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NEUROMODULATION OF NEURONAL CIRCUITS: BACK TO THE FUTURE

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Abstract

All nervous systems are subject to neuromodulation. Neuromodulators can be delivered as local hormones, as cotransmitters in projection neurons, and through the general circulation. Because neuromodulators can transform the intrinsic firing properties of circuit neurons and alter effective synaptic strength, neuromodulatory substances reconfigure neuronal circuits, often massively altering their output. Thus, the anatomical connectome provides a minimal structure and the neuromodulatory environment constructs and specifies the functional circuits that give rise to behavior.

Introduction

Neuromodulation adds extraordinary richness to the dynamics that networks can display. It also adds confounds of many kinds that require that we relinquish our wish for simple and linear answers to how brain circuits work. In this review, my goal is to summarize many of the take-home lessons from old and new work on neuromodulation that can inform the trajectory of future work on circuits, large and small.

Historians say that we should study history to avoid repeating the mistakes of the past. Remarkable advances in anatomical methods, genetics, optogenetics and optical recordings are providing extraordinary opportunities for understanding circuit structure and function in brains, large and small. The present era of circuit exploration is tremendously exciting. At the same time, I see numerous examples of today's researchers effectively "reinventing the wheel", albeit elegantly enough for publication in our elite journals, partially because the new work is done with state-of-the art techniques, and partially because the pioneering work on modulation and dynamics of small circuits has been partially obscured by the mists of time. Those interested in how circuit dynamics arise from the properties of neurons and their connections should read Getting 's prescient 1989 review (Getting, 1989).

Studies of some of the substances that we now term neuromodulators have a long and venerable history. The pharmacologists who worked 80 and 100 years ago already knew that there were multiple receptors for acetylcholine and norephinephrine (Dale, 1935), and that these were pharmacologically separable. By the early '70's it was already clear that different classes of neurons released different neurotransmitters (Barker et al., 1972; Carraway and

Leeman, 1973; Chang and Leeman, 1970; Kerkut and Cottrell, 1963; Kerkut and Walker, 1966; Otsuka et al., 1967; Walker et al., 1968), and that there were a large number of signaling molecules used in the brains of all animals including ACh, dopamine, norepinephrine, GABA, glycine, glutamate, serotonin, histamine, octopamine, and neuropeptides.

Although the diversity of signaling molecules was fascinating neurochemists of the day, many of the earliest workers interested in the neuronal circuits that gave rise to behavior saw no relevance of what they called “pharmacology” or “neurochemistry”. Instead, many of the early circuit electrophysiologists came from the traditions of engineering and electronics, and sought to develop a connectivity diagram (or connectome in today’s parlance) that would be the biological equivalent of an electronic circuit diagram, taking advantage of the identifiable neurons in invertebrate sensory and motor circuits (Burrows, 1975a, b; Calabrese and Peterson, 1983; Getting, 1981; Heitler and Burrows, 1977; Kristan and Calabrese, 1976; Kristan et al., 1974; Mulloney and Selverston, 1974a, b; Stent et al., 1978; Stent et al., 1979; Willows et al., 1973; Wilson, 1961; Wilson, 1966).

I was once told by one of the leaders in the field that the neurotransmitter that mediated a synaptic connection was irrelevant, and the only thing that mattered was the sign of the synapse, excitatory or inhibitory. Although today’s anatomists must know that neuromodulatory neurons can release their cotransmitters at a distance from their targets (Blitz et al., 2008; Brezina, 2010; Jan and Jan, 1982), the underlying assumption of today’s electron microscope connectome projects (Briggman et al., 2011; Chklovskii et al., 2010; Denk et al., 2012; Lichtman and Denk, 2011; Seung, 2011) is that the conventional close-apposition synapses provide most, if not all, of the information needed to characterize the circuit, the same assumption that was made 35 years ago the small-circuit physiologists.

The Early Era of Neuromodulation

In their preface, Kaczmarek and Levitan (1987) wrote that their book, **Neuromodulation: The Biochemical Control of Neuronal Excitability**, was intended to create a working understanding between electrophysiologists and biophysicists on one hand and neurochemists on the other hand, to understand the modulation of neuronal excitability and its consequences for neural processing. By 1987 it was clear that:

1. Neuronal intrinsic properties, action potential waveforms and membrane currents could be altered by manipulating the intracellular concentrations of second messengers such as cAMP (DeRiemer et al., 1985; Hockberger and Connor, 1984; Kaczmarek et al., 1986; Levitan, 1978; Siegelbaum et al., 1982).
2. Exogenous application of muscarinic agonists, amines and neuropeptides can increase or decrease the amplitude of a variety of voltage-dependent currents (Adams and Brown, 1980; Brown and Adams, 1980; Camardo et al., 1983; Dunlap and Fischbach, 1981).
3. Exogenous application of neuromodulators could alter the strength of synapses (Dudel, 1965; Glusman and Kravitz, 1982; Klein et al., 1982; Klein and Kandel, 1978), with implications for experience-dependent changes in behavior (Kandel and Schwartz, 1982).

