From the connectome to brain function

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In this Historical Perspective, we ask what information is needed beyond connectivity diagrams to understand the function of nervous systems. Informed by invertebrate circuits whose connectivities are known, we highlight the importance of neuronal dynamics and neuromodulation, and the existence of parallel circuits. The vertebrate retina has these features in common with invertebrate circuits, suggesting that they are general across animals. Comparisons across these systems suggest approaches to study the functional organization of large circuits based on existing knowledge of small circuits.

An animal's behavior arises from the coordinated activity of many interconnected neurons—"many" meaning 302 for *Caenorhabditis elegans*, 20,000 for a mollusc, several hundred thousand for an insect or billions for humans. Determining the connectivity of these neurons, via combined anatomical and electrophysiological methods, has always been a part of neuroscience. As we were writing this, these ideas were being revisited from the perspective of massively parallel methods for dense reconstruction, or 'connectomics'. One thread of this analysis involves the detailed, high-density mapping of point-to-point connections between neurons at synapses[1–4]. The specialized membrane structures and synaptic vesicles of synapses can be visualized with an electron microscope, and consequently dense reconstructions of nervous-system connectomes rely on electron microscopy of serial brain sections. In a complementary approach, detailed electrophysiological analysis shows how synapses and circuits function at high resolution, and is increasingly being applied to large numbers of interconnected neurons.

The first approaches used to map complete circuits came from studies of the smaller nervous systems of invertebrates. In the 1960s and 1970s, systematic electrophysiological recordings from neurons in discrete ganglia enabled the identification of neuronal components of circuits that generate specific behaviors[5–7]. In association with the recordings of these individually recognizable, identified neurons, the cells were filled with dye to visualize their structures and projection patterns via light microscopy[8–10]. In some cases, electron microscopy was used to observe the anatomical synapses in these small circuits[11–13]. But until the publication of the heroic electron microscopy reconstruction of the full nervous system of *C. elegans*[14] in the mid-1980s, it was unimaginable that the electron microscope could be used to determine circuit connectivity rather than providing ultrastructural detail to connectivity determined either with physiological or light microscopy–based anatomical methods.

Recent advances in electron microscopy and image analysis have made it possible to scale up this ultrastructural approach: to serially section and reconstruct pieces of both vertebrate and invertebrate nervous systems, with the stated purpose of using detailed connectomes to reveal how these circuits work[4,15–18]. Such large-scale projects will provide new anatomical data that will offer invaluable insights into the functional organization of the structures studied. An unbiased approach to data acquisition always reveals surprises and new insights. Moreover, because of the scope and size of these projects, such efforts will generate unprecedented amounts of data to be analyzed and understood.

Here we ask what additional information is needed beyond connectivity diagrams to understand circuit function, informed by the invertebrate circuits whose connectivity is known. For the prototypical case, the complete *C. elegans* nervous system, the anatomical connectome was largely established over 25 years ago[14]. In a variety of other invertebrate preparations, connectivity was established using combinations of electrophysiological recordings and neuronal tracing 30–40 years ago, which enabled researchers to generate a wiring map that incorporates activity information. Despite their different starting points from anatomy and electrophysiology, these two approaches have uncovered

