as genome sequencing). Perhaps a result, results sometimes had something of a short half life. This is perhaps most clearly visible in the clinical realm. Julius Wagner-Jauregg, for example, won the Nobel Prize in 1927 for his discovery of a cure for the paralytic dementia caused by late-stage syphilis. His cure involved infecting patients with malaria, and accordingly, this strategy rather quickly left the clinical repertoire (similarly to the therapy developed by his fellow Nobelist Egas Moniz). While these distant examples may seem quaint, even today, treatments for brain disorders are based on high-level behavioral observation and subjective reports, rather than on measurements of underlying circuit changes. This sometimes leads to surprises even about how known treatments might work -- as just one example, it was recently reported that the antidepressants sertraline and paroxetine, long taken to function primarily as selective serotonin reuptake inhibitors, are also potent sodium-channel blockers (Huang, 2006). Given that diseases of the brain often involve multiple, even distributed neural circuits, brain mapping tools will be required to pinpoint therapeutic targets. As described above however, we need to make sure we're mapping the mechanisms that are of importance. Thus the tension between brain mapping vs. new mechanism discovery might be acutely felt in the quest to solve brain disorders.

New mechanisms and the need for mapping them

In the first century of neuroscience, many fundamental mechanisms were revealed concerning the transmission of information from neuron to neuron via chemical synapses, in response to an action potential ("spike") in the presynaptic neuron. Not surprisingly, connectomics and dynomics are focusing on mapping the synaptic connectivity of neural networks, and the spiking activity of neural populations. Yet many other mechanisms of electrical and chemical computation and communication are routinely being discovered, often starting with specific cell types (e.g., interneurons) that strongly utilize a mechanism, or species (e.g., invertebrates) whose simplicity facilitates the discovery of new mechanisms. For example, there exist many non-spiking cells even in mammals that exhibit analog electrical signals (Zhou 1996), as well as cells that exhibit a mixture of spiking and graded potentials (Saszik, 2012). Even in spiking cells of the mammalian cortex, there is evidence that the analog membrane potential at the soma can modulate the impact of a spike on synaptic release (Shu, 2006; Alle, 2006). Thus, mapping the timing of discrete action potentials may reflect only part of the neural code, and full maps that reflect analog signals throughout neural circuits may require new technologies -- imaging, electrophysiological -- that do not yet exist.

Similar questions are being directed at the synapse. Direct electrical connections (mediated by proteins that make up gap junctions) can form local networks among interneurons with similar gene expression profiles (Galarreta, 1999; Gibson, 1999). Gap junctions have been shown to be behaviorally relevant, for example impacting the encoding of an animal's position in space (Allen, 2011). Mapping the dynamics of gap junctions is difficult with existing tools, and might benefit from new technologies. Direct electrical interactions between adjacent neurons -- so-called "ephaptic coupling" -- has been suggested to entrain the spiking of cortical neurons to extracellular electric fields (demonstrated in brain slice, (Anastassiou, 2011)), and has been also shown to support feedforward and lateral inhibition in the cerebellum (Blot, 2014). Ephaptic effects are also strongly implicated in mediating inhibition in the Drosophila olfactory system (Su, 2012). Such couplings of course might not be associated with any observable single anatomical feature (e.g., like a discrete synapse or gap junction), and might have to be understood through emergent analyses of whole circuit morphology (Kim, 2014). Classical neurotransmitters are of course of

great importance in neural communication. But retrograde signaling by cannabinoids and other diffusible messengers -- from postsynaptic to presynaptic neuron -- is now well established (Kreitzer, 2001; Wilson, 2001). Nitric oxide (NO) functions as a diffusible gaseous messenger that can pass through cell membranes and can induce synaptic plasticity upon coincidence within 10 ms of NO stimulation of a presynaptic neuron and calcium elevation in a postsynaptic neuron (Arancio, 2001; Lev-Ram, 1997). Even membrane lipids themselves can modulate the conduction properties of potassium channels (Schmidt, 2006). Indicators for gases and other hard-to-tag molecules might be needed to understand how these non-classical transmitters function in neural circuits.

