1 INTRODUCTION

Animals live long lives. Humans, sea turtles, elephants, and lobsters all may live for a good portion of a century. In these animals the nervous system that generates appropriate behavior consists, for the most part, of neurons that are born early in the animal’s life. During early development the complex neuronal circuits that mediate behavior are formed, and these circuits retain their functional integrity for most of the healthy animal’s lifetime. Several features of nervous systems make this particularly remarkable. (1) The nervous system must retain its essential flexibility and plasticity so that the animal can adapt to changes in its environment and learn. (2) Although individual neurons can live for tens or even one hundred years, all of the ion channels, receptors, and cell signaling proteins that give each neuron its characteristic structure and electrical properties turn over in minutes, hours, days, or weeks. Indeed, unlike electronic circuits which are built of static components, the nervous system consists of a relatively stable structure that is constantly rebuilding itself, as all of its constituent molecules are
replaced. Moreover, during normal behavior the properties of individual neurons and their synaptic connections are constantly varying, but do so in a manner that ensures functional changes in network output, and rarely leads to loss of network stability.

There are three fundamental and linked questions that we must address in understanding the nervous system:

1. How do short-term modifications of synaptic strength and neuronal properties lead to adaptive changes in network dynamics while avoiding regimes in which network stability is lost?
2. How is behaviorally relevant neuronal function maintained during growth of the animal and nervous system?
3. How does the nervous system maintain a relatively constant structure and function while constantly replacing its molecular structure?

As an example, in this chapter we will use a small model nervous system, the crustacean stomatogastric nervous system, that allows us to address these issues experimentally.

2 NETWORK DYNAMICS DEPEND ON THE INTERACTION BETWEEN SYNAPTIC AND INTRINSIC PROPERTIES

Years of both experimental and computational studies have demonstrated that the dynamics of networks depend both on the properties of the synapses connecting neurons and on the intrinsic properties of the network neurons. It is well accepted that synaptic connections vary in sign, strength, and time course [34], and that modifications of any of these properties can alter network dynamics. In contrast, it is less well-appreciated that modifications of neuronal intrinsic properties can also be important for alterations in circuit dynamics.

We call a neuron’s intrinsic properties its electrical properties when studied in isolation of all synaptic inputs (fig. 1). Some neurons are silent, others fire slowly, still others fire bursts of action potentials in isolation. Some neurons respond to strong inhibition with post-inhibitory rebound firing [35, 36]. Others show bistability and generate plateau potentials [29]. Still others show spike-frequency adaptation. These disparate firing properties depend on how many ion channels of different types an individual neuron has [12]. Because there are numerous forms of K⁺, Na⁺, and Ca²⁺ channels that differ in their voltage and time dependence [24] as well as density and location in the neuronal membrane, wide variations in the dynamics of firing produced by individual neurons are possible. Therefore, as will be seen below, the control of the number, kind, and distribution of the ion channels that give neurons their characteristic intrinsic properties is important. Because intrinsic neuronal properties determine how a neuron responds to a given synaptic input, modifications of any of the membrane currents
FIGURE 1 Intrinsic membrane properties of neurons. Upper panel: Neurons show different spontaneous activity in isolation from synaptic input. Lower panel: Voltage-dependent currents lead to nonlinear responses to input.

that shape a neuron’s intrinsic properties can alter network dynamics [17]. That said, it is important to recognize that there are many different mixtures of ion conductance densities that can produce similar activity patterns [12, 16]. Therefore, individual neurons with similar activity patterns may do so by utilizing different biophysical mechanisms.

3 THE STOMATOGASTRIC NERVOUS SYSTEM

The stomatogastric nervous system governs the movements of the crustacean foregut [18, 49]. It consists of a group of four ganglia that are linked by nerves (fig. 2(a)). The bilateral commissural ganglia (CoGs) each have 400–500 neurons including a number that project down the connecting nerves to modulate the stomatogastric ganglion (STG). The single esophageal ganglion (OG) consists of 14–18 neurons, several of which also project into the STG. The STG consists of about 30 neurons, and contains the motor neurons for two different motor patterns, the rapid pyloric rhythm and the slower gastric mill rhythm. These motor patterns can be recorded extracellularly from the motor nerves or intracellularly from the somata of the STG neurons themselves (fig. 2(a)). Figure 2(b) illustrates the triphasic pyloric rhythm. Shown are three simultaneous intracellular
FIGURE 2 The crustacean stomatogastric system. (a) The isolated stomatogastric nervous system placed in a dish. (b) Intracellular and extracellular recordings from cells of the pyloric system. (c) Simplified connectivity diagram for the pyloric rhythm. OG: oesophageal ganglion; CoG: commissural ganglion; STG: stomatogastric ganglion; LP: lateral pyloric (motor neuron); PY: pyloric; PD: pyloric dilator; AB: anterior burster; lvn: lateral ventricular nerve.