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Figure 1 | Connectivity of two well-studied invertebrate circuits. (**a**) Connectivity diagram of the crab STG based on electrophysiological recordings. Red and blue background shading indicates neurons that are primarily part of the pyloric and gastric circuits, respectively. Purple shading indicates that some neurons switch between firing in pyloric and gastric time, and that there is no fixed boundary between the pyloric and gastric circuits. Yellow highlights two neurons that are both electrically coupled and reciprocally inhibitory. Green highlights one of many examples of neurons that are coupled both monosynaptically and polysynaptically. (**b**) The connectome of *C. elegans,* showing all 302 neurons and their chemical synapses but not their gap junctions. Each neuron has a three-letter name, often followed by a spatial designator. This top-to-bottom arrangement (signal flow view) is arranged to reflect dominant information flow, which goes from sensory neurons (red) to interneurons (blue) to motor neurons (green). Reprinted from ref. 50.
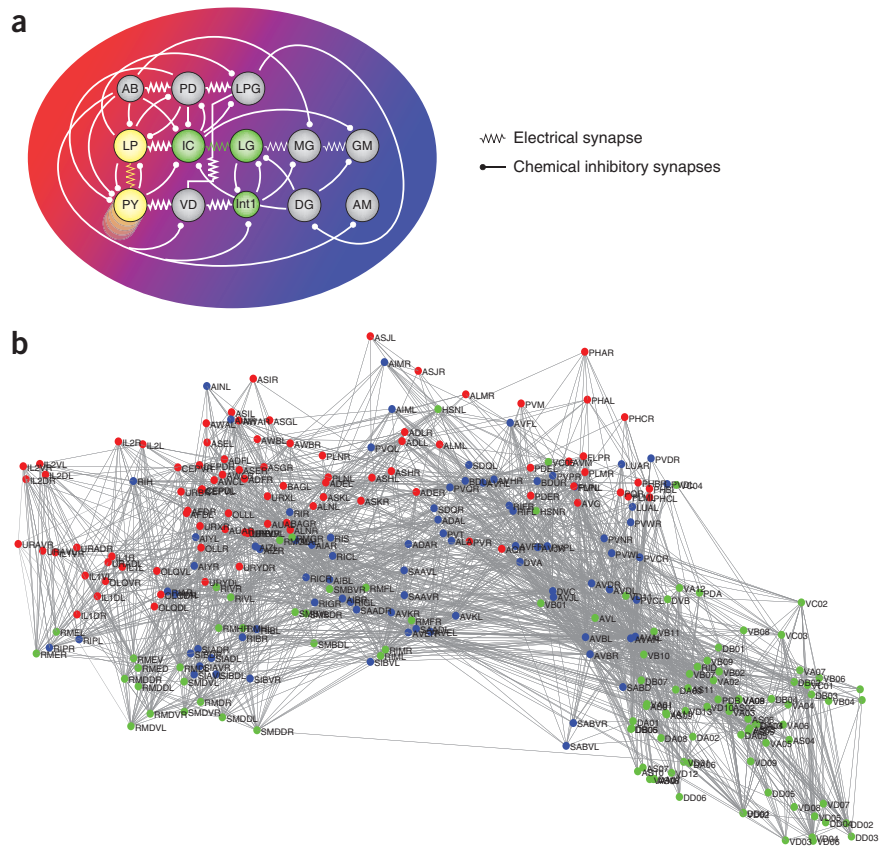
similar principles and similar puzzles as to how circuit function arises from the component neurons and their interactions.

What do functional and anatomical maps reveal?

We begin with the connectivity diagram of the stomatogastric ganglion (STG) of the crab, *Cancer borealis* (**Fig. 1a**) and a graph of the connectome of *C. elegans* (**Fig. 1b**). In each case, the number of neurons is small, ~27 neurons or 302 neurons, respectively, but the number of synaptic connections is much larger; the neurons are extensively interconnected. The basic function of each circuit is known: to generate rhythmic stomach movements for the crab STG and to control locomotion behavior in response to sensory inputs for *C. elegans.* The intellectual strength of the STG system is the ability to relate neuronal connectivity to neuronal activity patterns; the complementary strength of *C. elegans* is the ability to relate neuronal connectivity to whole-animal behavior.

The STG contains motor neurons and interneurons that generate two rhythmic motor patterns[19]. The pyloric rhythm is an oscillating, triphasic motor pattern that is continuously active and depends on a set of electrically coupled pacemaker neurons. The gastric mill rhythm is episodically active and depends on descending modulatory inputs activated by sensory neurons for its generation[19,20]. Although these rhythms are easily studied separately, a close look at the STG connectivity diagram reveals that the neurons that conventionally are thought to be part of the pyloric circuit (neurons AB, PD, LP, PY, VD and IC) are highly interconnected with those conventionally thought part of the gastric mill circuit (neurons DG, GM, LPG, MG, LG and Int1) (AM is part of a third circuit that we will not discuss here). Indeed, many

STG neurons switch their activity between the two rhythms[19], and the separation of the STG's connectivity into two discrete circuits, although convenient for those who study the network, does not really capture the highly interconnected reality of the ganglion's architecture.

Like all nervous systems, the circuit has many chemical synapses, in which a presynaptic neuron releases a chemical neurotransmitter to activate receptors on the postsynaptic neuron. Chemical synapses can be inhibitory or excitatory depending on the nature of the receptor and associated ion channels; the chemical synapses among STG neurons are inhibitory. Additional connections are created by the widespread electrical synapses, mediated by direct cytoplasmic communication through gap junctions, through which current flows depending on the voltages of the coupled neurons. In the STG circuit, there are many instances of neurons that are connected by electrical synapses as well as by chemical inhibitory synapses (**Fig. 1a**). There are also many instances of neurons connected by reciprocal inhibition. These wiring motifs contribute to circuit properties that are not easily predictable. In addition, there are many 'parallel pathways' in which two neurons are connected via two or more synaptic routes, one direct route and additional indirect routes (**Fig. 1a**). The complexity of this connection map poses the essential question: are all synapses important, or are some only important under certain conditions (as appears to be the case)[21]? How do we understand the importance of synaptic connectivity patterns that seem to oppose each other, such as the common motif of electrical coupling between neurons that also inhibit each other?

