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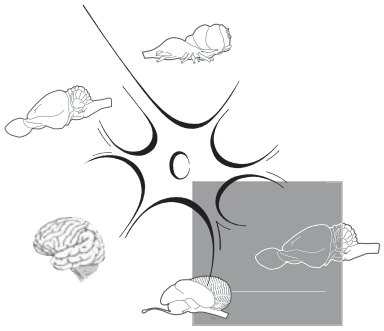

Panoptic Neuroanatomy: Digital Microscopy of Whole Brains and Brain-Wide Circuit Mapping

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Philosophers have pointed to the prevalence of the 'mereological fallacy' in contemporary neuroscience (attributing the properties of the whole to a part, a.k.a. the blind men and the elephant fallacy) [Bennett and Hacker, 2003]. Attention is often focused on the details of individual brain systems. Specific sensory or motor pathways are well studied and, increasingly, modulatory systems are as well (reward, sleep). However, integrative study of the whole nervous system is rare, and the knowledge remains fragmented. Overarching models, in the tradition of Skinner or Pavlov, are also oversimplified and therefore unable to grapple with the full complexity of brains and behaviors. In addition to focusing on brain subsystems, there is an increasing focus on specific model organisms, which makes this problem worse [Manger et al., 2008].

A basic reason for the difficulty of integrative study is instrumental. Despite technical advances, we do not have the means of simultaneously measuring all relevant variables (e.g. the state or activity of all neurons, including subcompartments). This is unlikely to change, due to basic physics/physiology limitations. Whole-brain imaging techniques (PET, fMRI and MEG/EEG) have fundamental spatial and temporal resolutions far removed from the individual neuron or action potential; relatively few electrodes (compared to the total number of neurons) can be put into the brain at a time due to mechanical/physiological constraints, and the live brain of any reasonably sized adult vertebrate can be probed with optical methods only over short distances because of optical scatter in live-brain tissue.

There is, however, one level of analysis at which studying the entire nervous system as a unit is possible: neuroanatomy. There is, in principle, no limitation to studying the entire ex vivo brain, while still being able to access microscopic detail down to the level of individual proteins or organelles. Addressing the integration problem in neuroscience is a primary reason for such whole-brain, multi-scale neuroanatomy. Just as genomes have provided an integrative framework for cellular/molecular biology, so can whole-brain anatomical datasets provide a unifying informational framework for neuroscience. Neuroanatomy has often been referred to in a derogatory sense as 'largely descriptive'. In contrast, digitized whole-brain datasets promise an era of quantitative analysis, while also providing a geometrical/spatial informational framework addressing the problem of discordant nomenclature [Bohland et al., 2009a] that hinders the integration of knowledge. Another rationale, also in parallel to genomic analysis, is uncovering the mechanistic basis of neurological disorders. Brain-wide digital neuroanatomy will produce reference datasets for brain circuits and cytoarchitecture that can be compared to the corresponding datasets for animal models of brain disorders. This will shed light on the largely unsolved problem of which neural circuit changes characterize neuropsychiatric disorders, and provide a valuable intermediate phenotype for genetic analysis.

Perhaps the most important scientific rationale for the new effort is the study of brain evolution. Comparative genomic analysis has fundamentally advanced our understanding of evolution; similarly, brain-wide digital datasets for circuits and cytoarchitecture could resolve long-standing controversies in brain evolution, such as the homologies between avian and mammalian brains [Jarvis et al., 2005]. With the addition of whole-brain developmental datasets in many species, we foresee a new era of quantitative 'evo-devo' style analysis in neuroscience, which also promises to counteract the increasingly narrow focus on a few model organisms.

The last decade has seen rapid developments in whole-brain digital neuroanatomy. Amongst the first such datasets were

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Edward G. Jones' rich inventory of comparative neuroanatomy at high resolution [Jones et al., 2011] (http://www.brain-maps.org) and the Allen Gene Expression Atlas of mouse brain [Lein et al., 2007]. Circuit mapping is inherently more complex than mapping gene expression, as connectivity information can grow with the square of the number of nodes. Complete mapping of the local microcircuitry in small portions of the neuropil is possible using electron microscopy, but this method is presently not scalable to whole brains except in very small brains (such as in flies or worms). Here, we confine our discussions to light-microscopy-based data [Helmstaedter and Mitra, 2012].

Recently, a proposal was made for whole-brain circuit mapping at a 'mesoscopic scale' of analysis, using tracer-based neuroanatomical tractography and light microscopy [Bohland et al., 2009b]. In this 'grid-based' approach that resembles genomic sequence assembly using a shotgun method, a number of injection locations are chosen to cover the entire brain, each location receiving a tracer injection in individual mice. The resulting datasets are subsequently coregistered to a common spatial framework to provide a brain-wide connectivity atlas. Three projects following this basic proposal are currently underway at the Cold Spring Harbor Laboratory (http://mouse.brainarchitecture.org), Allen Institute for Brain Sciences (http://brain-map.org) and University of California, Los Angeles (http://mouseconnectome.org). While employing the same overall grid-based strategy, the projects have significant differences in the tracers, genetic constructs and imaging strategies used that make the datasets complementary. In the Mouse Brain Architecture Project, a total of four different tracer types, two anterograde and two retrograde, are injected at 262 grid locations distributed over the left hemisphere of an adult male C57BL/6 mouse, with just a single tracer type and injection location per mouse. Preliminary datasets are available from the project website (http://mouse.brainarchitecture.org). Also available are auxiliary datasets employing a variety of cytoarchitectonic markers.

