Think again

Beyond the connectome: How neuromodulators shape neural circuits

Cornelia I. Bargmann

Powerful ultrastructural tools are providing new insights into neuronal circuits, revealing a wealth of anatomically-defined synaptic connections. These wiring diagrams are incomplete, however, because functional connectivity is actively shaped by neuromodulators that modify neuronal dynamics, excitability, and synaptic function. Studies of defined neural circuits in crustaceans, *C. elegans*, *Drosophila*, and the vertebrate retina have revealed the ability of modulators and sensory context to reconfigure information processing by changing the composition and activity of functional circuits. Each ultrastructural connectivity map encodes multiple circuits, some of which are active and some of which are latent at any given time.

Keywords:
C. elegans; *Drosophila*; neural circuits; neuropeptides; retina

Introduction

A bold goal of modern neuroscience is to enumerate the synapses that make up the connectivity of the brain. By analogy with the complete molecular catalogs of genomics and proteomics, this venture has been called connectomics. As a first step toward that goal, ultrastructural analyses of simple brains and small brain regions that contain tens of thousands or millions of synapses are being conducted. This systematic approach will uncover organizing principles and quantitative features that cannot be obtained from smaller-scale ultrastructural analysis.

A question remains: what will we learn from these complete anatomical descriptions, and what will still be missing? Studies of anatomically-characterized circuits in crustaceans and in *Caenorhabditis elegans* suggest that it will not be possible to read a wiring diagram as if it were a set of instructions. Instead, the anatomical connections represent a set of potential connections that are shaped by context and internal states to allow different paths of information flow. Context and internal states are often represented molecularly by neuromodulators, small molecules that activate G protein-coupled receptors to modify neuronal dynamics, excitability, and synaptic efficiency. These modulators effectively change the composition of a neuronal circuit, recruiting new neurons, or excluding previous participants [1]; recognizing their importance will be central to decoding circuit function.

This essay will describe modulatory mechanisms that allow circuits to change their properties rapidly, dynamically, and reversibly. Neuromodulators permit a fixed complement of neurons to give rise to many different patterns of activity. For the scientist examining neuronal connectivity, however, neuromodulators are a hidden challenge. The essay will first describe the classical modulatory principles discovered by electrophysiological analysis of the crustacean stomatogastric ganglion (STG), and then move to circuit modulation inferred from behavioral studies of *C. elegans*. Studies in flies and mice show that gating of sensory inputs by internal states is an important modulatory mechanism across animals, and context modulation of circuits is another. These roles of neuromodulators allow any wiring diagram to include a variety of circuits, whose properties and composition can be reconfigured during active behaviors.

DOI 10.1002/bies.201100185

Howard Hughes Medical Institute, The Rockefeller University, New York, NY, USA

Corresponding author:
Cornelia I. Bargmann
E-mail: cori.bargmann@rockefeller.edu

Abbreviations:
fMRI, functional magnetic resonance imaging; **NPF,** neuropeptide F; **STG,** stomatogastric ganglion.

Bioessays 34: 458–465,

Circuits within circuits: Modulation of the crustacean stomatogastric ganglion

The STG of lobsters and crabs, a motor circuit of 30 neurons that generates rhythmic output associated with feeding, provided the first compelling evidence for the importance of neuromodulation [2]. The usefulness of this system derives from its ability to generate rhythmic activity patterns when isolated in culture, which allows electrophysiological and pharmacological access in the context of functional circuitry. Within the STG are two subcircuits: a subcircuit that drives alternating muscle groups to constrict and dilate the pyloric valve once a second, and a subcircuit that generates the slower gastric mill rhythm, which oscillates with a period of 5–10 seconds (Fig. 1).

At one level, the pyloric rhythm of the STG arises directly from the properties of its intrinsic neurons. One neuron called AB oscillates as a pacemaker for the one-second rhythm. It forms electrical synapses to PD neurons that drive one muscle group, and both AB and PD release inhibitory transmitters onto the follower LP and PY neurons that drive other muscle groups, providing a simple mechanism of alternation.