Another mapping effort is the quest to enumerate the kinds of building blocks of the brain; one of the early flagship projects of the BRAIN initiative is to assemble a list of neuron types, for example. Glia, historically viewed merely as support cells for neurons, participate in neural computation, including sculpting neural codes, shaping memory consolidation, and affecting the impact of sleep (Araque 1999; Perea 2009; Lee 2014; Halassa, 2009). Tools for mapping glial circuits might easily complement those for mapping circuits of neurons. For neurons, mapping transcriptomes, for example, has been proposed, as indicators of which genes are "on" or "off" in a cell, and thus providing a basis for classifying cell types. But on and off is perhaps not enough: genes may be alternatively spliced, e.g. in pain neurons calcium channels are spliced so as to increase N-type calcium current (Bell, 2004). Alternative splicing of some neurexin gene products has been shown to modulate AMPA receptor trafficking and cycling (Aoto, 2013), and may influence whether such a gene product ends up at glutamatergic vs. GABAergic synapses (Chin, 2006). Beyond static transcriptomic snapshots, some evidence suggests that cells can change their type over time, perhaps gently calling into question the notion of cell type itself. Indeed, the splicing of genes ranging from neurexin-1 (Iijima, 2011) to the BK potassium channel (Xie, 2001) are regulated by neural activity and calcium signaling. In addition to splicing as a mechanism for transcriptomic variation, neurons in adult animals can alter which neurotransmitters they use for signaling in response to environmental cues, leading to behavioral consequences including altered mood (Dulcis, 2013; Dulcis, 2008). Beyond cells and their interconnections, the extracellular matrix has been shown to play a role in the stability of acquired fear memories (Gogolla, 2009; Hylin, 2013). It has been suggested that new tools to probe the extracellular matrix may be very helpful for understanding memory (Tsien, 2013). Along these lines, new circulatory systems in the brain are being discovered: recent years have seen the discovery of novel structures, like the glymphatic system, which generates a flow of the cerebrospinal fluid through the brain (Iliff, 2012), which seems to operate to clear waste products during sleep (Xie, 2013). Mapping tools should, ideally, be able to take into account these various non-neuronal building blocks as they are found.

Mapping tools that cannot take into account new mechanisms are essentially making the assumption that they are not needed to understand the problems at hand. Certainly this may be the case for some problems, e.g. understanding a few seconds of neural dynamics might not require detailed understandings of how that neural activity regulates downstream gene expression over timescales of hours to days. But, when developing new tools, it is useful to at least consider whether they can easily be extended to new problems involving other mechanisms. A related issue is that assumptions are built into the experimental methodologies, technologies, and approaches employed by neuroscientists. Fluorescent calcium indicators, for example, that are commonly used to optically monitor the dynamics of neuronal electrical signaling, bind to calcium and hence lead to a buffering of the intracellular calcium concentration (Maravall, 2000), which may alter neural activity or its observability. Fixation, permeabilization, and antibody staining in fixed tissue can result in artifactual alterations of protein localization compared to the living state (Schnell, 2012). Acute brain slices can sprout new synapses after slicing (Kirov, 1999), potentially confounding interpretation of slice-based network studies, and chilling of the brain may be at least partly to blame (Kirov, 2004); cutting slices without chilling has been reported to not cause synaptic density to change as much (Bourne, 2007), and more protective cutting solutions have been proposed and

utilized (Zhao, 2011). Many studies of neural coding have been performed in the anesthetized brain, but comparisons of neural codes in awake versus anesthetized brain have suggested mechanistic differences in awake vs. anesthetized brain, e.g. a much stronger role of inhibition in the awake brain (Haider, 2013), and (it goes without saying) profound changes in behavior (e.g., (Iwata, 1987)). Of course, awake animals may be more likely to have variability in behavioral state, requiring methods for controlling for variation in behavioral or physiological responses. Likewise, many cells in the brain spike only infrequently, and are effectively "silent" most of the time (Shoham, 2006); any method of neural recording that must first search for a strong signal before it can begin capturing reliable data – such as many common methods of single-unit recording – will be biased towards the minority of loud cells over the majority of silent cells. Designing brain mapping technologies and experimental approaches to avoid methodological assumptions may be difficult, but may reduce the amount of questioning of mechanisms in different species or circuits, and increase the reliability of the maps thus obtained.

Tools for assumption-free brain mapping

Analyses suggest that we are only near the beginnings of the scaling curves for brain mapping technologies (Marblestone, 2013; Zador, 2012; Pollock, 2014). Thus, the timing is right to elucidate design principles for neurotechnologies that work backwards from the fundamental properties of the brain, and are equal to the challenge of mapping their mechanisms, rather than working forwards from what we know how to do. In particular, we want to design technologies to take new mechanisms in stride, minimizing the reliance on assumptions that may later be shown to be false.