recordings from the lateral pyloric (LP), pyloric (PY), and pyloric dilator (PD) neurons and an extracellular recording from the lateral ventricular nerve (lvn) which shows the activity of all three.

Figure 2(c) is a simplified connectivity diagram for the pyloric rhythm. The anterior burster (AB) neuron is an interneuron that is intrinsically oscillatory when isolated from other pyloric neurons [48]. The AB neuron is electrically coupled to the PD neurons, and this electrical coupling causes the AB and PD neurons to burst synchronously although the PD neurons do not generate rapid bursts in the absence of the AB neuron. Together the AB and PD neurons inhibit the LP and PY neurons. The LP neuron recovers from inhibition before the PY neurons [21, 22], and the reciprocal inhibition between the LP and PY neurons produces their alternation.

The reader will note that this circuit depends critically on synaptic inhibition and electrical synapses. The pacemaker kernel consists of the electrically coupled PD and AB neurons, and the timing of the firing of the LP and PY neurons is set by the synaptic and intrinsic processes that govern the dynamics of the postinhibitory rebound bursts [10, 19, 22].
4 THE PROBLEM POSED BY MODULATION OF THE ADULT NERVOUS SYSTEM

Neuromodulators are substances that can dramatically alter the strength of synapses and/or the intrinsic properties of the individual neurons in a circuit [17]. A great deal of work over the years has shown that the STG is modulated by at least twenty different substances found both in identified input neurons or released as circulating hormones [35, 43, 44]. These include amines such as dopamine [30], serotonin [3], octopamine [2], and histamine [6, 42]. Additionally, a very large number of neuropeptides are found in fibers that project to the STG [35], and other small molecule neurotransmitters and gases also are likely modulators of the STG [47, 53].

What do neuromodulators do to the network dynamics of the STG? The changes in network dynamics produced by either the application of these substances in the bath, or by stimulating the neurons that contain them have been extensively characterized [35, 36, 43, 44]. These experiments have shown unambiguously that each neuromodulator and neuromodulatory neuron can evoke characteristic and different changes in the pyloric rhythm [35, 37, 40, 43, 44]. These involve changes in rhythm frequency, in the number of action potentials fired by each neuron in each burst, and in the relative timing or phase relationships of the constituent neurons within the motor pattern.

How do modulators produce changes in network dynamics? Neuromodulators can alter the strength of synapses [26] and/or modify the intrinsic excitability of individual neurons [11, 25]. Years of work on the effects of neuromodulatory substances on the STG have shown: (a) every neuron in the pyloric rhythm is subject to modulation by multiple substances [20, 52, 53], (b) every synapse in the pyloric network is subject to modulation [28], and (c) each modulator alters a different subset of parameters in the circuit.

It is beyond the scope of this contribution to review in detail these modulatory actions, but the general principle is illustrated in the cartoon schematic shown in figure 3. Each modulator acts on a different subset of possible targets for modulation, and consequently produces a different altered circuit output. This organization poses a deep design problem: with so many circuit components subject to modulation, in so many different combinations, how is it possible that the circuit that produces the pyloric rhythm, and other like circuits, are stable? Why does this rich pattern of modulation not result in “overmodulation” or circuit “crashes”? What protections are there so that functional circuit outputs are maintained together with all of this potential for flexibility?