Neuromodulation was part of a paradigm shift in the study of small circuits- the first “beyond the connectome realization”

By the end of the 1980’s there was an almost complete paradigm shift in the study of small circuits for six reasons:

1. It saw the end of the hope that similar motor patterns found in different species would be generated by similar circuits (Getting, 1989). By this time, enough was known about the specifics of rhythmic pattern generation in different animals to show that the details of each circuit was different, but there were certain canonical principles, or "building blocks" across preparations (Getting, 1989).
2. It brought the realization that it was going to be extremely difficult to obtain data sufficient to constrain detailed models of all but the simplest circuits (Selverston, 1980). This remains one of the most thorny problems in understanding biological circuits today. Because the output of all biological circuits results from the interaction of many non-linear elements, computational models are needed to understand them. How realistic do these models need to be, and what data are needed to constrain these models? How will modulation alter these processes?
3. It gave us the beginnings of the cellular mechanisms underlying neuromodulation of excitability (DeRiemer et al., 1985; Dunlap and Fischbach, 1981; Kaczmarek et al., 1986; Levitan et al., 1979).
4. It was the beginning of the understanding that neuronal dynamics and neuromodulatory mechanisms reconfigure circuits so that they could no longer be viewed as "hard-wired" (Eisen and Marder, 1984; Getting, 1989; Marder, 1984; Marder and Hooper, 1985), but capable of variable outputs under modulator control.
5. It brought the realization that circulating hormones and local neurohormones could alter behavior by acting at every level from sensory neuron (Pasztor and Bush, 1987), to central circuits (Harris-Warrick and Kravitz, 1984; Hooper and Marder, 1984; Marder and Hooper, 1985), to neuromuscular junctions and muscles (Lingle, 1981; Schwarz et al., 1980). This raised the possibility that the same modulator could act at different sites within a circuit to keep outputs coordinated, or that different modulators could compensate for changes at one site with changes elsewhere, or that modulators can effectively change the gain of one portion of a circuit or process without altering others (Brezina, 2010).
6. It demonstrated the prevalence of cotransmission in neurons of all kinds, including diffuse modulatory projection neurons that can liberate their transmitter at some distance from receptors (Adams and O'Shea, 1983; Bishop et al., 1987; Jan and Jan, 1982; Kupfermann, 1991; Nusbaum and Marder, 1989a; Siwicki et al., 1987).

Diffuse projections, hormones, and local hormones determine the modulatory tone of the brain

One of the most remarkable features of biological systems is that they are endlessly adaptable while usually maintaining their functional integrity. Moreover, many brain disorders, such as schizophrenia, depression, and epilepsy, are likely associated with some degree of dysfunction in modulatory control systems. Many of the other contributions in this issue will deal with the modulation of disparate regions of the vertebrate brain by the diffuse aminergic projections, local interneurons with peptide cotransmitters, and peptidergic systems that are important for pain regulation and other physiological processes. In their outstanding review in this issue, Taghert and Nitabach (2012) describe much of the wonderful recent work in flies and worms describing the roles of neuropeptides in specific behaviors. Consequently, in this review I will focus on "take-home messages" that have come from the study of neuromodulation primarily using crustacean and molluscan systems, and I draw heavily on specific examples from the crustacean stomatogastric nervous system.

Intrinsic versus Extrinsic Modulation

It can be useful to distinguish between neuromodulation that is intrinsic to the system or circuit being considered, and modulation that is delivered from an extrinsic source (Cropper et al., 1987; Katz, 1995; Katz and Frost, 1996; Morgan et al., 2000). In the former case, the modulatory substance is released by one of the circuit components, while in the latter case the modulatory substance is released from a source not directly part of the circuit at hand (Fig. 1). In the simplest case, a neuron that releases a cotransmitters that alters the excitability of its postysynaptic targets is intrinsic (Cropper et al., 1987; Katz and Frost, 1995a, b; Weiss et al., 1992; Weiss et al., 1978), while a neurohormone that is liberated by a neurosecretory structure and travels through the circulation is unambiguously extrinsic (Christie et al., 1995). While at some level this is an artificial distinction, it points out neurons can alter the configuration of the networks with which they are active in complex and rich ways (Katz and Frost, 1995a, b). Moreover, if the cotransmitters liberated from the same neuron are differentially released as a function of the dynamics of presynaptic activity (Brezina et al., 2000a; Karhunen et al., 2001; Peng and Horn, 1991; Peng and Zucker, 1993), this can alter the extent to which these substances influence postsynaptic function under different conditions.

Circuits are multiply modulated

Neuromodulation of circuit function has been studied for more than 40 years in crustaceans and mollusks. The crustacean stomatogastric ganglion (STG) contains ~30 neurons and the crustacean cardiac ganglion contains only 9 neurons. Both are central pattern generating circuits that generate fictive motor patterns when removed from the animal, and both are modulated by a large number of different substances (Blitz and Nusbaum, 2011; Cruz-Bermudez and Marder, 2007; Johnson et al., 2011; Marder and Bucher, 2007; Stein, 2009; Wiwatpanit et al., 2012).

Figure 2 summarizes a partial list of what is known about the neuromodulatory control of the crab STG. These data were accumulated over the years by many laboratories using a combination of immunocytochemistry and biochemical techniques. Most recently, mass spectrometry has allowed the identification and characterization of many individual members of a number of different peptide families (Dickinson et al., 2009; Ma et al., 2009a; Ma et al., 2009b; Ma et al., 2009c; Stemmler et al., 2010). Many of the same substances are released both by descending modulatory neurons and by neurosecretory structures as hormone.

It is unlikely that the STG is unusual in the number of its modulatory inputs. A large number of neuromodulators are known to have important functions in the Aplysia feeding circuits (Brezina and Weiss, 1997; Furukawa et al., 2003; Koh and Weiss, 2007; Li et al., 2001; Proekt et al., 2005; Sweedler et al., 2002; Vilim et al., 2010; Wu et al., 2010), another system in which the search for modulators has been intense. And certainly, the number of important peptide modulators known in C. elegans and Drosophila is also large (Bargmann, 2012; Taghert and Nitabach, 2012). In contrast, there are relatively few vertebrate circuits, in which there have been determined attempts to find all of the modulatory inputs to the circuit. But, whether there are 5 or 12 or 25 modulators that can influence the output of a given circuit in the brain, no circuit is likely to be modulated by only one or two substances, no matter how tempting it is to think that a single substance is solely responsible for controlling a significant piece of the brain.