The *C. elegans* wiring diagram was assembled in the near-complete absence of prior functional information. It allowed an
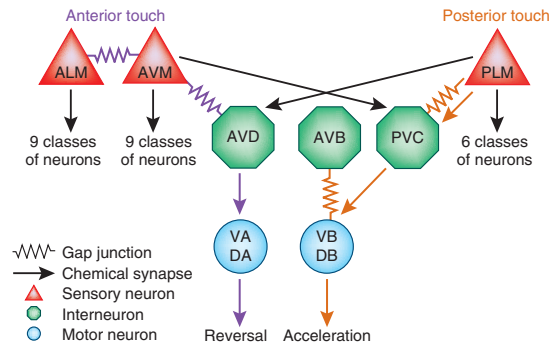
Figure 2 | *C. elegans* neurons essential for avoidance of light touch. Inferred connections necessary for anterior and posterior touch avoidance are in purple and orange, respectively; other synapses are in black. The 'essential' synapses here shown in orange and purple comprise less than 10% of the output synapses of the mechanosensory neurons. Image based on ablation data from ref. 22.

immediate classification of neurons into large classes: sensory neurons (with distinctive sensory dendrites and cilia), motor neurons (with neuromuscular junctions) and interneurons (a term that is used in *C. elegans* to describe any neuron that is not evidently sensory or motor, encompassing projection neurons and local neurons)[14]. In each group, neurons were subdivided into unique types with similar morphologies and connections, collapsing the wiring diagram from 302 neurons to 119 neuronal types. The flow of information through chemical synapses is predominantly from sensory to interneuron to motor neuron, with many parallel pathways linking neurons both directly and indirectly (as in the STG), as well as gap junctions that may form electrical synapses (~10% of all synapses). Most neurons are separated from each other by no more than two or three synaptic connections.

The *C. elegans* map was immediately used to define neurons required for the touch-avoidance response, which is still the most completely characterized of the animal's behaviors[22]. Light touch to the head elicits a reversal, and light touch to the tail elicits a forward acceleration. The neurons required for the touch-avoidance response were identified by killing cells with a laser microbeam and assessing the behavioral repertoire of the worms. Guided by the wiring diagram, this analysis revealed essential mechanosensory neurons in the head and tail, key interneurons required to propagate information, and motor neurons required for forward and backward movement (**Fig. 2**). The success of this approach inspired similar analyses of chemosensory behaviors, foraging, egg-laying, feeding and more. At this point, over 60% of *C. elegans* neuron types have defined functions in one or more behaviors.

This notable success, however, hides a surprising failure. For *C. elegans,* although we know what most of the neurons do, we do not know what most of the connections do, we do not know which chemical connections are excitatory or inhibitory, and we cannot easily predict which connections will be important from the wiring diagram. The problem is illustrated most simply by the classical touch-avoidance circuit[22] (**Fig. 2**). The PLM sensory neurons in the tail are solely responsible for tail touch avoidance. PLM forms 31 synapses with 11 classes of neurons, but only one of those targets is essential for the behavior—an interneuron called PVC that is connected to PLM by just two gap junctions and two chemical synapses. An even greater mismatch between

the number of synapses and their importance in behavior is seen in the avoidance of head touch, where just two of 58 synapses (again representing gap junctions) are the key link between the sensory neurons (ALM and AVM) and the essential interneuron (AVD). This general mismatch between the number of synapses and apparent functional importance has applied wherever *C. elegans* circuits have been defined. As a result, early guesses about how information might flow through the wiring diagram were largely incorrect.

Clearly, the wiring diagram could generate hypotheses to test, but solving a circuit by anatomical inspection alone was not successful. We believe that anatomical inspection fails because each wiring diagram encodes many possible circuit outcomes.

