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Toward large-scale connectome reconstructions

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Recent results have shown the possibility of both reconstructing connectomes of small but biologically interesting circuits and extracting from these connectomes insights into their function. However, these reconstructions were heroic proof-of-concept experiments, requiring person-months of effort per neuron reconstructed, and will not scale to larger circuits, much less the brains of entire animals. In this paper we examine what will be required to generate and use substantially larger connectomes, finding five areas that need increased attention: firstly, imaging better suited to automatic reconstruction, with excellent *z*-resolution; secondly, automatic detection, validation, and measurement of synapses; thirdly, reconstruction methods that keep and use uncertainty metrics for every object, from initial images, through segmentation, reconstruction, and connectome queries; fourthly, processes that are fully incremental, so that the connectome may be used before it is fully complete; and finally, better tools for analysis of connectomes, once they are obtained.

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Introduction

A resurgence of interest in high-throughput, high-resolution quantitative neuroanatomy, known as connectomics, has been accompanied by a passionate debate [1–5]. The proponents of this approach believe that knowing all synaptic connections in the brain will lead to understanding in ways that any lesser detail cannot. The opponents of this approach argue that it is largely a distraction which will not lead to advances in our understanding of brain function. Until recently, the debate about the role of connectomes in neuroscience has been largely theoretical as very few connectomes of biologically interesting circuits have been actually reconstructed.

In the last couple of years, new 'experimental data points' have been obtained and can provide experimental grounding to the debate. Thanks to pivotal technological advances, several connectomes of biologically interesting circuits have been reconstructed. The prime example is the connectome module of the fruit fly medulla [6••], which allowed identification of the neurons and the circuit motif involved in motion detection thus setting the stage for finally answering the sixty year old question about the biological implementation of the elementary motion detector (EMD). Others are the connectome of *Caenorhabditis elegans* male [7•], and the reconstruction of the mouse retina [8••].

In this review, we first revisit the connectomics debate in the light of new experimental data and demonstrate that stereotypical connectomes can indeed provide insight into neural computation when used in combination with other approaches. We further argue that, in less stereotypical connectomes such as those in the mammalian cortex, connectomics can help identify stereotypical features such as circuit motifs. One such famous but still experimentally unproven circuit motif has been proposed by Hubel and Wiesel (HW) to explain the emergence of orientational selectivity in the visual cortex from orientationally nonselective thalamic inputs. As the relevant cortical neurons span the volume larger than has been reconstructed before, we next discuss technological developments necessary to obtain this 'experimental data point'.

What are connectomes good for?

One obvious use of connectomes is as brain atlases: to identify neuronal pathways, individual neurons, and their upstream and downstream synaptic partners. Such information helps focus investigations using other approaches on relevant targets. The *C. elegans* connectome has been irreplaceable in guiding the work in the field. For example, the knowledge of connections allowed reverse engineering the circuit for forward and backward movement in the worm by ablation of identified neurons [9]. Of course, the usefulness of connectome atlases is restricted to the circuits highly stereotypical among animals, as it is in *C. elegans*, the visual system of *Drosophila*, and the long-range connections, such as those among cortical areas, in vertebrates.

How much can connectomes tell us about the function of neural circuits? The *C. elegans* connectome [10,11] is often given as a negative example of not revealing much about the mechanism of neural computation up until now. However, there are several reasons, specific to *C. elegans*,

