

Specificity and randomness: structure–function relationships in neural circuits

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A fundamental but unsolved problem in neuroscience is how connections between neurons might underlie information processing in central circuits. Building wiring diagrams of neural networks may accelerate our understanding of how they compute. But even if we had wiring diagrams, it is critical to know what neurons in a circuit are doing: their physiology. In both the retina and cerebral cortex, a great deal is known about topographic specificity, such as lamination and cell-type specificity of connections. Little, however, is known about connections as they relate to function. Here, we review how advances in functional imaging and electron microscopy have recently allowed the examination of relationships between sensory physiology and synaptic connections in cortical and retinal circuits.

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Introduction

One of the major challenges in neuroscience is to make and test realistic models of information processing in neuronal networks. The problem remains largely unsolved, even for networks such as the retina, arguably the best understood part of the central nervous system, or the visual cortex, perhaps the most studied cortical area. The biggest impediment to our understanding is that we have little idea how connections between neurons in a local circuit carry out specific computations. Examining the relationship between cellular function and network structure — between sensory physiology and specific synaptic connectivity — has the potential to address this problem.

What we discuss as ‘specificity’, however, should be clear. Most neural circuits have *topographic specificity*, for

instance when axons selectively target brain regions or neuronal lamina. Specific connectivity can alternatively be interpreted as the selective targeting of synapses onto particular cell types or sub-cellular compartments: examples of *cell-type specificity*. Here, we concentrate on *functional specificity*, as it relates to information processing in neuronal circuits.

For example, do neurons specifically connect to others respecting functional properties, or is the network haphazardly connected with respect to function? In the visual cortex, the question might be whether neurons with similar orientation, direction, or spatial preferences preferentially synapse with one another. In the retina, it may be how the function of output neurons — retinal ganglion cells — is produced by connections from bipolar or amacrine cells, specifically selected from other neurons of the same class with overlapping arbors. Here, we review recent reports examining structure–function relationships in neuronal networks. We focus mainly on the mammalian visual system where sensory physiology has been combined with techniques for assessing connectivity: *in vitro* electrophysiology and large-scale electron microscopy (EM).

Connectivity and function in cortical circuits

Although some have argued that the local cortical network is for the most part randomly connected, other than topographic specificity [1], detailed examination of cortical connectivity supports the idea that synaptic connectivity is specific at several different levels. Although at least one axonal and dendritic apposition is observed for every pair of pyramidal neurons sharing the same cortical column in somatosensory cortex, only a fraction of appositions have synaptic boutons reflecting synaptic connectivity [2,3]. Similarly, neurons are synaptically connected to only a small fraction of their neighbors, and such synaptic connections are specific to both cell types and sub-cellular compartments, and thus have cell-type specificity [4–8]. Therefore, whereas the potential connectivity between neurons in the cortex is dense, the actual synaptic connections are sparse and may be functionally specific. Finally, statistical analysis of simultaneous recordings between a seemingly homogeneous population of layer 5 (L5) pyramidal cells has shown that connections are far more clustered than expected from a random network [9,10] suggesting the presence of highly interconnected sub-networks embedded in local circuits. Indeed, lateral connections in L2/3 are frequently observed only between cells that received common inputs from L4 [11] and connections

between L5 pyramidal cells are dependent on their long-range targets [12]. Unfortunately, we still do not understand how the specificity of these connections underlies information processing *in vivo*.

Until recently, study of the relationship between identified neurons and their *in vivo* function has only been possible one cell at a time. Most commonly, the physiology of individual cortical neurons was recorded, and neurons were labeled to determine their axonal and dendritic morphology. With this approach, the general features of cortical microcircuits were described [13–19]. A new combination of technologies is poised to build upon this foundation.