The answers to the above questions are not obvious. Nonetheless, some beginning insights can be drawn from what we know about the cellular mechanisms underlying neuromodulation. Many neuromodulators act through second messengers to indirectly open or close ion channels [24, 32]. This sets the stage for several neuromodulators to converge onto the same signal transduction pathway.
or on the same ion channel. The consequence of such convergence is that modulators can saturate and occlude each other’s actions. This is seen in the crab STG, where six different modulators converge on the same voltage-dependent inward current [53], called the proctolin current, because it was first described in studies of the action of the neuropeptide proctolin [13]. The LP neuron is a direct target for all of these substances. Nonetheless, if one of the modulators has strongly activated the LP neuron, a second or third modulator will have little or no effect. Thus, the convergent action of neuromodulators at the cellular level provides a “ceiling effect” that prevents overmodulation. This is one of the cellular correlates of “state-dependent neuromodulation,” modulation that depends on the initial state of the network when the modulator is applied. These modulatory peptides are found in different combinations in specific projection neurons [4], and because these substances act on different network targets that display receptors for a subset of them, projection neurons that release different combinations of neuropeptides still evoke different motor patterns.

The voltage dependence of the proctolin current provides a second cellular mechanism that protects against overmodulation [38, 50]. When an oscillatory neuron is depolarized by constant current or the application of a conventional excitatory neurotransmitter, the baseline membrane potential and the membrane potential at the peak of the slow wave are both depolarized (fig. 4(a)). As the amplitude of the depolarization is increased, eventually the oscillator may be blocked in the “up” state. In contrast, the proctolin current’s voltage dependence

FIGURE 3  Modulation of neuronal networks. Each modulator acts on a different subset of possible targets for modulation, and consequently produces a different altered circuit output.
FIGURE 4  Different effects of modulation. (a) Applying an agonist to a ligand-gated channel delivers a tonic enhancement of the current through this channel. The baseline membrane potential is shifted and higher concentrations of the agonist can “crash” the rhythmic activity. (b) Modulating a voltage-gated channel via second messenger systems will only take effect in the phase of the cycle when the channel is activated. Therefore, only the amplitude of activity in a specific phase is altered and the baseline membrane potential is not affected.

means that at the trough of the oscillation there is no depolarization, but at the peak there is a large effect. Therefore, the baseline of the oscillator is not depolarized, but the amplitude of the oscillation is much enhanced (fig. 4(b)). Again, this allows the frequency and the amplitude of the pacemaker to be modulated, but prevents the pacemaker kernel from losing its ability to generate bursts.

There are undoubtedly numerous other cellular and circuit mechanisms that also protect against overmodulation. One of the challenges of the future will be to understand how these mechanisms function together to ensure that modulators can alter circuit behavior so effectively and safely.

5 THE PROBLEM POSED BY GROWTH

In most animals many of the circuits that underlie behavior must function continuously during the animal’s lifetime. In lobsters, the STG is already active by midway through embryonic life [5, 46], and even small juvenile lobsters produce
adult motor patterns. Figure 5(a) shows the relative sizes of a small juvenile lobster and a fully mature adult lobster. Figure 5(b) shows an intracellular dye fill of a PD neuron in the STGs of a juvenile and an adult animal shown on the same scale. First, it can be seen that the difference in the size of the STG is smaller than the difference in the size of the animals. Nonetheless, figure 5(b) shows that the growth in the animal is accompanied by significant growth in the size of the STG and in the size of the individual neurons that produce the STG's motor patterns. Despite these dramatic changes in cell and ganglion size, figure 5(c) shows that the motor patterns produced by the small and large animals are virtually indistinguishable.

It is easy to appreciate that it is important for animals to be able to produce constant motor patterns despite their growth. That said, it is not intuitively obvious how this is possible. Neurons are not isopotential. Rather, electrical signals propagate through the processes of a complex structure as a function of the number and distribution of channels [7, 28]. In principle, it is possible for a single neuron to grow in such a manner as to maintain its passive electrophysiological properties [23, 45]. However, for this to translate into constant activity, not only would the structures need to expand at the appropriate size ratios, but the number and placement of the voltage-dependent conductances would have to be properly controlled. In at least one system, changes in synaptic efficacy accompany neuronal growth [9], as synaptic sites change their relative positions in the neuronal arbor.

Among unanswered questions posed by the data shown in figure 5 are: (a) Are the motor patterns in young and old animals produced by the same underlying mechanism? It is possible that although the motor patterns are the same, different combinations of synaptic and intrinsic properties could give rise to these. (b) What are the control principles used to maintain constant physiological processes despite the large changes in the structure of the neurons that produce the circuit output?