Neuromodulators and neuromodulatory neurons alter circuit dynamics

The exogenous application of neuromodulatory substances and the stimulation of modulatory projection neurons can significantly alter circuit output (Blitz et al., 2004; Blitz et al., 1999; Blitz et al., 1995; Blitz et al., 2008; Dando and Selverston, 1972; Dickinson et al., 2001; Dickinson and Marder, 1989; Dickinson et al., 1990; Dickinson and Nagy, 1983; Eisen and Marder, 1984; Flamm and Harris-Warrick, 1986a, b; Hooper and Marder, 1984; Hooper and Marder, 1987; Nagy and Dickinson, 1983; Nagy et al., 1988; Nusbaum and Marder, 1988; Nusbaum and Marder, 1989a; Nusbaum and Marder, 1989b; Saideman et al., 2006; Saideman et al., 2007).

When the effects of descending modulatory projection neurons on the STG are removed by either cutting or blocking the input nerve to the STG, the fast pyloric rhythm either stops completely or slows down (Fig. 3, control). Under these conditions, exogenous application of a large number of different substances can elicit a triphasic motor pattern (Fig. 3), although each substance produces a different form of the rhythm. These data were initially interpreted as showing that the same neuronal circuitry can be reconfigured differently by each of a large number of neuromodulators. That interpretation still holds. But these data also make a second point: there are a large number of different neuromodulators that can activate the network. To some extent these constitute degenerate mechanisms that can, as a first approximation substitute for each other, if it is more important that a rhythm exist than its exact form. This is especially the case, if the neuromuscular junctions activated by these motor neurons act as a temporal filter (Brezina, 2010; Hooper and Weaver, 2000; Morris and Hooper, 1998). Modulators may also stabilize motor patterns (Zhao et al., 2011).

In addition to the fast pyloric rhythm, the STG also expresses two slower rhythms, the gastric mill rhythm and the cardiac sac rhythm. These rhythms require descending modulatory inputs for their expression. Figure 4A shows a cartoon comparing the effects of stimulating three different proctolin-containing modulatory projection neurons on the pyloric and gastric rhythms of the crab. While each of these neurons contains and releases proctolin, the cotransmitter complement of these three neurons is different (Blitz et al., 1999), and stimulation of these neurons elicits different motor patterns from the STG. A full gastric rhythm is elicited by MCN1, MPN increases the frequency of the fast pyloric rhythm, while MCN7 activates still a different rhythm.

Not only can modulators alter the motor patterns produced by a single circuit, but they can also combine elements from two circuits into one. The schematic shown in Figure 4B shows that the neuropeptide, Red Pigment Concentrating Hormone (RPCH) strengthens synapses from the IVN neurons to STG network neurons and creates a single, conjoint rhythm from neurons that ordinarily are part of the cardiac sac and gastric rhythm (Dickinson et al., 1990). This is one of many examples of circuit switching in the STG, in which neurons switch from being part of the pyloric or gastric circuits (Weimann and Marder, 1994; Weimann et al., 1991).

While some aspects of the effects of a cotransmitter-containing projection neuron may be recapitulated with bath application of one of its substances, it is unlikely that exogenous bath applications will reproduce the concentration profiles that are produced by neural stimulation. In contrast, there are substances that only reach the neuropil of the STG as circulating hormones (Saideman et al., 2006; Weimann et al., 1997). In this case, bath-applications at realistic concentrations are far more likely to elicit responses similar to those evoked in vivo.

Determining the cellular mechanisms underlying circuit modulation

One of the goals of much of the work on the modulation of the STG has been to determine the mechanisms that account for the changes in circuit performance elicited by modulators on the basis of the modulator's action on specific cellular and synaptic targets (Eisen and Marder, 1984; Flamm and Harris-Warrick, 1986a, b; Hooper and Marder, 1987; Marder and Eisen, 1984a). In these experiments pharmacological blockade of the glutamatergic inhibitory synapses was combined with photoinactivation of specific dye-filled neurons (Miller and Selverston, 1979) to isolate individual neurons for study.

These studies demonstrated: a) electrically coupled neurons could respond differently to the same modulatory substance (Marder and Eisen, 1984a), b) a given neuron could be a direct target for multiple modulatory substances (Flamm and Harris-Warrick, 1986b; Hooper and Marder, 1987; Marder and Eisen, 1984a; Swensen and Marder, 2000), c) multiple circuit neurons were simultaneous targets of the same neuromodulator (Flamm and Harris-Warrick, 1986b; Harris-Warrick and Johnson, 2010; Hooper and Marder, 1987), d) all circuit neurons are the subject of modulation (Harris-Warrick and Johnson, 2010; Swensen and Marder, 2001).

The effects of dopamine on membrane currents and receptors in STG neurons has been extensively studied (Clark and Baro, 2006, 2007; Clark et al., 2008; Harris-Warrick et al., 1995a; Harris-Warrick et al., 1995b; Harris-Warrick and Johnson, 2010; Peck et al., 2006; Zhang et al., 2010). An unexpected result from this work is that dopamine modulates several currents in the same neuron, and that the same current can be modulated differently in different target neurons (Fig. 5A).