The advent of optical methods to record the activity from populations of neurons at cellular resolution *in vivo*, allows investigators to simultaneously interrogate the functional properties of hundreds or thousands of cells [20–22]. Much of this recent work has focused on functional organization of neurons at cellular resolution [22–31], accompanied by more recent attempts at examining the functional properties of different cell types [32,33,34,35,88]. A logical next step is to examine the fine-scale connectivity underlying these local circuits. *In vivo* multi-photon calcium imaging is ideal for studying functional organization and cell type-specific physiology because its inherent spatial information provides the ability to precisely locate and identify cells with known physiological properties. This also makes the technique well suited to being combined with several different approaches to measure network connectivity.

Excitatory functional connectivity

While the study of cortical connectivity may at the surface seem an anatomical endeavor, *in vitro* physiology has been the most effective approach in recent years. Because of the relative ease of the experiments and the strong signal-to-noise ratio of the data, dual-cell recordings have become a fast and reliable method of assessing synaptic connectivity [36]. Indeed, most of what we know about the probabilities of connectivity — the likelihood that any pair of neurons exhibit the electrophysiological signature of a synapse — within and between cortical layers was determined by paired recordings between known cell types [4–8,12,37,38].

Recently, Ko and colleagues combined *in vivo* calcium imaging with subsequent recordings *in vitro* to ask if neurons with similar functional properties were preferentially connected [39]. They used simultaneous whole-cell patch-clamp recordings in acute slice preparations to test for monosynaptic connections between pairs of neighboring visual cortical neurons whose receptive fields had been mapped before tissue sectioning. Nearby neurons with similar orientation selectivities exhibited a connection probability of 0.38, whereas

neurons whose orientation preference was dissimilar (i.e., nearly orthogonal) had a connection probability of 0.17. Moreover, neurons with highly correlated stimulus-driven activity were connected 50% of the time. This is in contrast to conventional slice recordings, blind to *in vivo* function, that typically find 1 in 10 nearby pairs of pyramidal cells connected in the superficial layers of the cortex [4,37]. Taken together, these results suggest that subnetworks are embedded in the visual cortex and that some of their connectivity is related to processing similar features, or to correlated activity. Although very powerful, slice recordings suffer from a some shortcomings. First, connectivity testing is limited to the volume of the slice. More importantly, the number of cells from which one can record is limited by the health of the slice over time and the technical difficulties of simultaneous electrophysiological recordings. In comparison, several emerging anatomical circuit tracing methods may offer some complementary advantages.

Circuit connectivity by large-scale electron microscopy

Since its origins in the 1950s, serial-section EM [40] has proven the only way to image all the dense neural tissue of the central nervous system. With increases in computing power, high-speed data acquisition, and laboratory automation, there has been a resurgence of interest in the method [41–47]. It is now possible to extend this fine-scale analysis of neural tissue — in which virtually every single axon, dendrite and synapse can be visualized — to large volumes: circuit-scale ultrastructural anatomy. Previous reviews have previewed technology and methods for large-scale EM [42,43,45] and championed development of the methodology and what might be gleaned [46,47]. For the remainder of this review, we focus on recent applications of such techniques and their results in the mammalian retina and cerebral cortex.