In physics, it is easy to solve the hydrogen atom mathematically: calculating the orbitals and energy levels is done in university quantum mechanics courses. At the other extreme, for gases with 10^{23} identical particles, statistical mechanics allows one to derive simple relationships like the ideal gas law. But physics struggles with "mesoscale" problems, e.g. calculating how a protein folds, or the exact fluorescence spectrum of an organic dye, or how a pile of sand tumbles after one final grain is added. That is because mesoscale problems have too many different and distinct components and interactions to be amenable for a purely statistical analyses, and yet that diversity of components and interactions makes an exact solution also incalculable. The brain presents some of these difficulties: it is organized with nanoscale precision, yet neural circuits can span vast regions, even tens of centimeters or larger. Individual signaling events can last milliseconds, yet learning or development or disease progression can take years. Thus neuroscience is a kind of "mesoscale biology". To map a mechanism in a neural circuit, a mapping technology must both be precise spatiotemporally (e.g., recording millisecond events, observing nanoscale connections) and yet scale to tens of centimeters (e.g., the circuits involved with perception or memory or attention).

For the case of neural activity, it will likely be important to map neural activity at the single neuron level or even more precisely. It has been shown that stimulating even a single neuron with the right pattern and in the right context can influence behavior (Houweling, 2007) or change whole brain dynamics (Cheng-yu, 2009). Observing the propagation of neural activity through parts of neurons, e.g. in the dendritic tree, may also be required to understand how neurons integrate inputs towards their neural code outputs. For example, dendritic spikes (Smith, 2013; Palmer, 2014) can achieve local computations, the significance of which to neural outputs is still being explored. Dendritic signals in cortical interneurons that reflect specific properties of visual stimuli have also been observed (Chen, 2013), and individual dendritic branches of specific retinal amacrine cells have been shown to reflect direction selectivity (Euler, 2002). Despite the need for such resolution, however, it is also clear that neurons in widely distributed circuits are operating in close coordination, and thus technologies for brain activity mapping must span these large spatial scales. As just a few out of a large number of examples: the striatum changes earlier than, and may serve a training role, for the prefrontal cortex, during associative learning tasks (Pasupathy, 2005);

primary auditory cortex neurons exhibit oscillations that are reset by somatosensatory inputs (Lakatos, 2007); replay of memory-associated spike patterns during sleep are coordinated between cortex and hippocampus (Ji, 2006); conditioned fear behaviors involve distributed plasticity, all the way to the facilitation of the very first synapses in the olfactory bulb (Kass, 2013; Abraham, 2014), and so forth. Thus, activity maps might need to have spatial precision at scales as fine as, or even finer than, single neurons, yet span entire circuits. The temporal precions is also demanding, one millisecond or even better. It has been shown that rats can discriminate electrical pulses to the barrel cortex timed with ~1 ms precision, for example (Yang, 2012), and physiological phenomena ranging from spike-timing dependent plasticity (Markram, 1997) to reproducible spike timing by single neurons (Mainen, 1995) also depend on millisecond-timed events. Other evidence is provided by anatomical findings which are surprising -- for example, the equal axonal conduction delays of olivocerebellar axons throughout the cerebellum suggests an anatomical mechanism for equalizing timing across a region (Sugihara, 1993). Thus, increasing the temporal precision as well as the spatial precision of imaging as well as electrophysiology tools continues to be a high priority, ideally to single-cell or even subcellular resolution, at the millisecond timescale.

In the connectomic and molecular mapping space, there is similarly a problem of achieving fine discrimination of synaptic, gap junction, and other communicatory apparatuses, while scaling to the spatial extent of behaviorally or disease-relevant circuits. The requisite spatial resolution for assumption-free structural brain mapping is likely in the tens of nanometers or even better (if the goal is to resolve individual proteins, important to understand synaptic strength and dynamics for example). Even if proteins are not under consideration in a mapping tool, it is clear that axons often are <100 nm in diameter (Mishchenko, 2009), and closely apposed dendritic processes in neuropil often come within this distance of one another (Michael, 2007). Resolving single synapses in dense neuropil from their neighboring synapses also appears to require 50-100 nm spatial resolution (Mishchenko, 2010; Micheva, 2007). Thus nanoscale imaging systems that can scan quickly will be required; rather than just going for precision, or speed, of an imaging system, the ideal systems will need to do well along both performance axes.

Along the lines of molecular mapping, mechanisms ranging from prion-like effects for memory encoding (Si, 2003; Si, 2010) to epigenetic effects on memory consolidation (Levenson, 2005) to post-translational modifications of synaptic receptors (Lee, 2000) to molecular switching within aggregated kinase multimers (Lisman, 2002), and beyond, have been described as potential mechanisms relevant to neural operation. The ability to systematically map these and other molecular mechanisms will likely require new kinds of observable tags and imaging systems.