Every synapse is subject to neuromodulation

The dynamics of circuit modulation in the STG also involves modulation of synaptic strength (Dickinson et al., 1990; Eisen and Marder, 1984; Harris-Warrick and Johnson, 2010; Johnson et al., 2011; Johnson and Harris-Warrick, 1990; Kloppenburg et al., 2000; Thirumalai et al., 2006; Zhao et al., 2011). Figure 5B shows that the same synapse is subject to modulation by dopamine, serotonin, and octopamine. Additionally, the extent of the modulation is altered as a function of synaptic depression (Johnson et al., 2011). This shows that there is an interaction between neuromodulation and other use-dependent processes that also influence synaptic strength during ongoing circuit activity.

The interaction between basal neuromodulatory tone and phasic activation of neuromodulatory inputs

Many of the same substances are delivered by specific modulatory projections into the STG and also are released into the hemolymph from neurosecretory structures such as the pericardial organs (Figure 2). This same dual function is a general feature of many nervous systems (Keller, 1992). The concentration of neuromodulators in the hemolymph are in the nanomolar range, while release from nerve terminals can produce substantially higher concentrations, at least for short periods of time in response to bursts of presynaptic activity (Rodgers et al., 2011a; Rodgers et al., 2011b). Neurons in the STG show DA receptors at non-synaptic regions (Oginsky et al., 2010), consistent with their role as signaling a tonic modulatory tone. Moreover, tonic low concentrations of DA seem to be important for maintaining circuit basal function, while phasic, higher concentrations produce shorter-term modulation (Rodgers et al., 2011a; Rodgers et al., 2011b).

How can highly modulated circuits be stable in the face of parameter changes brought about by modulation?

One of the most puzzling questions arising from extensive neuromodulation is how the integrity of the modulated circuits is maintained, although so may circuit parameters can be altered? If one tries to build a computational model of either a single neuron, or a circuit, it can be quite hard to find a set of parameters that are consistent with the desired output. Indeed, random assignment of parameters to a single neuron or a circuit will lead to significantly more failures than successful models (Prinz, 2010; Prinz et al., 2003a; Prinz et al., 2004; Taylor et al., 2009). Nonetheless, there are many different sets of parameters that can produce similar output patterns (Goldman et al., 2001; Prinz et al., 2004; Taylor et al., 2009). There are circumstances in which neuromodulators are used to qualitatively transform the behavior of a circuit, such as during transitions from sleep to wakefulness (McCormick, 1989, 1992; McCormick and Bal, 1997), or when a hormonal pathway is used to trigger eclosion (Kim et al., 2006) or molting (Webster et al., 2012). There are also neuromodulatory influences that reshape networks during ongoing behavior, and the sets of parameters that are produced by neuromodulator action must be consistent with stable and appropriate cellular and circuit function (Goldman et al., 2001).

Understanding how circuits can be stable in the face of ubiquitous neuromodulation is an important and deep problem. Why don't the circuits important for behavior become "over-modulated" more often, and what mechanisms might protect against over-modulation? The answers to this question may be partially idiosyncratic to each circuit, but I suggest some general mechanisms that may play a role in maintaining functional circuit performance during modulation.

Stability Mechanism #1- Modulators that Coordinately Act on Opposing Processes

Harris-Warrick and Johnson (2010) suggest that the pattern of dopamine modulation of STG neurons at the cellular level (Fig. 5) is ideally suited to maintain stable function. Specifically, by acting on both inward and outward currents, dopamine actions can keep individual neurons, and therefore the network, within their operating range (Harris-Warrick and Johnson, 2010).

Stability Mechanism #2- Voltage-dependence of modulator actions

The importance of the voltage-dependence of the NMDA receptor for the induction of LTP is well-appreciated, but the ability of the NMDA receptor to induce oscillations in the spinal cord is less well-known (Sigvardt et al., 1985). The neuropeptide proctolin elicits a voltage-dependent inward current similar to that evoked by NMDA (Golowasch and Marder, 1992). This current is blocked at hyperpolarized membrane potentials by extracellular Ca^{2+}, and has a reversal potential about 0 mV. Consequently, the peak inward current activated by proctolin is close to threshold (Golowasch and Marder, 1992).

Because of its voltage-dependence, the current activated by proctolin increases the amplitude of the oscillations generated by bursting neurons without producing a depolarization of the baseline (Fig. 5A). The same effect is seen with muscarinic agonists such as pilocarpine or oxotremorine (Marder and Paupardin-Tritsch, 1978; Swensen and Marder, 2000). In contrast, nicotine which activates a conventional nicotinic receptor (Marder and Eisen, 1984b; Marder and Paupardin-Tritsch, 1978), depolarizes the baseline of the oscillator (Fig. 5B), and can result in a depolarization block. Thus, the voltage-dependence of the current elicited by proctolin and muscarinic agonists has a built-in brake that maintains the integrity of the burst generating mechanism in the pyloric pacemaker neurons.

Stability Mechanism #3- Convergence of many modulators onto the same voltage-dependent current

In addition to proctolin and muscarinic agonists, a large number of other peptides including Crustacean Cardioactive Peptide (CCAP), RPCH, TNRNFLRFamide, SDRNFLRFamide, Cancer borealis Tachykin-Related Peptide (CabTRP1a) activate the same voltage-dependent current (Swensen and Marder, 2000), and act on some of the same neurons (Fig. 7A). Because these modulators converge onto the same current, they occlude each other's actions (Fig. 7B) (Swensen and Marder, 2000). Thus, if a neuron is already highly activated by one of these modulatory substances, a second of them will be relatively ineffective.

