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Homeostatic Plasticity of Excitability in Crustacean Central Pattern Generator Networks

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Abstract

Plasticity of excitability can come in two general forms: changes in excitability that alter neuronal output (e.g. long-term potentiation of intrinsic excitability) or excitability changes that stabilize neuronal output (homeostatic plasticity). Here we discuss the latter form of plasticity in the context of the crustacean stomatogastric nervous system, and a second central pattern generator circuit, the cardiac ganglion. We discuss this plasticity at three levels: rapid homeostatic changes in membrane conductance, longer-term effects of neuromodulation on excitability, and the impacts of activity-dependent feedback on steady-state channel mRNA levels. We then conclude with thoughts on the implications of plasticity of excitability for variability of conductance levels across populations of motor neurons.

INTRODUCTION

Excitability refers to the influence of ionic membrane conductances of a neuron on the integration of inputs and the probability of generating action potentials. Neuronal excitability is most directly attributable to the identity and relative abundance of ion channels inserted into the membrane of the cell, as well as the activation/inactivation properties and kinetics of those channels. Regulation of channel properties [1,2], the spatial distribution and localization of channels [3,4], or association of channels with modifying subunits [5,6] are potent ways to modify the output of cells, and are ubiquitous targets to generate plasticity in neuronal output via changes in intrinsic excitability. Changes in excitability that *stabilize* the output of a given neuron to maintain a given activity level are a form of “homeostatic” plasticity.

One of the earliest demonstrations of homeostatic plasticity of intrinsic excitability was in cultured neurons of the lobster stomatogastric ganglion (STG; [7]). The canonical output of the STG derives from rhythmic bursting activity of motor neurons that generate movement of the teeth (gastric mill) and foregut (pylorus) allowing for chewing and filtering of food in the crustacean stomach. When isolated from their modulatory and synaptic inputs, STG

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neurons lose their bursting output and tend to fire tonically. However, Turrigiano et al. [7] discovered that chronically isolated lobster STG neurons in cell culture generate endogenous bursting as a result of changes in expression of their intrinsic membrane conductances [8]. These changes are activity-dependent and mediated at least in part by intracellular calcium concentration [7], and have been reported in crab STG as well [9], indicating this to be a common feature of the robustness of these neurons. Thoby-Brisson and Simmers then demonstrated that following loss of descending modulatory projections, lobster STG neurons spontaneously regained rhythmicity after several days in culture. This recovery of function was associated with downregulation of K^+ and upregulation of H- currents [10,11]. This phenomenon has since been reported in multiple species of crustacean STGs [12–14], albeit to differing levels of loss and gain of function [15].

Since these first studies were published, crustacean central pattern generator networks have become a key model system in the study of plasticity of intrinsic excitability – in particular with regards to homeostatic plasticity. Because many of these studies rely on removal of descending inputs and therefore neuromodulation of STG neurons, it can be difficult to separate the impacts of metabotropic modulatory signaling from activity-dependent changes in excitability [12,16]. Furthermore, the underlying mechanisms of changes in excitability are still poorly understood. Here we discuss the contributions of the STG and another crustacean preparation, the cardiac ganglion (CG), to our current understanding of plasticity of excitability.

ACTIVITY-DEPENDENT CHANGES IN EXCITABILITY

Compensatory changes in neuronal excitability tend to occur as a result of prolonged changes in firing activity [2], and neurons of the STG are no exception. For example, several hours of periodic depolarizing stimuli result in compensatory increases of both calcium-activated and A-type K^+ currents in IC neurons of the STG [17]. This change in I_A is calcium-dependent, but does not occur in response to hyperpolarizing stimuli. The same depolarizing stimuli do not elicit these changes in all STG cells, indicating that homeostatic plasticity can occur in a cell-type specific manner.

A similar example of compensatory plasticity has been reported in large cell (LC) motor neurons of the crustacean cardiac ganglion (Figure 1). When I_A is blocked with 4-aminopyridine (4AP), LCs respond within an hour with an increase in I_{BKCa} (Figure 1A). This compensatory response restores excitability (Figure 1A) and is dependent on intracellular calcium and calcineurin activity (Figure 1B; [18]). Interestingly, compensation is bidirectional: if I_{BKCa} and I_{Kd} are blocked with tetraethylammonium (TEA), LCs respond with an increase in I_A . TEA-induced compensation depends on a distinct signaling mechanism that is not calcium-dependent, but the precise pathway has not been identified [18]. Further, due to intrinsic variability across LCs, TEA causes LCs to lose conserved output and produce divergent voltage responses [19], and in an ongoing network rhythm LC burst waveforms become desynchronized. Synchrony is then restored within 1 hour via at least 2 conductance changes acting synergistically [19,20]. The compensatory increase in I_A contributes to resynchronization, along with a compensatory increase in electrical coupling among LCs [20].