Parallel and antagonistic pathways complicate circuits
Both of the wiring diagrams shown in **Figure 1** are richly connected. In the STG, a large fraction of the synapses are electrical synapses. In some cases, the electrical synapses connect multiple copies of the same neuron, such as the two PD neurons in the STG. Notably, many electrical synapses connect neurons with different functions. Almost invariably, the combination of electrical and chemical synapses create 'parallel pathways', that is to say, multiple pathways by which neuron 1 can influence neuron 2 (**Fig. 1a**). For example, in the STG, the PD neuron inhibits the IC neuron through chemical synapses but also can influence the IC neuron via the electrical synapse from LP to IC. Parallel pathways such as those in the STG can be viewed as degenerate, as they create multiple mechanisms by which the network output can be switched between states[23] (**Fig. 3**). A simulation study[23] shows a simplified five-cell network of oscillating neurons coupled with electrical synapses and chemical inhibitory synapses. The f1 and f2 neurons are connected reciprocally by chemical inhibitory synapses, as are the s1 and s2 neurons. This type of wiring configuration, called a half-center oscillator, often but not universally causes the neurons to be rhythmically active in alternation[24]. In this example, two different oscillating rhythms are generated, one fast and one slow. The hub neuron at the center of the network can be switched between firing in time with the fast f1 and f2 neurons to firing in time with the slow s1 and s2 neurons by three entirely different circuit mechanisms: changing the strength of the electrical synapses, changing the strength of the synapses between f1 and s1 onto the hub neuron, and changing the strength of the reciprocal inhibitory synapses linking f1 to f2 and s1 to s2 in the half-center oscillators.

An example from the *C. elegans* connectome illustrates another twist of circuit logic: divergent circuits that start at a common point but result in different outcomes. In this example, gap junctions and chemical synapses from ADL sensory neurons generate opposite behavioral responses to a *C. elegans* pheromone (**Fig. 4a**). The chemical synapses drive avoidance of the pheromone, whereas the gap junctions stimulate a pheromone-regulated aggregation behavior[25]. Differing use of the chemical synapse subcircuit versus the gap junction subcircuit allows ADL to switch between these two opposing behaviors in different contexts. ADL illustrates the point that is not possible to 'read' a connectome if it is intrinsically ambiguous, encoding two different behaviors.

Parallel and divergent systems of synapses are widespread features of invertebrate and vertebrate networks alike, and can be composed of sets of chemical synapses as well as sets of chemical

Figure 3 | Similar changes in circuit dynamics can arise from three entirely different circuit mechanisms. (**a**) Circuit diagram (top) shows the 'control' condition in which f1 and f2 are firing in a fast rhythm (indicated by red shading) and the remaining neurons are firing in a slow rhythm (shaded in blue). hn is the hub neuron. In these diagrams, electrical synapses are shown as resistor symbols and chemical inhibitory synapses with filled circuits. Traces (bottom) show the voltage waveforms of the five neurons. (**b–d**) Responses when the strength of the chemical synapses to the hub neuron (g_{synA}) was decreased (**b**), when the strength of the electrical synapses (g_{el}) was decreased (**c**) and when the strength of the chemical synapses between f1 and f2 and between s1 and s2 (g_{synB}) was decreased (**d**). Image modified from ref. 23.
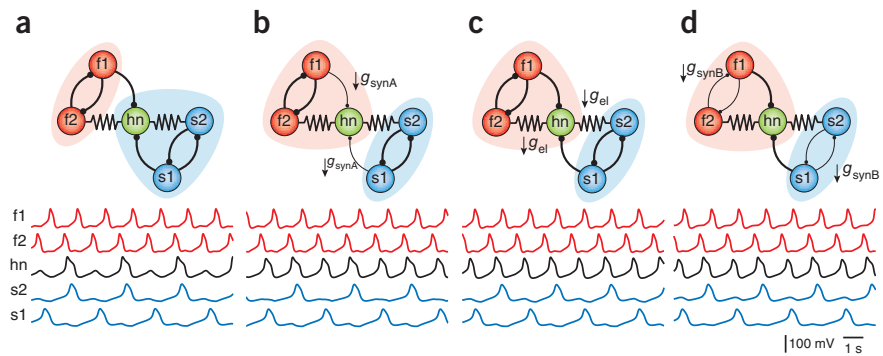
and electrical synapses. To understand information flow, there will be no substitute for recording activity. The methods for monitoring neuronal activity have improved dramatically in recent years, with development of new multi-electrode recording techniques and a suite of genetically encoded indicators that can be used to measure calcium, voltage and synaptic release at cellular and subcellular levels. However, improved methods are needed to detect electrical synapses, which can also be difficult to see in electron micrographs. The regulation of electrical synapses by voltage, neuromodulation, phosphorylation and small molecules is understudied[26,27]. A chemical method for measuring gap junctions, local activation of molecular fluorescent probes, is a promising new direction that should spawn innovation[28].