Stability Mechanism #4- Saturation of postsynaptic action: bigger synaptic inputs do necessarily produce larger effects on target neuron activity

Modulators can enhance the amplitude of synaptic currents many-fold. For example, RPCH produces several-fold increases in the amplitude of the inhibitory LP to PD synapse in the pyloric network of the lobster, Homarus americanus (Thirumalai et al., 2006). Although this synapse is the major feedback to the pacemaker of the pyloric rhythm, this increase in synaptic strength does not necessarily change the frequency of the pyloric rhythm (Thirumalai et al., 2006) because the effect of the inhibitory input to an oscillator often saturates as synaptic strength is increased (Prinz et al., 2003b). This saturation means that the network's activity is de facto protected against over-modulation of the feedback synapse to the oscillator.

Stability Mechanism #5- Modulators act coordinately on multiple targets to keep systems functionally "matched"

In motor systems central pattern generating networks drive muscles, and it is the muscle movement that is important for behavior. Brezina and colleagues (Brezina et al., 2005; Brezina et al., 2000b; Brezina and Weiss, 2000; Zhurov and Brezina, 2006) have argued that coordinate modulation of muscles, neuromuscular junctions and the central pattern generating circuitry ensures that the presynaptic activity generated in the motor neurons is appropriately matched to their muscle targets. This general principle, of correlated and coordinated modulation of multiple sites in a sensory-motor circuit is likely to be a general principle, found in many nervous systems (Taghert and Nitabach, 2012).

Can modulator action be robust and predictable despite variability in underlying conductances?

Much computational and experimental evidence shows that there can be considerably variability across animals or across neurons in the parameters that control neuronal excitability and network function even when the circuit output is maintained (Calabrese et al., 2011; Goaillard et al., 2009; Nerbonne et al., 2008; Norris et al., 2011; Prinz et al., 2004; Roffman et al., 2011, 2012; Schulz et al., 2006; Schulz et al., 2007; Sobie, 2009; Swensen and Bean, 2005; Tobin et al., 2009). This raises the question of whether it is possible for neuromodulation to be reliable across individuals, if each of them has a nervous system with different underlying parameters.

The answer to this question is complicated. First, even for modulators that have robust actions, there can be significant differences in their responses to threshold concentrations (Weimann et al., 1997). Second, many modulators show state-dependent actions (Nusbaum and Marder, 1989b; Szabo et al., 2011), so that the activity or prior history of activity of the network determines the extent or sign (Spitzer et al., 2008) of modulator action. Third, modulator action may depend critically on other modulators (Brezina, 2010; Dickinson et al., 1997). That said, many networks with different underlying parameters can respond

reliably to the same modulators (Grashow et al., 2009), although in this study, a small proportion of networks responded anomalously (Grashow et al., 2009). These data are reminiscent of what we see in the human population with pharmacological agents that produce anomalous responses in a small subset of people. Thus, although there are significant individual differences in circuit structures across individuals, the particular sets of network parameters found in the healthy population may be enriched for sets of parameters that permit reliable neuromodulatory control under most conditions.

Summary and Conclusions: Modulation and Connectomes

The discerning among you have already made the connection between the early belief that a connectivity diagram would be sufficient to bring understanding of how a circuit worked, and the some of the more lofty justifications made for the recent attempts to establish connectomes using anatomical methods (Briggman and Bock, 2012; Briggman and Denk, 2006; Briggman et al., 2011). Detailed anatomical data are invaluable. No circuit can be fully understood without a connectivity diagram. But, the experience of the small circuit community (Bargmann, 2012; Brezina, 2010; Getting, 1989; Jang et al., 2012; Marder and Bucher, 2007; Marder and Calabrese, 1996) demonstrates unambiguiously that a connectivity diagram is only a necessary beginning, but not in itself, an answer.

What then is the answer? The full answer will require a connectivity diagram that is supplemented with a complete description of all of the cotransmitters present in each neuron. It will require detailed information about the properties of the receptors to all of those substances. It will require having methods to record simultaneously the electrical activity of many circuit elements, to understand circuit dynamics. It will require systems that allow us to go back and forth from in vitro and in vivo preparations. It will require computational models that will help us to understand how behavior at one level emerges from the properties of a lower level.

But most critically, it will require a return to appreciating the benefits of working on disparate animal species. Each animal has devised extraordinary and baroque circuit mechanisms that employ neuromodulation to achieve important behavioral flexibility in the context of its environment, neuronal complement, and biomechanical constraints. Many of the circuit configurations that we will uncover may be weird and specific solutions to particular needs of that species. It will only be by looking for general principles across species that we will find the more general rules that govern the robust and stable neuromodulation needed for functional circuit activity in all animals.