In addition to its core synaptic circuitry, however, the STG is richly innervated with neurons that produce neuropeptides and biogenic amines [2]. These neuromodulators act through G protein-coupled receptors to generate dramatic effects on neuronal excitability and synaptic function. To give just one example, a mixed chemical-electrical synapse between LP and PY neurons switches from depolarizing to hyperpolarizing in the presence of dopamine [3]. The rules for modulation are remarkably complex: single modulators affect multiple neurons and the activity of multiple channels, and single target neurons respond to multiple modulators.

Superimposing the modulatory inputs onto the core alternating circuit of the pyloric rhythm changes its function in profound ways (Fig. 1B,C). First, modulation of the circuit can change *circuit dynamics*. For example, sensory stimulation of the stomach activates modulatory inputs to the pyloric ganglia that change the firing of the PY neurons with respect to the overall phase of the pyloric rhythm [4, 5].

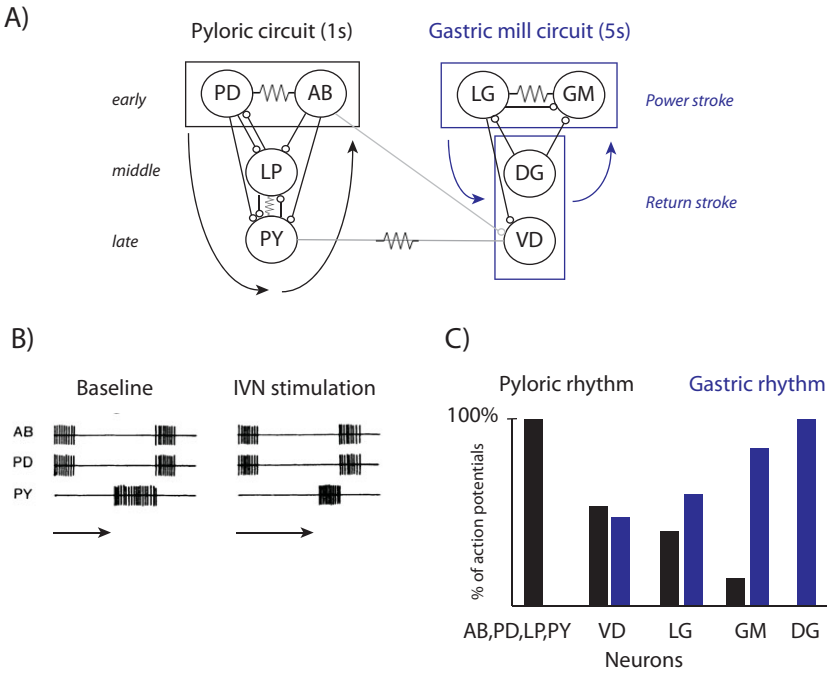

Figure 1. Principles of circuit modulation, illustrated by the crustacean STG. **A:** Simplified view of the two subcircuits of the STG, the pyloric and gastric circuit [2], showing a subset of connections within subcircuits (black) and a few of the connections between subcircuits (gray). Inhibitory synapses are stopped arrows; electrical synapses are jagged lines; arrows denote relative timing of neuronal firing during pyloric and gastric cycles. **B:** Sensory modulation of the pyloric circuit changes the phase of circuit component PY, altering circuit dynamics [5]. Action potentials in three classes of neurons are shown. The IVN nerve conveys sensory information from the stomach; its activation displaces PY action potentials to a later phase of the cycle (arrows). **C:** The degree to which individual neurons fire action potentials together with the pyloric rhythm (black) or the gastric rhythm (blue) during spontaneous activity [58]. Modulatory inputs can shift the membership of ''mixed'' neurons such as VD and LG between the pyloric and gastric mill subcircuits, altering circuit composition [6].

The roles of modulation extend beyond circuit dynamics, however, to include *circuit composition*. Dramatic changes are observed when comparing the activity of ''pyloric'' or ''gastric'' neurons under different conditions (Fig. 1C). A neuron called VD is normally active with the pyloric rhythm, but in the presence of neuromodulatory input can switch its input to join the gastric rhythm. Conversely, a neuron called LG that takes part in the gastric rhythm will fire with the pyloric rhythm when the gastric rhythm is silent. These neurons are not just passive followers of circuitry: the activity of either VD or LG has the ability to reset the phase of both gastric or pyloric rhythms [6]. At a functional level, the VD and LG neurons are full participants in two alternative circuits, depending on modulatory input and ongoing circuit activity states. These specific examples are just a few of the known mechanisms that can reconfigure STG circuits into different assemblies with different properties [7].