The retinal network anatomy of direction selectivity

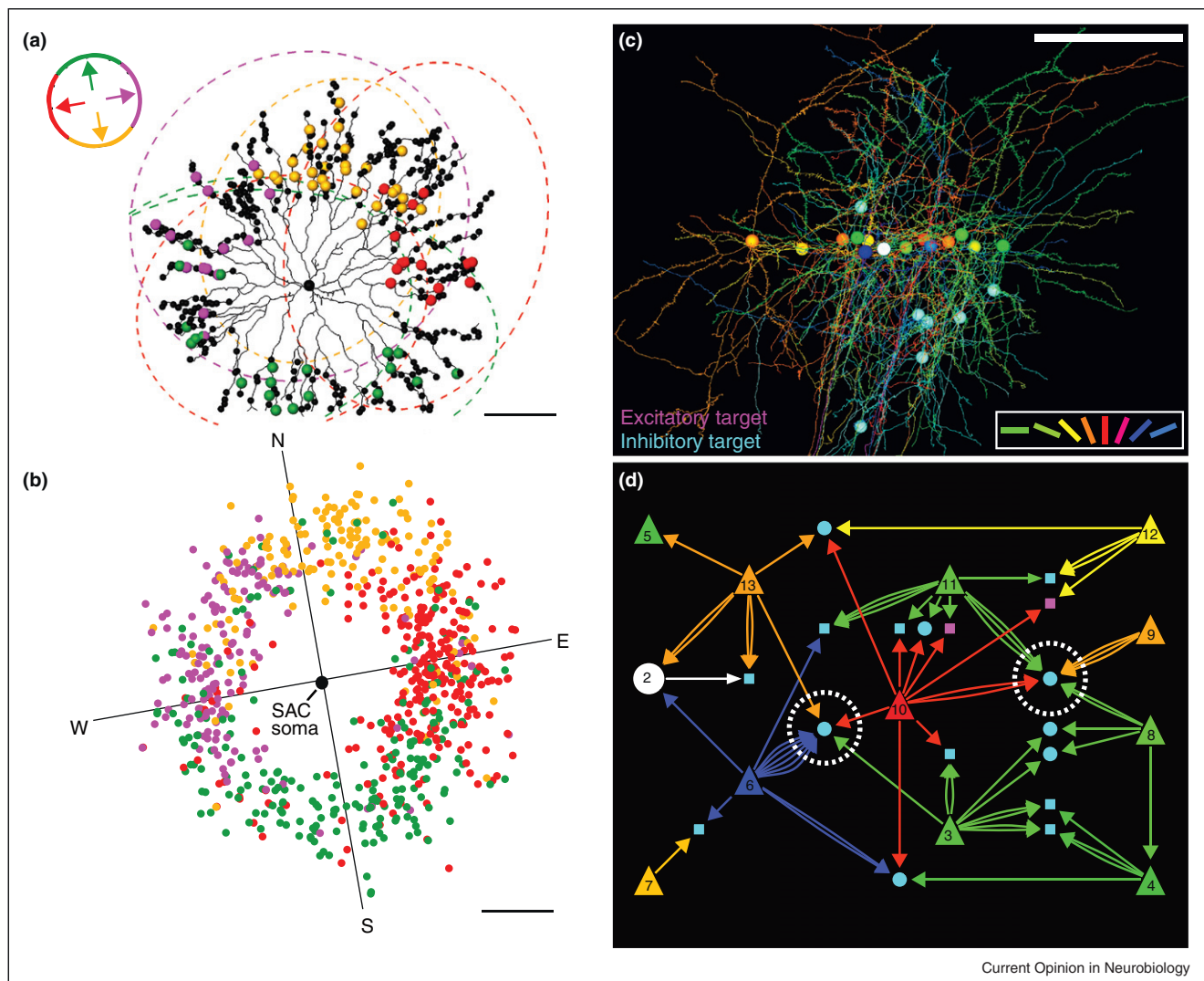
In the retina, the brain's window on the world, a wealth of information processing occurs. These are reviewed in this issue by Azeredo da Silveira and Roska [48] and Taylor and Smith [49], so we focus on one aspect — direction selectivity — that has received renewed attention with newer network mapping methods. Direction selectivity in some ganglion cells is one of the clearest examples of complex information processing that occurs in the retina. Direction selective ganglion cells in the retina exhibit preferential activity for motion of bars in a particular direction [50]. Mechanisms underlying this phenomenon have long been investigated and point toward inhibition of direction selective ganglion cells by starburst amacrine cells as a key component [51–53]. Starburst amacrine cells are retinal interneurons with radially symmetric stellate morphology that have varicosities on their distal processes contacting direction selective ganglion cells. Centrifugal motion along

their processes activates these dendrites [54], suggesting that starburst cells can inhibit ganglion cells when the direction of motion is away from the starburst's cell body. This inhibition, opposite to the preferred direction of the ganglion cell's, is thought to be a major mechanism underlying direction selectivity in the retina.