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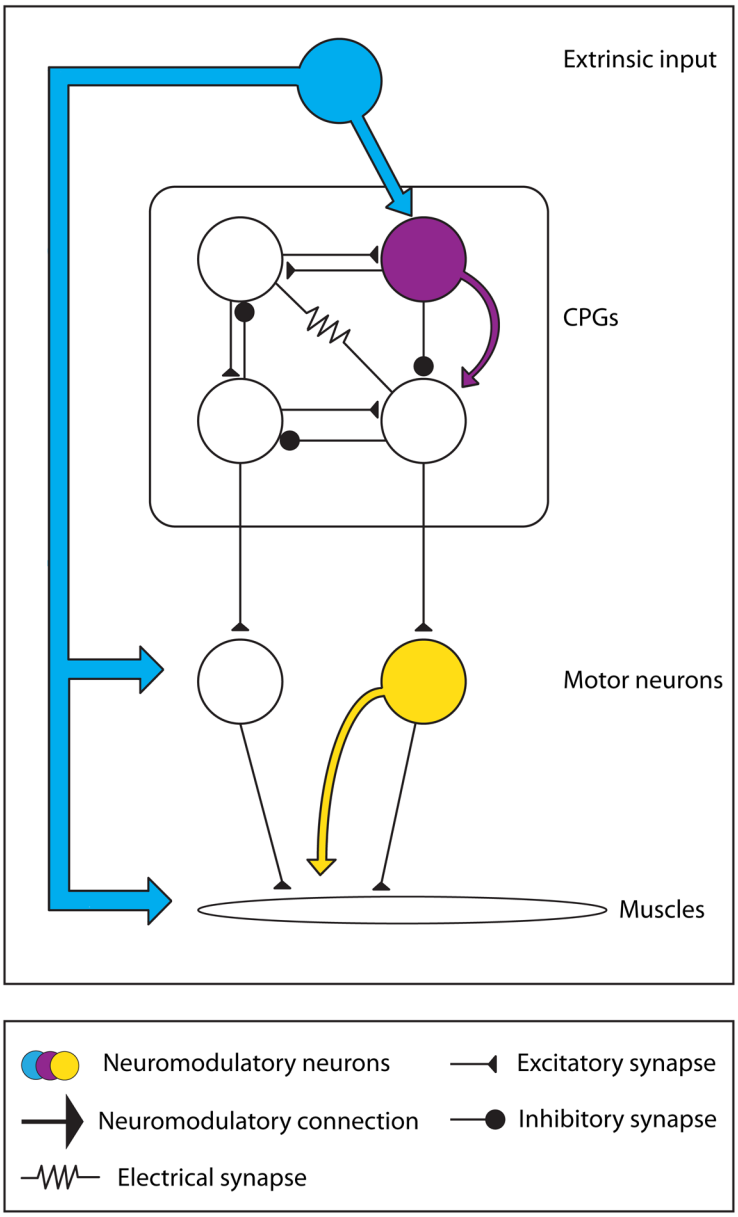

Figure 1.
Intrinsic and Extrinsic Modulation. Extrinsic modulation comes from outside the circuit or modulated target. Intrinsic modulation refers to neurons that are part of a circuit and release modulators that can alter the properties of other circuit elements. Drawing loosely after Katz and Frost (Katz and Frost, 1996).

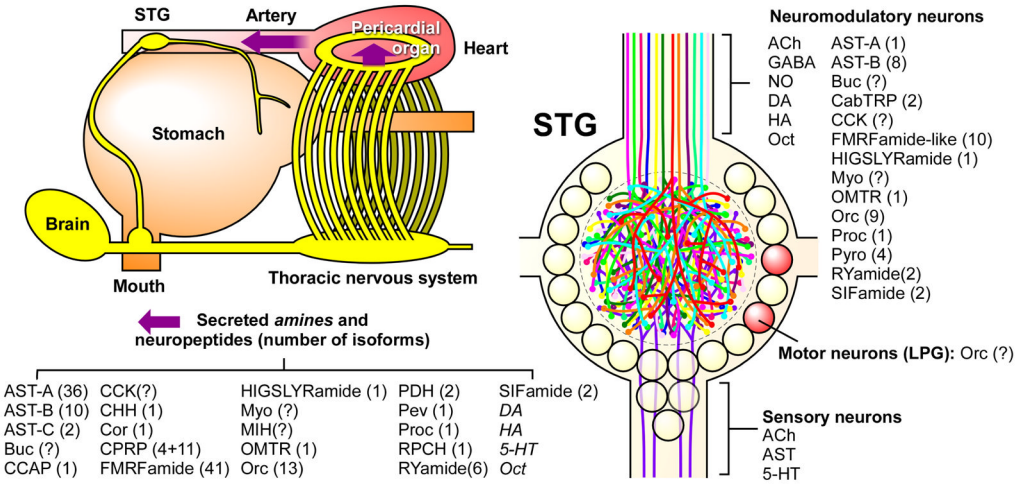

Figure 2.

Partial Summary of Neuromodulation of the crab stomatogastric ganglion (STG). The STG sits anterior to the heart within an artery that brings modulatory amines and peptides from neurosecretory structures such as the pericardial organs (bottom list). 25 pairs of descending modulatory neurons bring a host of substances into the neuropil of the STG (right). The number of family members of the neuropeptides are shown in parentheses. Figure was made by D. Bucher, summarizing work from the Li and Stemmler labs and numerous collaborators.