The modulation of circuit dynamics and circuit composition discovered in the STG creates a framework for understanding many neuronal circuits. Indeed, the more complete the connectome of a given circuit is, the more modulation must be invoked to explain its functional properties.

The *C. elegans* connectome: Simple anatomy, surprising functional complexity

Part of the rationale for developing the nematode worm *C. elegans* as an

experimental system was that its small nervous system would allow a complete anatomical reconstruction, which took the form of serial-section electron micrographs through the entire adult worm. Each neuronal process was laboriously followed, its neighbors identified, and the patterns of potential chemical synapses and gap junctions identified through their anatomical features. The draft connectome was published in 1986 [8], with a more complete version appearing over 20 years later [9].

Certain features of the nervous system were immediately understandable from the anatomy of the *C. elegans* wiring diagram [8]. For example, about a third of the neurons had specializations indicating a sensory function, another third had synapses onto muscles that identified them as motor neurons, and the final third had both inputs and outputs in abundance, suggesting roles in integration. The overall wiring diagram was largely hierarchical; sensory neurons were more presynaptic than postsynaptic, but for motor neurons the opposite was true. Certain groups of neurons were heavily interconnected, suggesting common functions.

At a more profound level, however, the wiring diagram was and remains difficult to read. The neurons are heavily connected with each other, perhaps even overconnected – it is possible to chart a path from virtually any neuron to any other neuron in three synapses. Modeling has failed, so far, to generate a unifying hypothesis that explains the overall structure of the wiring diagram, or even the functions of commonly observed synaptic motifs [9–13]. Circuit studies suggest a reason for this failure: there is no one way to read the wiring diagram.

One neuron, one behavior?

Targeted ablations of *C. elegans* neurons initially suggested that single neurons had simple, discrete, highly reliable functions [14]. Cell ablation defined a forward locomotion circuit and a backward locomotion motor circuit with largely non-overlapping sets of motor neurons and ''command'' interneurons [15]. Similar ablations defined neurons required for the sensation of mechanical, thermal, and chemical stimuli. Genetic and molecular analysis of sensory and motor functions seemed to dovetail with the ablation analysis: cell fate mutants affecting motor neurons required for backward locomotion preserved near-normal forward locomotion, and mutants with specific sensory deficits largely mapped to genes expressed in the predicted sensory neurons.

However, these sharp distinctions became blurry when applied to integrating neurons, and this issue became increasingly evident with more quantitative analysis of behavior. Careful analysis showed that forward locomotion and reverse locomotion are distributed through the integrating interneurons, not rigidly assigned to a single class [16]. Most neuronal contributions to locomotion and locomotion control are more closely approximated by distributed and quantitative neuronal functions, than by unique and qualitative neuronal functions [17, 18].

One behavior, several circuits

A profound violation of the one neuron-one behavior rule was uncovered by characterizing behaviors under different conditions. For example, avoidance of the repulsive odor octanol at particular concentrations can be generated by two different sets of sensory neurons. In well-fed animals, octanol avoidance is almost entirely mediated by the ASH nociceptive neurons, but after an hour of starvation, octanol avoidance is distributed between ASH, AWB, and ADL nociceptive neurons, revealing a change in circuit composition (Fig. 2A) [19]. Initial results indicated that the fed state could by mimicked by exogenous serotonin, a transmitter associated with food-related behaviors in *C. elegans* [20]. Further analysis has broadened the modulatory inputs to encompass dopamine, tyramine, and octopamine, as well as numerous neuropeptides [21, 22]. These amines and peptides are produced by a variety of neurons, and interact through mutually antagonistic relationships [23]. To a first approximation, the neuromodulators appear to switch the circuit between two alternative functional states: one driven by ASH alone, and one driven by ASH, AWB, and ADL. The actions of these modulators are anatomically and molecularly distributed across the nervous system: for example, part of the effect of serotonin is mediated by a G protein-coupled serotonin receptor on the ASH sensory neurons, whereas another part of the effect requires a serotonin-gated ion channel expressed by interneurons [24].