### Neuromodulation reconfigures circuit properties

Superimposed on the fast chemical synapses and electrical synapses in the wiring diagram are the neuromodulators—biogenic amines (serotonin, dopamine, norepinephrine and histamine) and neuropeptides (dozens to hundreds, depending on species)[29]. These molecules are often released together with a fast chemical transmitter near a synapse, but they can diffuse over a greater distance. Modulators also can be released from neuroendocrine cells that do not make defined synaptic contacts or can be delivered as hormones through the circulation. As a result, the targets of neuromodulation are invisible to the electron microscope. Signaling primarily through G protein–regulated biochemical processes rather than through ionotropic receptors, neuromodulators change neuronal functions over seconds to minutes, or even hours.

Many years of work on the effects of neuromodulators on the STG have revealed that the functional connections that give rise to a specific circuit output are specified, or in fact 'configured', by the neuromodulatory environment[29]. Every synapse and every neuron in the STG is subject to modulation; the connectivity diagram by itself only establishes potential circuit configurations, whose availability and properties depend critically on which of many neuromodulators are present at a given moment[29]. Under some modulatory conditions, anatomically 'present' synaptic connections may be functionally silent, only to be strengthened under other modulatory conditions. Likewise, modulators can qualitatively alter the neurons' intrinsic properties, transforming neurons from tonic spiking to those generating plateau potentials or bursts[29]. These effects of neuromodulators can activate or silence an entire circuit, change its frequency and/or the phase relationships of the motor patterns generated.

C. elegans has over 100 different neuropeptides as well as biogenic amine neuromodulators. The integration of neuromodulation into its fast circuits appears to selectively enhance the use of particular connections at the expense of others. For example, a 'hub-and-spoke' circuit drives aggregation of *C. elegans* by coupling multiple sensory inputs through gap junctions with a common target neuron, RMG (**Fig. 4b**). Neuromodulation of RMG by the neuropeptide receptor NPR-1 effectively silences this gap-junction circuit, while sparing other functions of the input sensory neurons that are mediated through chemical synapses[30].

Neuromodulators are prominent in all nervous systems, and act as key mediators of motivational and emotional states such as

Figure 4 | Two views of a multifunctional *C. elegans* circuit. (**a**) Ambiguous circuitry of the ADL sensory neurons, which drive avoidance of the ascaroside pheromone C9 through chemical synapses onto multiple interneurons (right) but can also promote aggregation (attraction toward pheromones) through gap junctions with RMG (left). Image modified from ref. 25. (**b**) Neuromodulation separates overlapping circuits. Multiple sensory neurons form gap junctions with the RMG hub neurons and promote aggregation through this circuit, but each sensory neuron also has chemical synapses that can drive RMG-independent behaviors. The neuropeptide receptor NPR-1 inhibits RMG to suppress aggregation. Image modified from ref. 30.
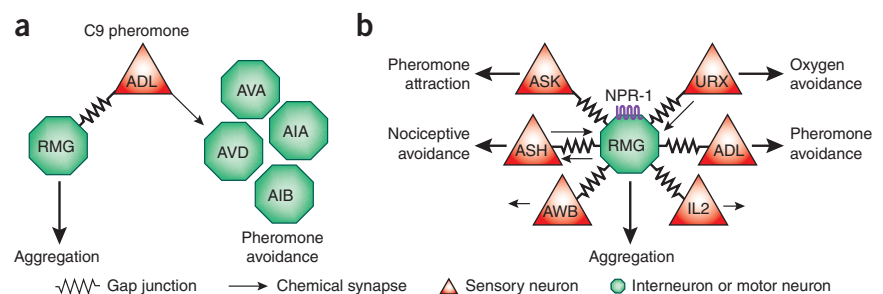
sleep, arousal, stress, mood and pain. To understand their release and their effects on circuits, new methods are needed to monitor neuromodulation *in vivo*. Electrophysiology remains the best tool for characterizing the functional effects of neuromodulators but is low-throughput. Biochemical methods can be used to reveal the presence of neuromodulators in tissue or in bulk extracellular fluid but are less effective for detecting them near a particular synapse or release site. A new genetic method can be used to read out the neuromodulatory state directly by monitoring receptor activation but with a timeframe of hours, whereas endogenous modulation can change within minutes or seconds[31]. Progress is needed in all of these domains and beyond: there is a need to move from individual neurons and modulators to physiologically relevant modulatory states, which are likely to include multiple neuromodulators acting at many sites.