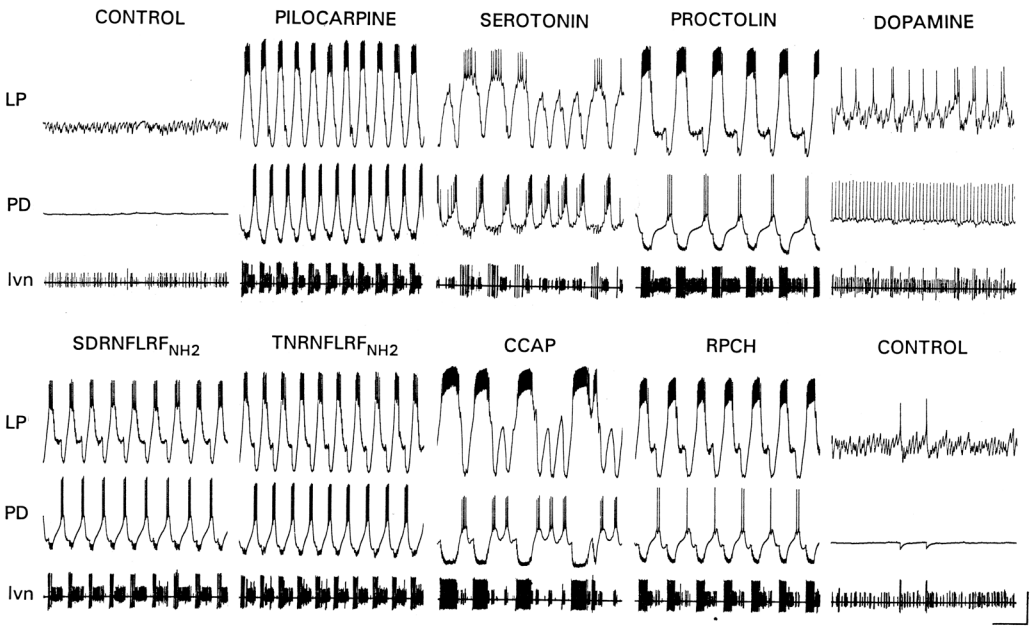

Figure 3.
Multiple neuromodulators can activate different forms of the pyloric rhythm. In each panel the top two traces are intracellular recordings from the Lateral Pyloric (LP) and Pyloric Dilator (PD) neurons. The bottom trace is an extracellular recording from the lateral ventricular nerve (lvn) that carries the axons of the LP, PD, and Pyloric (PY) neurons. (Marder and Weimann, 1992).

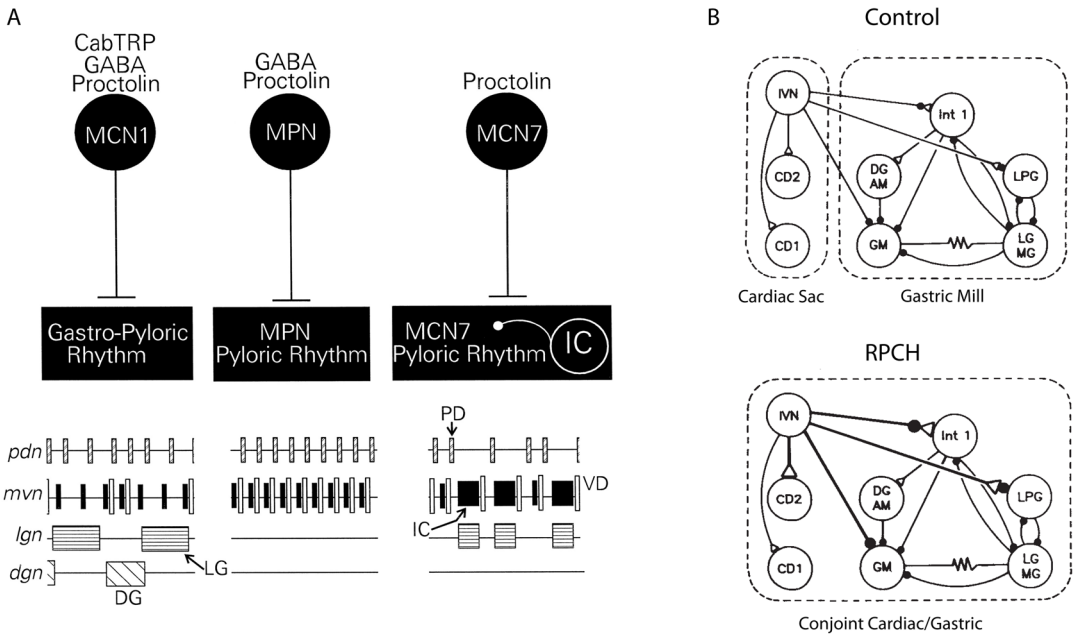

Figure 4.

Modulatory reconfiguration of circuits. A) Three different proctolin-containing modulatory neurons each evoke different changes in STG motor patterns. (Blitz et al., 1999; Nusbaum et al., 2001). B) Bath application of RPCH constructs a conjoint rhythm from previously separate cardiac sac and gastric mill circuit elements. Modified from Dickinson et al (1990).

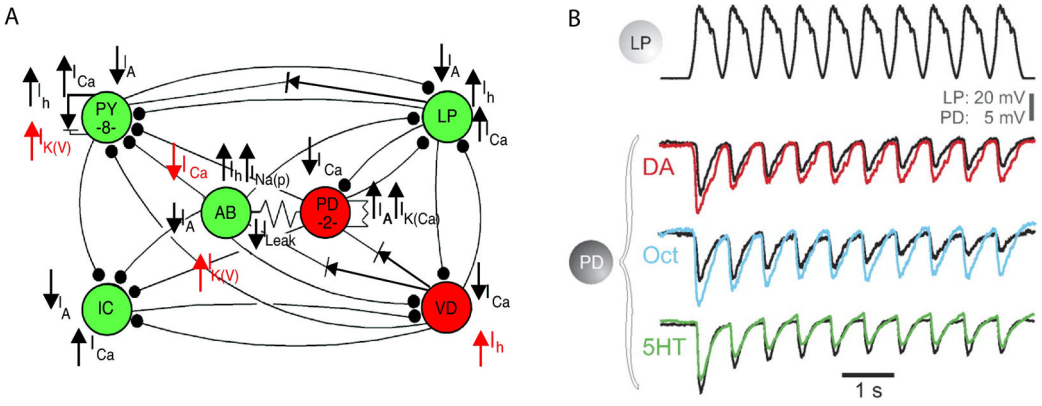

Figure 5.