Food changes the composition of a circuit for oxygen preference behavior (aerotaxis) as well, and the regulation by food is itself subject to second-order modulation. Aerotaxis is more robust in starved than in well-fed animals, due to the activity of multiple neuromodulators (Fig. 2B) [25–27]. A neuropeptide input mediated through the G protein-coupled receptor *npr-1* is one important signal that imposes food regulation on the circuit; another is a TGFbeta-related peptide. These food inputs antagonize a second group of neurons that potentiate aerotaxis, although only a subset of these neurons sense oxygen directly [27]. The circuit for oxygen preference behavior is further altered by rearing animals in hypoxia, which makes them insensitive to the effects of food and reconfigures circuit composition to a smaller set of sensory neurons [28]. In the latter case, a hypoxia-induced transcription factor triggers the circuit change.

One neuromodulator, multiple behaviors

The *npr-1* neuropeptide receptor that affects aerotaxis also regulates a second behavior, the aggregation of animals into feeding groups. Aggregation is triggered by a number of sensory neurons, including the nociceptive ASH neurons and oxygen-sensing URX neurons [29, 30]; it involves a regulation of pheromone-sensing neurons and a large-scale shift from pheromone repulsion to attraction [31]. All of these inputs are integrated by one pair of *npr-1*-expressing neurons called RMGs [31]. The circuit for aggregation is a hub-and-spoke gap junction circuit, in which the RMG hub is linked to the nociceptive, oxygen-sensing, and pheromone sensory neuron spokes by gap junctions (Fig. 2C).

Surprisingly, *npr-1* can differentially affect two behaviors initiated by a single sensory neuron. It is a critical regulator of aggregation triggered by ASH, but it is not essential to nociceptive avoidance mediated by the same neuron [25, 27].