6 THE PROBLEM POSED BY CHANNEL TURNOVER

Even in the adult animal undergoing no further growth, protein turnover is ongoing. Therefore, all of the channels that are important for excitability are constantly being replaced. Thus, it is not sufficient for neurons to establish their intrinsic properties once, but rather neurons are synthesizing, inserting, removing, and degrading ion channels and receptors continuously. What controls this constant flux of membrane proteins? If this flux were not properly controlled, then the characteristic intrinsic membrane properties of network neurons would be subject to fluctuations that might be deleterious to network function. In a series of theoretical studies, we argued that neurons can use intracellular concentrations of Ca\(^{2+}\) or other signal transduction molecules as activity sensors [1, 14, 15, 31, 33, 51]. In these models, the intracellular activity sensors
were then used to control the densities of each kind of ion channel in the membrane, using simple negative feedback rules to achieve homeostasis [39]. These models had several essential features that made them extremely attractive: (a) model neurons with dynamically regulated current densities could self-assemble from random initial conditions [33], and (b) when perturbed by an extrinsic input, model neurons with dynamically regulating conductances could recover their initial pattern of activity, even with an ongoing perturbation [31, 33]. Since these models were first proposed, a number of experimental studies have been conducted that are consistent with the spirit of these models.

Figure 6(a) shows the results of experiments that suggest that isolated STG neurons can cell-autonomously acquire the balance of conductances necessary to produce an activity state similar to the one shown in the intact ganglion. In the intact network, all STG neurons fire rhythmically, although most of these neurons do so because of the rhythmic synaptic drive that they receive. When single STG neurons are removed from the ganglion and placed in dissociated cell culture they are initially relatively inactive [57, 58]. The top panel of figure 6(a) shows that on day 1 of culture they typically fire small, rapidly inactivating action potentials. On day 2 of culture, they tonically fire full action potentials (middle panel). On day 3 of culture, they typically fire in bursts, the pattern of activity most similar
FIGURE 6 Plastic changes in intrinsic membrane properties. (a) STG neurons in dissociated cell culture are actively regulating their current densities to rebuild their rhythmic activity state. (b) Rhythmic activity of the STG resumes some time after modulatory inputs have been removed.

to that which they show in the intact STG. The histograms in figure 6(a) show the results of voltage clamp measurements of the currents in these neurons on these days. The neurons are actively regulating their current densities to rebuild their rhythmic activity state. Note that as the current densities of the inward currents are increasing, those of the outward currents are decreasing. Such changes will tend to make the neurons more excitable and more oscillatory [58].

The premise of the theoretical models described previously was that neurons use their own activity to regulate conductance densities. Consistent with this interpretation are a number of experiments. First, when STG neurons are rhythmically stimulated, their K⁺ current densities are altered [14, 15]. Second, when vertebrate cortical neurons are placed in TTX to block all action potentials, they upregulate their Na⁺ conductances and downregulate their K⁺ conductances [8], as predicted by the earlier models.

Thus far, the models and the data we have discussed refer to regulation of the properties of single neurons. However, what matters for behavior is how whole networks of neurons are controlled. Figure 6(b) shows the results of experiments that demonstrate that network stability may be controlled as well [14, 15, 41, 55, 56]. In this experiment the top set of recordings are extracellular recordings of the pyloric rhythm in an experiment in which the anterior ganglia containing modulatory inputs were left intact. Then these inputs were removed, and the rhythmic activity was lost. However, approximately 1 day later, rhythmic activity
resumed. In this case, the rhythmic activity was independent of the modulatory inputs. A model of this recovery process [14, 15] showed that cell-autonomous activity sensors were sufficient to produce network stability. These data strongly suggest that sensors of either activity and/or the release of neuromodulatory inputs may be important for the regulation of channel density necessary for stable network function. Moreover, these data argue that similar network function can be produced by different underlying mechanisms: in the control case the rhythm strongly depends on currents that are up-regulated by modulators whereas after recovery there have been changes in the network that allow it to function in the absence of neuromodulatory inputs.

7 CONCLUSIONS

Robustness of neuronal structure and function depends on all of the complexity of biological structures. Every cell in a biological structure contains thousands of control pathways that allow differentiation and appropriate responses to the environment while maintaining cellular homeostasis. The nervous system uses all of this miraculous biochemical and molecular control to implement complex networks that themselves maintain precise organization while at the same time all of the constituent molecules are in a constant state of flux. It is hard to reconcile the vast molecular and cellular complexity displayed by neurons and nervous systems with the fact that they work so well.

REFERENCES