Aminergic modulation of pyloric circuit elements. A) The actions of dopamine on ionic currents in the indicated neurons are shown. From (Harris-Warrick, 2011). B) Graded IPSPs evoked in the postsynaptic PD neuron by depolarization of the LP neuron in control (black traces), dopamine (red), octopamine (blue) and serotonin (green). Modified from Johnston et al (2011).

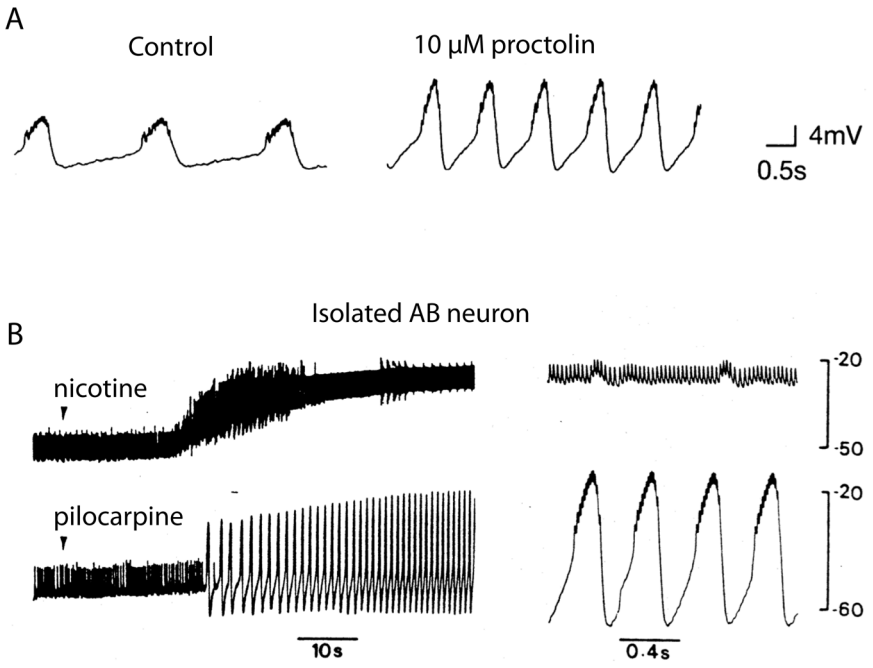

Figure 6.
Effects of modulatory substances on a bursting pacemaker neuron, A) Intracellular recordings from the isolated Anterior Burster (AB) neuron in control and proctolin. Modified from Hooper and Marder (1987). Notice the increase in amplitude without change in baseline. B) AB neuron in response to nicotine and pilocarpine.

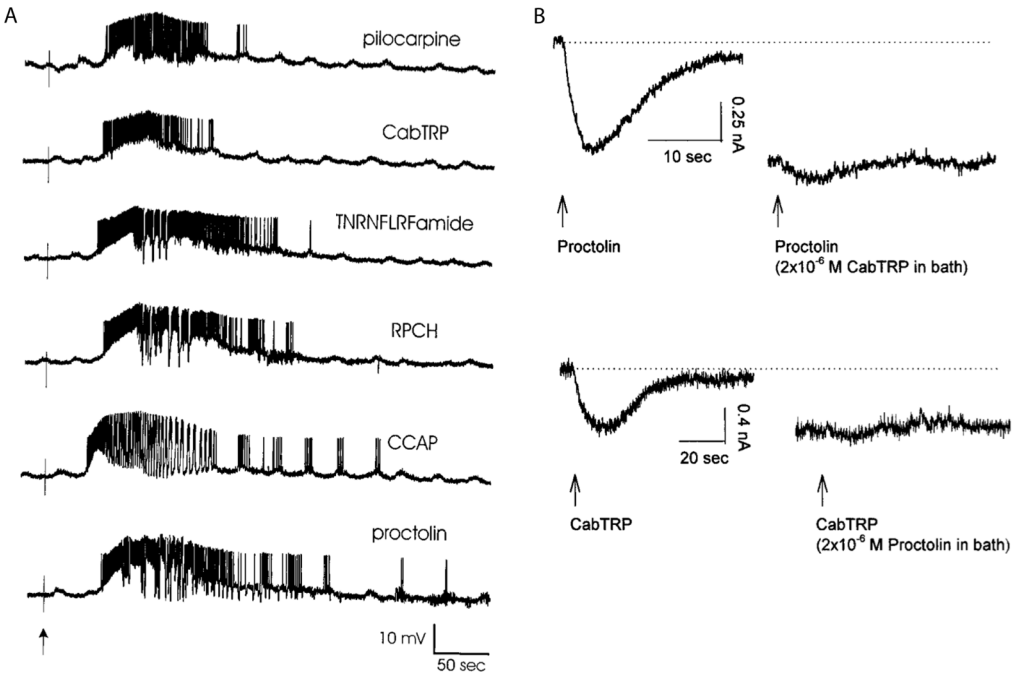

Figure 7.

Multiple modulators act on the same neuron and converge onto the same current. A) Puff applications of the modulators indicated onto intracellularly recorded LP neuron. (Swensen and Marder, 2000). B) Voltage-clamp recordings of inward currents evoked by proctolin and CabTRP1a. Top traces, puff of proctolin elicited an inward current. When CabTRP1a was placed in the bath, eliciting a steady-state inward current, a puff of proctolin produced only a very small additional inward current. Bottom traces, reverse experiment. (Swensen and Marder, 2000).