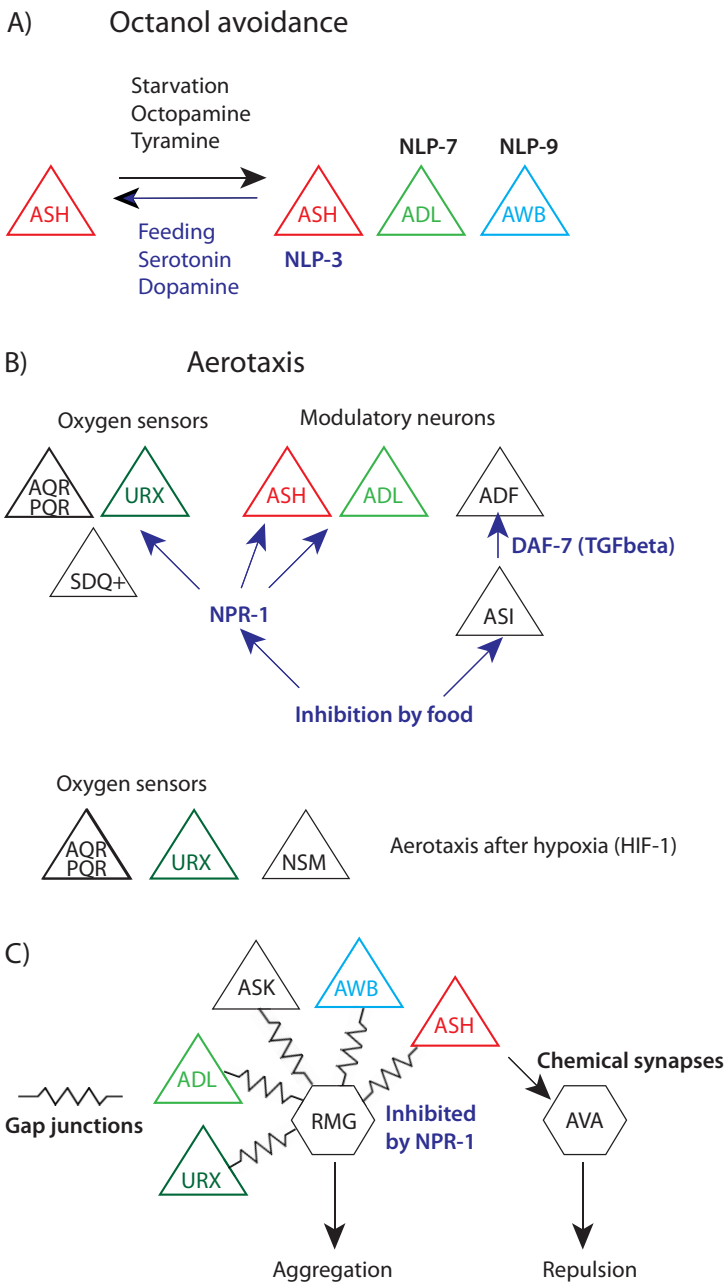

Figure 2. Alternative, overlapping circuits for *C. elegans* sensory behaviors. Triangles are sensory neurons (arbitrary colors are used to highlight the neurons that appear in several panels); hexagons are integrating neurons. In each panel, pathways for food regulation appear in dark blue. **A:** Sensory neurons and neuromodulators that affect octanol avoidance in starved and well-fed animals. Blue, modulators or neuropeptides that enhance or accelerate avoidance; black, modulators that delay avoidance. Neuropeptides within this sub-circuit are shown adjacent to the neuron that produces them; biogenic amines derive from external sources [23]. **B:** Alternative sensory circuits for aerotaxis behavior. Aerotaxis is inhibited by food through inhibitory peptide signaling onto oxygen-sensing and modulatory neurons [25–28]. After growth in hypoxia, transcriptional regulation via HIF-1 collapses aerotaxis into a smaller circuit that is resistant to food regulation. **C:** ASH chemical synapses are required for acute avoidance behavior, whereas ASH gap junctions in a hub-and-spoke circuit regulate aggregation; these two classes of ASH synapses are differentially regulated by the NPR-1 neuropeptide receptor [31–33].

The explanation for this result can be inferred by examining ASH circuitry. The avoidance of noxious repellents is driven by glutamatergic chemical synapses from ASH to interneurons that control backward locomotion [19, 32, 33]. Aggregation, however, is driven by ASH gap junctions with the RMG neurons, not by ASH chemical synapses [31]. *npr-1* action in RMG uncouples the aggregation circuitry, but leaves the avoidance circuitry intact. This allows ASH to generate different behaviors in two neuromodulatory states: avoidance occurs regardless of modulation, and aggregation occurs only when *npr-1* activity is low.

Neuromodulators define a set of active circuits among potential, latent circuits

The implication of these studies is that information flow through *C. elegans* circuits depends on neuromodulatory states. The anatomical wiring diagram encodes the potential for multiple behaviors, but only a subset of those behaviors are accessible at any given time. AWB and ADL may participate in octanol avoidance, or they may not, depending on amines and peptides. The RMG hub-and-spoke circuit may drive aggregation, or it may be functionally silent, depending on *npr-1*.

These results indicate that neuromodulation in the *C. elegans* nervous system selects a set of functional synapses among a greater number of anatomically-specified possibilities. This understanding of nervous system function creates a profound problem for the wiring diagram. First, the anatomical wiring diagram is ambiguous. Its connections contain the potential for different behaviors, but the neuromodulatory state determines whether this potential is available at a particular time. The known effects of biogenic amines and peptides regulated by food are probably just a few among many ways of sculpting the potential connectivity. Second, the wiring diagram is incomplete. Neuropeptides and biogenic amines are not necessarily associated with anatomically-defined synapses that can be recognized in the wiring diagram. They can be released synaptically or extrasynaptically, and they can act on targets locally or at a distance, depending on the amount of

modulator that is released, the efficacy of the receptors at different sites, and the rate of degradation. Serotonin and dopamine are synthesized by only three major sets of neurons each in *C. elegans*, but serotonin and dopamine receptors are present and probably functional on sensory neurons, interneurons, and motor neurons that receive no direct synapses from the modulatory neurons [34, 35]. But there is no reliable way to assess the complete modulatory state within any animal, including *C. elegans* – it is the dark energy of the nervous system, inferred but not measured.

The *C. elegans* wiring diagram consists of just 302 neurons, but the *C. elegans* genome encodes over 200 neuropeptides, suggesting that the potential for modulation is considerable. Nematode nervous systems evolve very slowly at the cellular level – the foot-long parasitic nematode *Ascaris* has just 298 neurons, and most of these can be recognized as one-for-one homologs of the 302 *C. elegans* neurons, despite entirely different lifestyles and an estimated 100 million years of evolutionary time [36]. With this low level of cellular diversity, it is not surprising that neuronal circuits would have multiplexed functions driven by neuromodulation. Having said that, the results described below argue that even complex nervous systems make use of multiplexing to generate their functions, and that neuromodulation is an essential component of their flexibility.