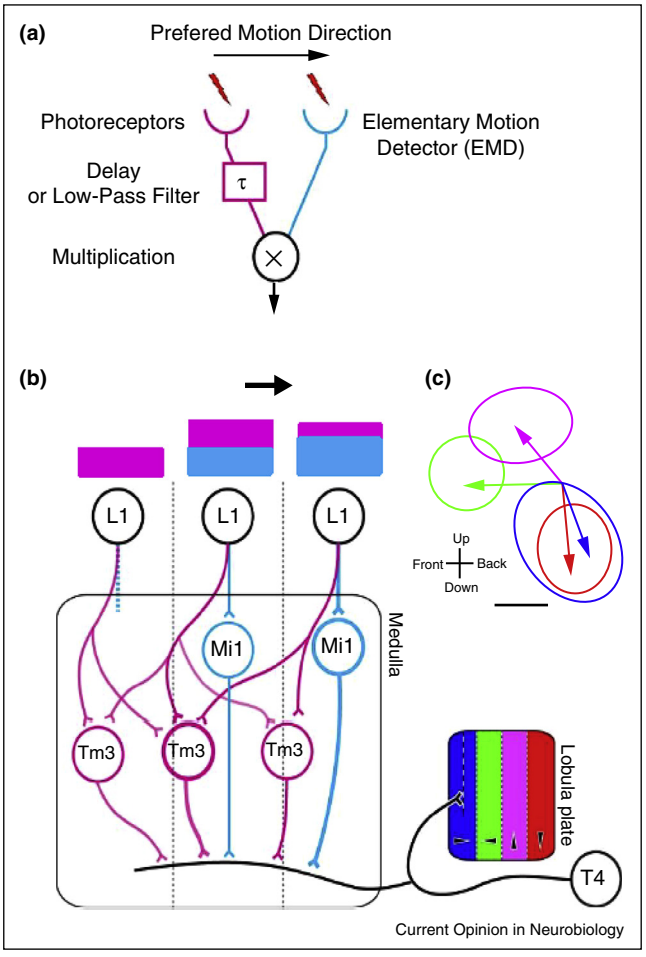
that could account for this: firstly, up until recent advances in Ca imaging, the difficulty of electrophysiology in the worm led to the deficit of physiological data, which is necessary to inform and test computational models; secondly, because of the low processing depth of the *C. elegans* nervous system, many behaviors are generated by a combination of neuronal activity with mechanical properties of the body and the surrounding medium. This necessitates complex multisystem models which are only partially informed by the connectome; and finally, evidence exists that worm neurons are highly compartmentalized and perform local computations, thus having not only multiple inputs but also multiple output signals. In such a situation, the wiring diagram where nodes represent whole neurons is less informative than for neurons with a single output signal, as is often the case in vertebrates.

Perhaps the most impressive example of connectomes playing a central role in reverse engineering neural computation is the discovery of an EMD circuit motif. More than half a century ago, Hassenstein and Reichardt (HR) [12] proposed a famous but anatomically inexplicit model of motion detection, Figure 1(a). Despite intensive experimental and theoretical investigations [13], it has not been clear whether this model is a correct mechanistic description of the biological circuit. Indeed, behavioral data are not sufficient since the same behavioral output can be generated in many ways. Because electrophysiology is difficult in small insects and there are many different cell types in the relevant part of the fly brain, their responses could not be fully characterized. Even if optophysiology could be used to record the activity of all the neurons, one would not know how to interpret it without knowing the connectivity.

Even demonstrating that some of the inputs conform to the motif is not sufficient, since it remains possible that the operation is also influenced by some yet unmapped input. Therefore, a full mapping of synaptic inputs or a dense connectome is necessary. In turn, the full connectome cannot be determined using light microscopy and requires laborious serial electron microscopy (EM) reconstruction. Light-level anatomy and morphology of the neurons is not sufficient since these cannot map out circuit motifs, or even determine the cells involved, with certainty.

To identify cell types implementing the two arms of the EMD circuit motif [6••] used the reconstructed connectome module along with the identity of cell types providing input to the EMD [14,15]. Because the HR EMD must combine signals arriving from different points in the visual field they predicted the displacement between the anatomical receptive fields implemented by these cell types. This prediction was confirmed by additional tracing, providing strong support for the suggested circuit

Figure 1



Theoretically proposed and anatomically discovered EMD. **(a)** Schematic circuit of the rightward motion component of the Hassenstein–Reichardt (HR) EMD. Light input (lightning bolt) into the left photoreceptor is signaled with a delay (magenta channel) relative to the right photoreceptor (cyan channel). For a rightwards moving object, signals from both photoreceptors will arrive at the multiplication unit closer in time to each other, and therefore become nonlinearly enhanced (and vice versa for leftward moving objects). As a result, the network responds preferentially to rightward motion. **(b)** 1D cartoon of a biological circuit for motion detection in the fly optic medulla [6••] L1 neurons conduct signals from the photoreceptor to the EMD but are not directionally tuned. T4 is a directionally tuned neuron serving as an output of the EMD. According to the connectome, Mi1, Tm3 neurons are the two conduits between L1 and T4 resembling the two arms of the HR EMD. Unlike in the HR EMD (a), in the actual connectome each T4 receives inputs via Mi1s and Tm3s from multiple L1s and hence from multiple locations in the visual field. After tracing their synaptic connections we found that Mi1 and Tm3 mediated components (cyan and magenta bars on top) of a T4 anatomical receptive field are displaced in the direction of motion preference of that T4 determined by its arborization layer in the lobula plate. **(c)** Mean displacement vectors averaged over all T4s terminating in each lobula plate layer are consistent with the direction preference of the 3 out of 4 layers. Ellipses show confidence intervals due to tracing errors. Scale bar: 0.1 of the inter-ommatidial angle.