Two great advantages of the retina are that its visual physiology can be studied thoroughly *in vitro* [55] and it

has a relatively orderly, laminated anatomy with well-defined cell classes [56]. Historically, many have exploited this accessibility and anatomical simplicity, including two groups who have recently used these advantages to investigate its fine-scale network anatomy [57,58,59**]. Briggman and colleagues provided circuit-scale ultrastructural evidence that direction selectivity can be derived from wiring between these cell-types with functional specificity [59**]. By combining two-photon

Figure 1



Inhibitory specificity and randomness in visual circuits, as demonstrated with functional network anatomy. **(a)** An example of an inhibitory starburst amacrine cell (black skeleton), with presynaptic varicosities (small black dots). Synapses onto dendrites of direction-selective ganglion cells (outlined with dashes) are indicated by large dots, colour-coded by the preferred direction (key, top left). **(b)** The locations of synapses onto direction-selective ganglion cells (colour-coded as in a), plotted relative to the somas of 24 starburst amacrine cells (SAC). Scale bars, 50 μm . Ganglion cells receive input from SAC dendrites that radiate from the SAC in the direction opposite to the ganglion cell's preferred direction. **(c)** Three-dimensional rendering of the dendrites, axons and cell bodies of functionally characterized visual cortical neurons (coloured according to their *orientation* preference; key, lower right), along with postsynaptic inhibitory targets (cyan) that receive convergent input from multiple functionally characterized neurons. Scale bar, 100 μm . **(d)** A subset of the network diagram showing the connections in c. Presynaptic pyramidal cells are triangles colour-coded by preferred orientation (as in c). Multiple arrows indicate several synapses from the same pyramidal cell. Postsynaptic targets that could be traced to cell bodies are drawn as circles; post-synaptic dendritic fragments are drawn as squares. Dashed white circles highlight interneuron targets that received multiple excitatory inputs with a broad range of preferred orientations (a, b reproduced with permission from [59**] and c, d from [68**]).

calcium imaging and serial block-face scanning EM they show that starburst amacrine cells selectively target most of their putative inhibitory synapses onto direction selective ganglion cells of the appropriate preferred direction. That is to say that, despite their morphological symmetry, they have specific asymmetrical functional connectivity whereby their distal inhibitory synapses target ganglion cells with the opposite preferred direction of motion of the starburst neurite (Figure 1a, b). This demonstrates that inhibitory afferents can have exquisite synaptic specificity with a critical spatial component to shape cell physiology.

Recently, the early postnatal development of directionally selective ganglion cells was closely examined in two studies that demonstrated a rapid and activity-independent development of asymmetric inhibition from starburst amacrine cells [60[•],61[•]]. In addition to electrophysiological recording, one group mapped connectivity with two promising techniques: photostimulation and virally mediated trans-synaptic tracing to demonstrate direct connectivity between direction selective ganglion cells and starburst amacrine cells [61[•]]. This viral technique uses a replication-deficient virus to specifically label only neurons that provide synaptic input to an isolated target cell in which the deficient viral components are expressed [62–64]. The anatomical approach in particular will likely prove powerful in cortical experiments in which the *in vivo* physiology of a single neuron and all its presynaptic inputs could be compared.

Cortical network anatomy and the functional role of interneurons

The adult visual cortex is comprised of two broad classes of neurons 80% of which are excitatory pyramidal cells with the remainder inhibitory interneurons. Visually responsive pyramidal cells are generally highly selective for specific stimulus properties. Currently, there is debate in the field over the tuning properties of inhibitory interneurons. Recent studies have found inhibitory interneurons generally appear much more broadly tuned than pyramidal cells [32,33[•],65,66,88,89]. Several others, however, report different interneuron subtypes with quite selective tuning [34[•],67], while still others describe examples of intermediate selectivity [35[•]].