Integrativeness of tools

Above we discuss activity maps and connectomic maps in isolation. But an ideal technology would be able to map many kinds of variables (anatomical, molecular, physiological) on the same instantiation of a nervous system (e.g., a single animal's brain). There is an increasing degree of evidence for fine structure within individual nervous systems, for example, which suggests that averaging unimodality observations over many animals may not always lead to an accurate depiction of the nervous system (Marder, 2011). Surprising organizational features of the connectivity of circuits may only be apparent after looking at many neurons within a single circuit and their topology of connectivity. For example, neurons that are connected to each other in the rodent cortex, may also be more likely to have common inputs from other neurons within the cortical microcircuit (Song 2005; Yoshimura, 2005). Pairwise connectivity analyses would not detect these three-way correlations: the Song et al. paper analyzed quadruple patch clamp data, and the Yoshimura et al. paper presented experiments with dual cell patch clamp in conjunction with optical stimulation of cells through cortical slices -- requiring great skills or novel technology to achieve. As another example, there exist strong correlations between the expression levels of potassium channel genes in PD1 vs. PD2 neurons within individual crabs' stomatogastric ganglia, even though the gene

levels are highly variable across animals (Schulz, 2006; Schulz, 2007). Thus, measuring PD1 physiology in one set of crabs, and PD2 physiology in a second set of crabs, would not reveal this subtle coordination of potassium currents. Such correlations might arise, perhaps unsurprisingly, from homeostatic tuning rules that help circuits self-organize (O'Leary, 2013). Of course, within a circuit, self-organization via plasticity mechanisms occurs to insure network operation within the evolutionarily selected bounds of behavior, but neurons in two different brains would not experience any such interaction. Such mechanisms of multi-variable homeostasis could prove to be important organizing principles of neural circuitry.

Correlations between multiple variables can of course be seen even at the population level. It has been observed that gene expression pattern and projection pattern are linked variables for neurons in the cerebral cortex, for example (Sorenson, 2013); technologies that only reflect connectomic or only gene expression patterns, and not both, would miss such linkages. As mentioned before for the case of gap junction connected interneurons, interneurons expressing key genetic markers are more likely to be gap junction connected to each other, than interneurons of different genetic classes (Galarreta, 1999; Gibson, 1999). Studies linking cell shape and gene expression pattern have also revealed rich interdependencies, although the mapping is often not one-to-one between single markers and overt morphologies (Markram, 2004), raising the question of how best to represent the geometry of a cell for informatic analysis, and the converse question of how many genetic markers it takes to define a cell type. More complete descriptions of cell shape and gene expression, as well as mechanistic links between the two, would be valuable to map in intact circuits. Ideally, of course, we could map molecular, connectomic, and activity patterns -- including new mechanisms governing or contributing to each -- through circuits. Integrative mapping technologies, must be compatible with each other -- e.g., if you want to acquire an activity map from a brain, and then obtain its molecular and anatomical maps, you ideally would not alter the molecular or anatomical maps in the first experiments on activity mapping. One may hope that integrative technologies, because they must work together, will also be modularly applicable, and thus extensible to the mapping of new mechanisms.

Towards systematic, assumption-free investigation of the brain

In some situations, solving a bigger problem can be easier – not harder – than solving a smaller one. Because neuroscience is not a single goal – after all, which is more important, solving Alzheimer's or understanding memory? – there has been a tendency to fragment investigations across a wide variety of systems, problems and approaches, each tackling only a small subset of brain mechanisms. But even if we understand most of the mechanisms along most axes of brain function, the brain may remain fundamentally unpredictable, as well as unexplainable, until we achieve comprehensiveness. This idea can be crudely visualized in terms of a high-dimensional space of brain functionality. In this analogy, if the brain has N dimensions of functionality, and we understand 80% of the mechanisms along each dimension, then the total "volume" of brain function which can be explained is only 0.8^N, or 20% for N = 7. The amount of human effort required to analyze the brain element by element may be smaller than that needed to engineer scalable mapping technologies which would enable analyses of the entire system; likewise the effort needed to engineer a scalable mapping method capable of mapping one property – like synaptic connectivity – may be equal to or even greater than that required to engineer a more integrative technology that can integrate measurements of multiple co-varying properties at once – like connectivity and gene expression.

While hypothesis-driven research is important and will ultimately be essential to derive powerful explanatory and predictive theories of brain function, at the present time there is also great value in hypothesis-independent, yet highly systematic, mapping and exploration of the brain. Until we understand appreciate the full range of biological variables that govern brain computations, the testing of specific

hypotheses may sometimes lead us to lose track of the forest for the trees. Systematism, in this context, means quantitativeness and comprehensiveness, but not necessarily the testing of a specific pre-defined hypothesis.