Neuronal dynamics shape the activity of circuits

The existence of parallel circuits and neuromodulation means that connectivity alone does not provide adequate information to predict the physiological output of circuits. Even without these factors, the behavior of neurons over time is unpredictable from anatomy because neuronal behavior is sensitive to intrinsic channels and electrical properties that vary within and between cell types. Channels, synapses and biochemical processes interact to generate explicitly time-delimited features, or dynamics, in neurons and circuits.

The importance of neuronal dynamics in circuit function can be seen most simply in a two-cell circuit (**Fig. 5**). Two isolated neurons from the STG that are not normally synaptically coupled were connected using the dynamic clamp, a computer-neuronal interface that allows a user to manipulate biological neurons with conductances that imitate ion channels and synaptic connections[32]. The neurons are connected reciprocally by dynamic clamp-created inhibitory synapses so that the neurons rhythmically alternate their activity[24]. The dynamic clamp allows the investigator to change the strength of the synapses as well as the amount of one of the membrane currents, hyperpolarization-activated inward current (I_h)—either of which dramatically alters the period of the circuit oscillation (**Fig. 5**). Thus, a given wiring diagram can produce widely different dynamics with different sets of circuit parameters, and conversely, different circuit mechanisms can give rise to similar oscillation dynamics. Without knowing the strength and time course of the synaptic connections as well as the numbers and kinds of membrane currents in each of the neurons, it would not be possible to simply go from the wiring diagram to the dynamics of even two neurons. Synaptic connectivity alone does not sufficiently constrain a system.

Understanding neuron-specific and circuit-specific dynamics will be essential to understanding mammalian circuits as well as invertebrate circuits. In some cases, unique dynamic properties are characteristics of particular cell types—for example, different classes of inhibitory cortical interneurons are distinguished as much by their dynamics as by their connectivity[33]. In other cases, neuronal dynamics are variable among similar cells or even within one cell type. For example, pyramidal neurons in specific areas of the cortex exhibit persistent activity associated with working memory[34], and neurons in brainstem modulatory systems switch their properties between tonic and phasic firing modes depending
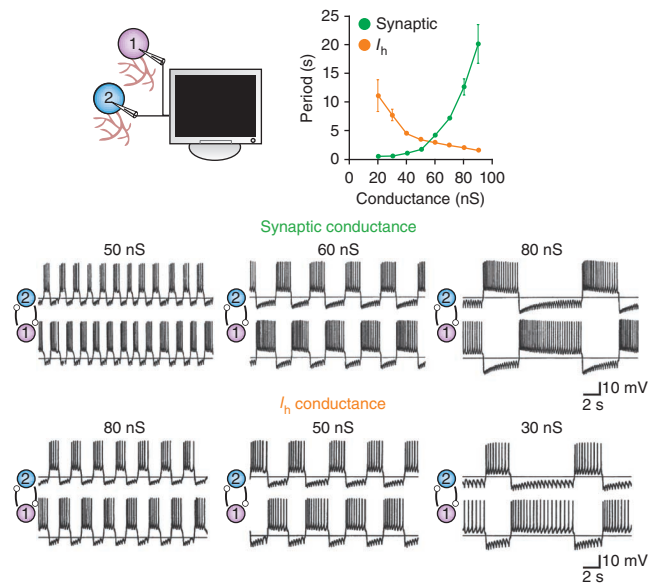
Figure 5 | Changing either intrinsic neuronal properties or synaptic properties can alter network function. The dynamic clamp, a computer-neuron interface (top) was used to vary either the strength of synaptic connections between two neurons (synaptic) or the amount of an intrinsic hyperpolarization-activated inward current (I_h), in one neuron as graphed (right). Traces (bottom) show the action potentials generated by the alternating, oscillating neuron pair as those properties were varied. Image modified from ref. 24.

on behavioral states[35]. Finally, synaptic plasticity can occur on rapid timescales to strengthen and weaken synapses based on use, adding complexity to circuit-level dynamics[36].

Analyzing neuronal dynamics often requires the circuit to be simultaneously monitored and manipulated, as shown in the example of the dynamic clamp. Emerging techniques of optogenetics and pharmacogenetics can be combined with recording as well, but a limitation of all of these methods is that they act at the level of neurons or groups of neurons. To understand functional connectivity, it will be useful to develop methods to silence or activate specific channels and specific synaptic connections between two specified neurons, without affecting all other functions of the same cells.