While the mouse has become the most widely studied model species due to the wide availability of transgenetic constructs and pharmaceutical research, studies of other species are essential to enable comparative and evolutionary analysis. Just as in genomics, it is imperative that we also have whole-brain neuroanatomy projects characterizing the circuits and cytoarchitecture of other species, covering major taxa on the phylogenetic tree. Efforts in this direction have begun, pioneered by the late E.G. Jones [Jones et al., 2011] (http://brain-maps.org), but require significantly more support. Gene expression maps for the human brain are now available (http://brain-map.org). A project to map gene expression in the avian brain is underway (http://zebrafinchatlas.org) and whole-brain cytoarchitectonic datasets for the Zebra Finch are available (http://zebrafinch.brainarchitecture.org). A digital whole-brain circuit-mapping project in the common marmoset monkey *(Callithrix jacchus)* is also in progress (http://marmoset.brainarchitecture.org), enabled by a high-quality collection of digitized histological materials (http://marmoset-brain.org). Marmosets are ideal nonhuman primate species for whole-brain digital neuroanatomy [Paxinos et al., 2012], and may provide a much-needed model for understanding the development of brain circuits involved in cognition, given their short developmental cycle and existing technology for generating stable transgenic lines [Sasaki et al., 2009].

Whole-brain neuroanatomy and circuit mapping are consequent to three major technological advances:

(1) The exponentially decreasing costs of storage and computation. Digitizing a mouse brain at a resolution of approximately 1 μm generates about a terabyte worth of data (a human brain at the same resolution is a petabyte of data). Twenty years ago, a terabyte of disk storage would have cost close to USD 1 million. At present it costs less than USD 100.

(2) Increasing automation, with digital slide-scanning microscopes or serial block-face sectioning microscopes being employed to acquire the primary image data. These, as well as automated on-slide histochemistry and cover-slipping machines developed for use in clinical histopathology are serendipitously enabling whole-brain neuroanatomy by automating repetitive labor-intensive tasks.

(3) Molecular biology methods allow definition of specific cell types for analysis, through the insertion of transgenes into neurons, using transgenic animals, intracerebral injection of viral vectors carrying the transgenes or fluorescent reporters into the brain, or a combination of the two. These fluorescent reporters provide bright intrinsic labeling, obviating the complexities of histochemical processing.

In addition to the experimental acquisition, digital analysis and computational integration of the data is equally important. The challenges include detection of cells and processes, coregistration of sections from a single brain or brains across individuals, statistical characterization of cytoarchitecture and stereological considerations, quantification and analysis of individual variations from brain to brain for the large, anisotropic data volumes. All of these have to be done on petabyte scale data, requiring 'big data' computational infrastructure. In addition, the web infrastructure required for making these data available through the internet is substantial. Progress has begun on all these fronts, and we can expect these computational issues to increasingly take a central role as the field advances.

Given the current lacuna in our knowledge about the complete circuit diagram of any vertebrate brain, the need for mapping out brain circuitry at an anatomical level may be obvious. Nevertheless, skeptics will point out that without further 'functional' characterization (in terms of the activity of the neural network, the traditional subject of neurophysiological investigations), even having the whole brain circuit diagram will not be adequate. Often cited in this context is the example of the nervous system of the roundworm *Caenorhabditis elegans*, which has been available for decades from analysis of electron microscopy image data, but arguably has not led to an 'understanding' of how this worm's brain works. In addition, it is pointed out that the brain is plastic, responding to experience, raising questions about a static circuit diagram as well as bringing up the important issue of individual variability.

However, 'function' can be layered on to the underlying informational scaffolding of the circuit geometry and topology. This can be done using the same ex vivo techniques that permit detailed whole-brain analysis by adding information about neurotransmitters and receptors through the usage of immunohistochemistry or transgene labels. Physiological methods can be combined with whole-brain neuroanatomical methods by first performing electrophysiological studies or optogenetic fMRI to map neuronal activity (though at a coarser resolution) and then registering these functional data to digital templates generated from neuroanatomical analyses.

Individuals belonging to the same species are expected to share a common, genetically determined, species-typical neuroanatomical substrate, which is modulated by effects of phenotypic or adult plasticity. In fact, this is part of the rationale for meso-scale circuit mapping: it is at this level, familiar with the brain atlases of classical neuroanatomy, that one expects a species-typical circuit diagram that is robust in the face of individual variation and (normal) environmental perturbations. Digital whole-brain datasets will permit both the determination of this underlying, 'reference' circuitry and an unprecedented quantitative study of the individual variations around the species-typical reference.

Similar concerns were also raised in the early days of whole-genome sequencing, with gene transcription or translation playing the roles of neurophysiological activity. In that case, whole-genome analysis of RNA transcription and regulation (as in the ENCODE project) has naturally followed the initial whole-genome sequencing efforts. Once whole-brain neuroanatomy provides an informational reference framework, whole-brain physiological analysis will naturally follow. Coupled with high-throughput behavioral analysis, this will enable us to address the 'blind men and the elephant' problem in neuroscience, of excessive focus on narrow aspects of the nervous system. Such analysis is already underway for specific brain circuits; the generalization to entire brains is a logical next step.

This new era of computationally enabled, brain-wide neuroanatomy at light microscope resolution, a 'pan-optic neuroanatomy' of entire brains, will help us bring the same integrative approach to the study of the nervous system as the nervous system itself brings to the world, binding the multifaceted and complex array of activities of neurons into one experiential whole.

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