A related issue is the need to attack a given hypothesis or problem at the right time, when the necessary technical, conceptual, and empirical foundations are in place. It would not make sense to attempt a large-scale project to land on the moon, if the year is 1600. A prize for a moonshot design, in this case, might be a distraction - perhaps leading researchers to explore fast-track methods of tying kites to chairs or balloons to carts – rather than letting them explore the fundamentals that are ultimately needed to make moon landing a possibility (i.e., calculus, classical mechanics, aerodynamics, thermodynamics). When Kennedy announced the moon project, note well: he was not advocating a "high risk" or unplanned exploration. Rather, engineers had already sketched out much of the fundamental paradigms that are needed to make space travel possible (and indeed, already accomplished a number of missions of various kinds). The moon shot would be based on known physics, and Kennedy in his speech hinted that if the United States didn't go, others could probably get there first. For the brain, one might argue that a foundation in the engineering of scalable technologies for mapping, recording and controlling whole brain circuits is a necessary foundation for future efforts. Encouragingly, we can already begin to sketch the forms of such technologies using known principles of physics and engineering.

Tightening the loop between discovery and mapping

How can one design assumption-resistant, scalable, brain mapping technologies that can be extended to new mechanisms as they are found? It is important to work backwards from the properties of the brain that need to be mapped, and then to design the technology to meet that need. But this can be limiting if it ignores other mechanisms not yet found. One strategy is to forge new models of collaboration that bring together people from different backgrounds so that technologies are designed without overtly ignoring any potential mechanisms that might need to be considered. It also requires systematic thinking in design: for example, making roadmaps of all possible directions, before picking a path, has in our experience helped narrow focus on paths that obey physical laws and match the complexity of the brain. In this "architecting" strategy, we actively recruit experts on different potential technology building blocks, bringing them together to consider not just the quantitative evaluation of the power of a path, but what creative ideas or intuitions might apply in the context of an integrative technology. For example, we recently completed a study of how different modalities -- optical, radiofrequency, ultrasonic, biomolecular, and so forth -- might contribute to brain activity mapping (Marblestone, 2013). Working across 14 different departments and organizations, we collectively mapped out a variety of paths. Subsets of the collaborative then went on to achieve specific milestones, e.g. the adaptation of lightfield microscopy to whole-organisms neural activity imaging in C. elegans (Prevedel, Yoon, et al., 2014). As a second example, fusing robotics and automation to one of the most powerful, yet most art form-like skills in neuroscience, whole cell patch clamp neural recording, yielded a collaboration that invented an algorithm and robot for automated in vivo whole cell patch clamping in live mouse (Kodandaramaiah et al., 2012). Thus, although neurotechnology may seem omnidisciplinary and thus daunting, requiring tool inventors to know a significant fraction of the engineering enterprise, bringing together the right teams has already proven itself to yield impactful technologies. "Architecting" works best often when people from solution-providing engineering fields and problem-driven scientific fields are brought together in the right combinations, as all the incentives are naturally in place to encourage people to work together (e.g., engineers want more impact; scientists want solutions).

A curious direction for the future is whether new neurotechnologies or at the very least technology building blocks might "hiding in plain sight" in the literature. After all, neurotechnology is not a fundamental engineering discipline like mechanical engineering and chemical engineering; rather, it

ideally dips into all these other disciplines as needed in order to solve the problem. It is interesting to examine, even a decade or more before a tool came to prominence, the precurors to the tool. For example, the use of light-activated ion pumps (microbial opsins) to control a eukaryotic cell was actually achieved in 1994, in a paper where yeast were genetically engineered to produce chemical energy in response to light -- a primitive form of photosynthesis, if you will (Hoffman, 1994). This paper preceded the publication that kicked off optogenetic control of neurons, by a full decade (Boyden, 2005), and has been cited (at this moment) only 0.6% as many times. Similar stories apply to other inventions of importance in biology and medicine, such as the polymerase chain reaction, which was described in outline form in a paper (Kleppe, 1971) a full decade before the physical implementation at Cetus (Saiki, 1985). New tools that allow surprises to be mined from the literature, perhaps software based, may be of use in the future for helping generate new technologies. In the meantime, teaching engineers not only about the big problems in neural circuits that we want solved now, but about the ambiguities and unknowns that will require new technologies, may help them make better inventions not only now, but going forward into the future.

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