Neuromodulation in flies and mammals

Some of the strongest mechanistic insights that link behavioral states, neuronal activity, and circuit function have been obtained in *Drosophila* and mammals. Although these animals lack the single-neuron resolution of the STG or the worm, they benefit from a combination of anatomy, physiology, molecular analysis, and behavior that can provide global insights into modulatory function.

Sensory inputs are gated and modulated by internal states

One mechanism by which neuromodulators reconfigure circuits is to change the gain of peripheral sensory inputs. A familiar example of this kind of plasticity is stress-induced analgesia, an acute suppression of pain responses that has been characterized in rodents and to some extent in humans [37]. During stress, enkephalin and other peptides (endogenous opioids) are released by descending brainstem circuits and peripheral cells, and temporarily inhibit pain sensation by activating G protein-coupled opioid receptors on the presynaptic terminals of primary nociceptive neurons and spinal cord resident neurons [38]. The opioid receptors diminish synaptic neurotransmitter release by nociceptive neurons, inhibiting the perception of pain. Stress-induced analgesia is a dramatic example of the uncoupling of a sensory stimulus by a neuromodulator, and demonstrates that the principle of flexible circuit composition extends to mammals.

The synaptic terminals of sensory neurons are important sites of neuromodulation, and gating of sensory inputs by neuromodulators appears to be a common principle across systems. Circuit modulation by feeding and starvation provides numerous examples of this principle, as shown in leech mechanosensory neurons [39], and perhaps most completely by recent work in *Drosophila*. Starvation increases the sensitivity of a fly's behavioral response to sugar, an effect that depends on the endogenous modulator dopamine and the expression of a specific dopamine receptor in taste receptor neurons [40] (Fig. 3). Mechanistically, dopamine enhances presynaptic calcium influx into the taste receptor neuron, gating sensory input. In addition, an inducible molecular reporter for dopamine receptor activation showed that dopamine is released onto taste neurons during starvation in vivo [40]. The reporter was comprised of a dopamine receptor indirectly coupled to a transcriptional regulator (DopR-TANGO); in the future, similar reporters for other modulators, particularly peptides, have the potential to greatly facilitate measurements of internal modulatory states.

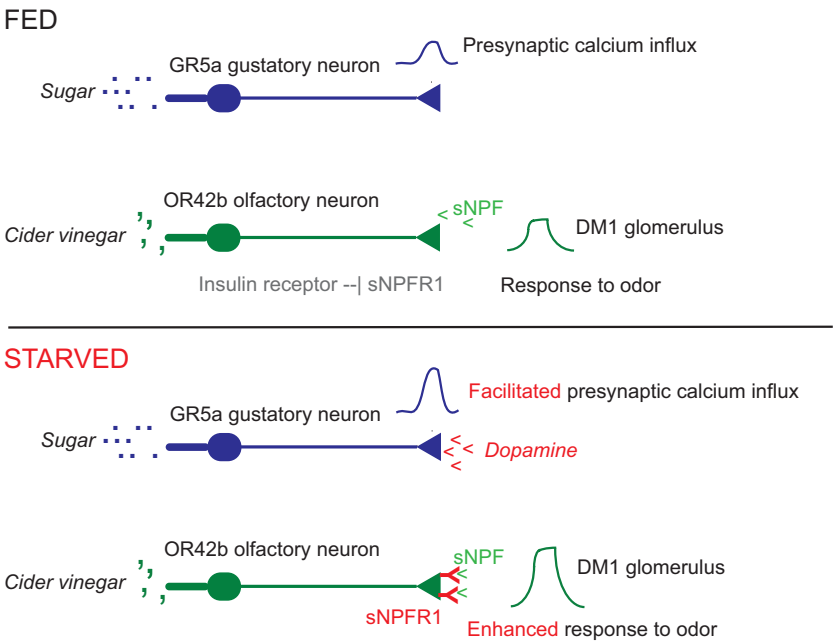

Figure 3. Modulation of *Drosophila* sensory gain by feeding state [40, 41]. In well-fed flies, sugar and cider vinegar stimulate gustatory (GR5) and olfactory (OR42) neurons, respectively. Presynaptic calcium influx into GR5 axon terminals was measured directly; OR42 presynaptic function was inferred from responses in the postsynaptic dendrites of the DM1 glomerulus. The neuropeptide sNPF is released locally in olfactory tissues, but expression of its receptor is suppressed by insulin-like peptides that are present in fed animals. In starved flies, dopamine release onto GR5 presynaptic terminals facilitates calcium influx, and the sNPF receptor is induced on OR42 neurons, leading to presynaptic facilitation. In both gustatory and olfactory neurons, the gain of sensory input is increased by neuromodulation during starvation.