Vertebrate retina also has complex circuit properties

What lessons will emerge as connectomes are scaled up from small-scale to large-scale circuits? Many features will be common to small and large circuits. Vertebrate circuits, like invertebrate circuits, have multiple cell types with nonuniform intrinsic properties, extensive and massively parallel synaptic connectivity, and neuromodulation. The balance of these components varies between animals and brain regions (the STG has more electrical synapses than most vertebrate brain regions; *C. elegans* uses mostly graded potentials instead of all-or-none action potentials), but in reality, the diversity of circuits in the vertebrate brain is at least as great as the difference between any one vertebrate region and any invertebrate circuit. The essential distinction we see in vertebrate brains is not a particular microcircuit property but their repeating structure (for example, the many cortical columns) and their enormous scale compared to the worm brain and the STG.

Examination of the vertebrate retina has begun to reveal the relationships between performance of a large circuit and properties of a small circuit. The special power of studying the isolated retina is the ability to experimentally control visual input while simultaneously recording output—the spikes from retinal ganglion cells that project to the brain. The retina represents an intermediate degree of complexity with features of both a small circuit and a large circuit, and has been subject to the most complete anatomical and electrophysiological characterizations of any vertebrate brain region. Current connectomics studies of the retina, for example, include dense reconstruction of serial-section electron micrographs accompanied by analysis of the neurotransmitter phenotype and activity patterns of the reconstructed neurons[3,17]. The combination of structure and function, and a rich history of elegant experiments, make this the ideal system for understanding neural computations in detail.

The retina contains millions of neurons that fall into five major neuronal classes (photoreceptors, horizontal cells, bipolar cells, amacrine cells and retinal ganglion cells), which are subdivided into about 60 discrete cell types[37]. Each of the 60 cell types is arrayed in a near-crystalline two-dimensional array, so that any pixel viewed by the retina is covered by at least one neuron of each cell type. Ultimately, information leaves the retina through the 20 classes of retinal ganglion cells, each of which is considered to be a parallel but partially overlapping processing stream.

The first views of the retinal connectome show all of the properties that we highlight in small circuits: cellular complexity, extensive interconnectivity, parallel circuits with chemical and electrical synapses, and neuromodulation. The heterogeneity of the 60 retinal cell types is substantial, echoing the heterogeneity of individual neurons in the STG or *C. elegans*. Anatomically, some dendritic arbors cover only a tiny area of the visual field, but others arborize much more broadly. Their intrinsic physiological properties are also extremely diverse, with some neurons that spike (such as retinal ganglion cells), and many neurons that do not spike (such as photoreceptors and bipolar cells)[37]. There are even amacrine neurons that perform independent computations in different parts of their complex arbors[38].

Synaptic connections in the retina are extensive and diverse, and electron microscopy reconstructions have revealed many classes of synaptic connections that had not been observed in physiological studies[3,17]. There is a great variety of excitatory and inhibitory chemical synapses, and there are many electrical synapses, that all vary in their strength and their modification by experience. Both anatomical and physiological studies demonstrate that the retina, like small circuits, consists of many partly parallel circuits with overlapping elements. In particular, the retina operates over many orders of magnitude of light intensity, and the properties of its circuits change with its visual inputs. Within a few seconds in a new visual environment, retinal ganglion cells shift their properties to encode relevant features of light intensity, contrast and motion, drawing on different features of the network[39]. Subsets of retinal ganglion cells change their weighting of center and surround inputs in a switch-like fashion as light levels change[40]. A brief period of visual stimulation can even reverse the apparent direction-selectivity of retinal ganglion cells[41].

Finally, neuromodulation has a role in retinal processing that reshapes visual circuits. Dopamine is released from a subset of amacrine cells around dawn, under the control of acute light stimuli and circadian rhythm[42], and acts on cells throughout the retina to switch them from properties appropriate to night vision to day vision. The photoreceptors themselves are modulated, and their coupling through gap junctions decreases to increase their resolution but reduce their sensitivity. Downstream of the rod photoreceptors, which dominate night vision, dopamine closes the gap junctions between rod bipolar cells and AII amacrine cells, effectively diminishing rod input to the retinal ganglion cell output of the retina.