Is it possible that the underlying cortical network's anatomy might explain interneuron receptive field properties? Bock and colleagues addressed this question, while providing a framework for untangling cortical wiring by combining *in vivo* two-photon calcium imaging and large-scale serial-section transmission EM [68^{••}]. This study found that synapses from pyramidal neurons converged onto interneurons in their general vicinity irrespective of presynaptic function (Figure 1c, d). Pairs of pyramidal neurons with different preferred orientations were found to converge input onto inhibitory targets with

the same probability as pairs of inputs with the same preferred orientation. This result is consistent with generally haphazard inhibitory interneuron connectivity in light of the recent demonstration that Martinotti cells (an interneuron subtype) appear to provide synaptic input to nearly every local pyramidal cell in the superficial layers of the cortex [69[•]] and with the convergence of pyramidal output onto fast-spiking putative interneurons in other regions of the neocortex [37,70]. Thus, evidence from studies of connectivity are consistent with most interneurons receiving a broad array of differently tuned input, which would combine non-specifically to control the gain of excitation in a local circuit [33[•]].

Conclusions

The functional connectivity of mammalian neural circuits appears to span from specific to haphazard. Excitatory pyramidal cells in the visual cortex and inhibitory interneurons in the retina exhibit functionally specific connectivity, whereas inhibitory wiring in the cortex appears less so. What does this connectivity tell us about the function of circuits and their components? Broadly speaking, a circuit's structure develops to suit its purpose. Unlike the cortex, the retina, with its orderly tiling of cell types, appears more like a mosaic of individual processing units with minimal cross-talk between the same cell types, so that computations are localized to discrete retinotopic locations. A lattice of parallel processing pathways may demand specific connections within processing units to maintain order and efficiency. In the cerebral cortex, however, information being processed becomes more complex and integrated. It is therefore reasonable that functionally specific connections exist between excitatory neurons — the main thoroughfare for information processing — but that inhibitory interneurons, which largely appear to regulate local gain of a circuit and network oscillations, are promiscuous in their local wiring.

One benchmark of our understanding of network information processing is the extent to which we can predict neuronal function from connectivity. Attempts to do so with the wiring diagram of *C. elegans* [71] have not seemingly borne this out, but critically missing was a rich literature of single-cell physiology. Recent work using large-scale EM in mammalian systems has demonstrated that structure appears to confirm predictions from function in systems with well-understood physiology. This is reassuring, as it suggests that we may soon have sufficient understanding of how information flows and is transformed through some networks to make accurate predictions of their function from circuit structure.

Ultimately, a variety of approaches — and likely a synergy of methods — will be employed to reach a satisfying understanding of how neural circuits process information. Circuit-scale EM is now among the tools available [41,57,58[•],59^{••},68^{••},72]. EM datasets and their

analysis will get ever larger and richer. This richness can be in the form of more function [59^{••},68^{••},73–75], correlated labeling for cell identity or biochemical analysis [57,58[•],76,77], EM dense labeling [78–80] for information on long-range connections, and more complete 3D segmentation [45,81,82,90] for volumetric properties like synaptic strength as correlated to synapse size [83–85].

Large-scale EM datasets should also serve as resources that can be mined by multiple investigators. The EM dataset from Bock and colleagues is publicly available online from the UCSD Cell Centered Database [86] (<http://ccdb.ucsd.edu/>, accession number 8448) and browsable [87] from Open Connectome Project at Johns Hopkins University (<http://openconnectomeproject.org/>). A potentially useful analogy is with that of a perpetual slice experiment where investigators can test for synaptic connectivity between any cells in the volume. Higher-order patterns of connectivity can be found in such datasets as the reconstruction becomes increasingly dense, generating many-to-many graphs of network connectivity, currently unachievable with other methods. Finally, recently acquired EM datasets demonstrate our coming ability to routinely image at synaptic resolution the entire nervous systems of model organisms such as *C. elegans*, *Drosophila*, and zebrafish. Thus, with recent advances in imaging techniques — *in vivo* two-photon calcium imaging and large-scale serial-section EM — as well as traditional slice physiology and sophisticated transynaptic tracing methods, the stage is set to achieve a deeper functional and structural understanding of neuronal networks.

Note added in proof

Very recent reports show additional examples of broadly tuned interneurons in the adult visual cortex [88,89] and describe a new suite of annotation tools that will likely accelerate manual reconstruction of large-scale EM datasets with high-fidelity [90].

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