Starvation and satiety are complex signals that are represented by multiple neuromodulators. In *Drosophila*, starvation causes the release of neuropeptide F (NPF) and short NPF in the olfactory antennal lobe, which facilitates presynaptic neurotransmitter release from the olfactory neurons and stimulates food search during starvation [41] (Fig. 3). Fly feeding and satiety are associated with an antagonistic neuromodulatory signal, the secretion of insulin-related peptides that suppress transcription of the sNPF receptor in olfactory neurons [41]. Antagonistic groups of neuromodulators are also associated with feeding and satiety in mammals, where local peptidergic modulation in the hypothalamus interacts with long-range peptide signaling from the intestine and fat stores to modulate appetite [42, 43].

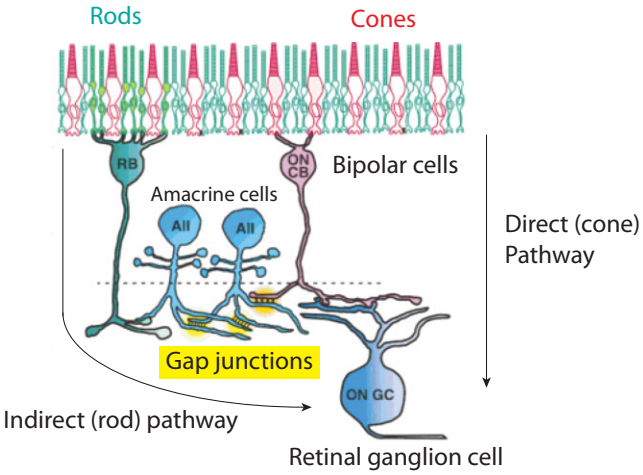

Figure 4. Overlapping circuits in the rabbit retina. Rod and cone inputs converge on cone bipolar cells, with cones taking a direct route and rods an indirect route through rod bipolar cells and AII amacrine cells. All amacrine cells form gap junctions with each other and with cone bipolar cells; these gap junctions (among others) are regulated by sensory input and neuromodulation (image modified from [46]).

Sensory context modulates circuit states

Circuits can change their properties rapidly in response to environmental context signals as well as internal cues, and some of these effects are also mediated by neuromodulators. The existence of alternative circuit states is elegantly demonstrated by the effect of sensory context on information processing in the mammalian retina (Fig. 4). Multiple properties of retinal circuits shift when an animal moves from low light levels, where retinal processing is dominated by rod photoreceptors, to higher light levels dominated by cone photoreceptors. Rods and cones synapse onto different bipolar neurons, but this information ultimately converges on common retinal ganglion cells that function differently in bright or dim light. The site of rod-cone convergence is the cone bipolar cell, which receives direct input from cones and indirect input from rods through rod bipolar cells and AII amacrine cells. The electrical synapses between AII amacrine cells and cone bipolar cells in the indirect pathway are regulated by light levels, circadian rhythm, and neuromodulators such as nitric oxide and dopamine. When light levels are high, gap junctions linking the AII amacrine cells and the cone bipolar cell are uncoupled, effectively cutting off information from the rod pathway [44–46].

More detailed analysis of retinal circuits has demonstrated that state-specific circuit function applies to a variety of conditions. For example, one class of retinal ganglion cells in the salamander responds to light offset (OFF) under baseline conditions, but responds to light onset (ON) immediately after a stimulus that mimics an eye movement, or saccade. This switch in circuitry is mediated by inhibitory amacrine cells that detect large-scale changes in the retinal scene to modify processing [47].