What differences are there between small and large circuits? The sheer size of the retina shows a sharp transition compared to the size of the STG and the worm brain, and the level of analysis moves from single cells to cell classes. Understanding a single pixel is not sufficient to understand the retina, and here the properties of simple and complex circuits diverge. For example, long-range communication allows groups of retinal cells to perform computations that a single cell cannot. Wide-field cells such as starburst amacrine cells can make judgments about motion that no single-pixel neuron could make but can then feed that information into narrow-field single-pixel neurons to bias their properties. The scaling from fine resolution to broad resolution and back again emerges from the diversity of spatial scales across the structure of the retina.

Circuits interact to generate behavior

The entire nervous system is connected, but reductionist neuroscientists invariably focus on pieces of nervous systems. The value of these simplified systems should not let us forget that behavior emerges from the nervous system as a whole. At the moment, obtaining the connectomes of even small parts of the vertebrate nervous system is a heroic task. However, establishing the detailed pattern of connectivity for a small part of the nervous system may not be sufficient to understand how that piece functions in its full context. By parceling out small regions, one invariably loses information about the long-range connections to and from that area.

The extent to which long-range connectivity clouds our understanding of connectomes will vary. For example, the vertebrate retina is anatomically isolated, functionally coherent and lacks recurrent feedback synapses from other brain areas that are prominent in most other parts of the central nervous system. We might imagine the retina as a two-dimensional circuit, whereas most vertebrate circuits are three-dimensional; new principles will certainly arise from connectomes that include recurrent inputs. In the amygdala, for example, the intermixing of multiple cell types with different long-range inputs and outputs would preclude a meaningful understanding based on local anatomy alone[43]. Choosing well among brain regions, and combining connectomes with molecular and functional information about the same cells, as is being done in the vertebrate retina[3,17], will lead to the most informative results.

How can we 'solve' the brain?

As we look to ways that other neural systems may be characterized with similar power to the three described here, we can draw certain lessons. One is that precise circuit mapping and specific neuron identification have had great importance for unifying structural and functional data from different laboratories. Extending this idea, other systems may not have individually

named cells, but all nervous systems have cell types distinguished by anatomy, connectivity and molecular profile that can serve as the basis of a common vocabulary. Improvements in molecular staining methods will only increase the power of a connectome anchored in cellular identity.

We can also see that connectomic endeavors will need to be supplemented by experiments that monitor, manipulate and model circuit activity. Monitoring and manipulation of circuit function have been considered above. To complement and inform these experimental approaches, the third step is to develop models that describe how a system's output results from the interactions of its components. There is a tension between the desire to study abstract models that are amenable to precise mathematical analysis and the desire to study models with sufficient biological realism to represent the system's underlying structures and functions. In small circuits, it is now possible to construct models and families of models that can be quite instructive[44]. In *C. elegans*, a few testable models emerged directly from analyses of anatomy. One was the concept of a motif, a set of connection patterns between three or four neurons that are over-represented in the wiring diagram compared to the statistical expectation based on individual connections[45]. Perhaps these motifs perform a canonical computation, or a few canonical computations, so that solving a few of them effectively solves a larger piece of the diagram.

But how should we approach building models of large networks without generating models that are as difficult to understand as the biological systems that motivated and inspired them? The beauty of the connectome is its precision and specificity, but it is hard to imagine useful network models that implement all of the details of cell-to-cell connectivity obtained with the electron microscope, when building such models would require enormous numbers of assumptions about other circuit parameters, and these parameters are likely to change in different modulatory states. So we face a conundrum: the new anatomical data will be instructive, but it is not yet obvious what kinds of models will best reveal the implications of these data for how circuits actually work.

We are in the midst of a fascinating international debate about whether it is the right time to embark on a 'big science' project to monitor and model large brain regions. There are those who argue that we are now at the point at which investments in large-scale projects will considerably advance the field in ways not possible by a distributed small-lab approach[46–48]. Big science works best when the goals of a project are well-defined and when the outcomes can be easily recognized. Both were true about the human genome project, but neither is true, yet, about large-scale attempts to understand the brain. Moreover, this is well-recognized, and all of the proponents of large-scale initiatives are acutely aware of the necessity to develop new technology[48] and of the extraordinary complexity of biological systems[49]. That said, the largest challenge we face in future attempts to understand the dynamics of large circuits is not in collecting the data: what is most needed are new methods that allow our human brains to understand what we find. Humans are notoriously bad at understanding multiple nonlinear processes, although we excel at pattern recognition. Somehow, we have to turn the enormous data sets that are already starting to be generated into a form we can analyze and think about. Otherwise, we will be doomed to creating a machine that will understand the human brain better than we can!

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