Neuromodulation at fast and slow timescales is observed across evolution

The ubiquity of neuromodulation at fast and slow timescales, in invertebrate and vertebrate brains, suggests that it will also apply to humans. Information processing in the human brain cannot be resolved at the level of individual circuits and synapses, but large-scale analysis of brain activity with functional magnetic resonance imaging (fMRI) data suggests that the functional connectivity of information flow in the human brain changes during alternative circuit states. The most extreme example of state-dependent activity is the difference between brain activity during sleeping and waking states, but there are more subtle examples as well. For example, exposure to stressful visual stimuli engages fMRI activity patterns distinct from those engaged by neutral stimuli, and this effect is inhibited by the beta-adrenergic antagonist propanolol, implying a role of noradrenergic neuromodulation [48].

Neuromodulation by noradrenaline and adrenaline represents one of the most widespread mechanisms for reconfiguring circuit function in the mammalian cortex. It is best known for its role in the "fight or flight" arousal response, but also has a more general role in decision-making [49]. The invertebrate neurotransmitters tyramine and octopamine, which are structurally similar to noradrenaline and signal through related GPCRs, also have roles in arousal and decision-making [20, 50, 51]. These analogies suggest that some forms of modulation may be conserved not only at a conceptual level, but also at a molecular level, across species.

Visible wiring diagrams will interact with neuromodulators

Anatomical reconstructions of neuronal circuits are ongoing in a variety of experimental systems [52]. The vertebrate retina is the subject of focused ultrastructural attack through serial-section electron microscopy combined with antibody staining that defines neurotransmitters and cell types [53]. Other circuits that are being subjected to intensive ultra-

structural analysis include the *Drosophila* brain and the mammalian cortical column, a ~1 mm^3 array of a few thousand neurons that is the fundamental unit of computation in the cerebral cortex [54, 55]. These efforts are complemented by parallel lower-resolution studies that will map long-range connections between brain areas [56].

Anatomical reconstruction has provided, and will provide, valuable information about brain function. Defining neuronal connectivity will help to delineate the functional classes of neurons; we cannot understand the brain until we know its parts. Ultrastructural analysis will help establish the rules for synaptic connectivity, which can only be incompletely inferred from electrophysiological techniques that glimpse a subset of connections at any given time. There is no other approach that can provide a full picture of the inputs even onto a single neuron, let alone groups of neurons.

Many results, however, suggest that anatomically-defined connections between brain areas are necessary but not sufficient to define patterns of brain activity. The ultrastructural synapses between neurons will encode the precise, millisecond-speed information flow that is essential to sensory perception, complex motor outputs, and cognition. These synapses will not represent the modulators that alter circuit dynamics and circuit composition, because most neuromodulatory inputs are extrasynaptic and many derive from diffuse long-range projections that will be invisible or uninformative in the first level of connectome analysis. In the simplest formulation, modulators will select a subset of the anatomically-defined synapses for activity under a given set of conditions. Through their effects on neuronal excitability and presynaptic efficacy, they will sculpt information flow on the seconds-to-minutes timescale of GPCRs and second messengers.

Conclusions and outlook: Overconnected circuits and latent circuits

The idea of flexible neuromodulation of circuit states makes predictions about what the anatomical wiring diagrams will show. It suggests that neurons will seem to be ''overconnected'', with a greater variety of synapses than seem necessary for their functions. These extra synaptic connections will not be random or learned – they will represent *latent circuits* for alternative modes of information processing. Indeed, initial ultrastructural analysis of the retina has revealed many synapses that were not known or predicted from physiological studies [53]. At a macroscopic level, structural approaches to large-scale brain connectivity, such as diffusion tensor imaging, also suggest a very high degree of interconnectedness between human brain regions [57]. It may be useful to regard these connections as a set of alternatives, not a set of invariant instructions.

Defining the connectome is like sequencing the genome: once the genome was available, it was impossible to imagine life without it. Yet both for the genome and for the connectome, structure does not solve function. What the structure provides is a better overview, a glimpse of the limits of the problem, a set of plausible hypotheses, and a framework to test those hypotheses with greater precision and power.

Acknowledgments

Many thanks to Eve Marder, David Anderson, Howard Fields, Andres Bendesky, Steve Flavell, Andrew Gordus, Alex Katsov, and anonymous reviewers for their comments and advice. C. I. B. is an Investigator of the Howard Hughes Medical Institute.

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