In contrast to activity-dependent plasticity of K^+ currents in the STG and CG, activity-independent homeostasis also has been reported. Overexpression of the *shal* transcript in STG neurons results in significant increases in I_A while having modest effects on neuron output as a result of a compensatory increase in I_H that is independent of activity [21,22]. These results suggest direct co-regulation between the Shal protein and I_H that stabilize the output of the cell [21]. Subsequent experimental and computational studies support this compensatory interaction between I_A and I_H in STG neurons [21,23], including naturally occurring correlations among H- and A-type conductances [24] as well as channel mRNA levels [25], establishing this is an important conductance relationship for these neurons.

NEUROMODULATOR DEPENDENT PLASTICITY OF EXCITABILITY

Neuromodulation is one of the most direct and potent means to alter cellular excitability, and often works to directly alter ionic conductances through second messenger mediated signaling pathways [26,27]. The STG has been a premiere system for the study of neuromodulation, going back many decades, and continues to yield interesting and timely insight into this important form of plasticity [28]. These direct and acute effects of modulation are themselves the subject of many extensive reviews [29–31], and are not the focus here. Rather, we discuss the longer-term impacts of neuromodulators on excitability, through metabotropic interactions that influence conductances over time scales extending beyond acute changes that alter neuron output over time scales of seconds to minutes.

The effects of tonic nanomolar dopamine in LP neurons of the lobster STG emphasizes that the distinction between activity-dependent and modulator-dependent regulation is not always clearly separable (Figure 2). In the micromolar range, low-affinity D1R receptors respond to dopamine to directly decrease I_A , which advances the LP activity phase [32]. However, dopamine also acts at high-affinity D1R receptors at far lower concentrations (~5 nM) to mediate a response that can regulate I_H in an activity-dependent manner, precisely compensating for decreased I_A via a concomitant calcineurin-dependent decrease in I_H which restores LP phasing [32–34]. These findings demonstrate a mechanism by which modulation can provide flexibility and stability through a self-stabilizing feedback loop to maintain the $I_A:I_H$ ratio, and that low levels of constitutive modulation can potentiate activity-dependent regulation of conductances [35]. Interestingly, these high-affinity D1 receptors also act on longer timescales to increase both of these ionic conductances through pathways that depend on transcription, translation, and RNA interference [36,37]. Similarly, new evidence implicates tonic nanomolar concentrations of other amines in regulating I_A and I_H in PD neurons, suggesting cell-type specific regulation [38] which occurs in part through tonic levels of modulation influencing expression of microRNAs [37,38].

Neuromodulators also can work over longer time scales to influence co-regulation of pairs of ionic currents and channel mRNAs in STG cells, resulting in correlated conductance densities in STG neurons [24,39,40]. In particular, both PD and LP neurons are known to have correlated levels of I_H , I_A , and I_{BKCa} [24,40], although their relative abundances differ across cell types [40]. Following removal of descending modulatory inputs, these conductance relationships are substantially altered or lost altogether in PD but not LP neurons [24,40]. By adding back exogenous neuropeptide modulation following removal of

modulatory inputs, Khorkova and Golowasch [24] were able to rescue the loss of correlated levels of membrane currents in PD neurons, even in the absence of rhythmic activity, suggesting that modulators can have direct metabotropic effects to coordinate conductance relationships in STG neurons.

PLASTICITY OF CHANNEL mRNA EXPRESSION

While these studies have been at the forefront of our understanding of homeostatic plasticity of excitability, there has been less work done on cellular mechanisms. The molecular underpinnings of excitability in STG neurons has been investigated at the single-cell level to determine cell-specific patterns of expression that presumably give rise to distinct outputs across cell types [25,41–45]. One of the hallmarks of these cell-specific patterns of expression in STG neurons is the presence of correlations among channel transcripts that differ across cell types [25,40,45,46]. Recently these findings have been incorporated into an elegant computational modeling framework that puts forth a hypothesis by which activity-dependent homeostatic regulation rules can generate correlations in the steady-state distribution of ion channels [47,48](Figure 3). This homeostatic regulation is based on a calcium-dependent model of self-regulating rhythmically bursting cells [50] (Figure 3A), generating distinct outputs via plasticity in conductance expression as a result of cell-type specific channel expression rates [48] (Figure 3B). These tuning rules give rise to correlated conductance levels that differ across neuron output types (Figure 3C), and are consistent with the biological data for STG cells [25,46]. This model framework provides directly testable hypotheses that will advance our understanding of the interplay between neuron and network homeostasis, channel expression, and conductance relationships important in the generation and maintenance of cell-specific output.

While neuromodulation can influence longer-term expression of channels in STG neurons (see above; [35–37,40]), only recently has there been evidence of direct activity-dependent feedback to the level of channel mRNAs. Temporal et al. [46] recently performed such a study that decoupled the effects of loss of activity and neuromodulation as a result of decentralization of STG neurons (Figure 3D). Decentralization results in not only a significant change in channel mRNA abundance in PD neurons, but also in the loss of characteristic channel mRNA correlations [25,46]. These correlations can be rescued by the application of a muscarinic agonist (pilocarpine) that restores activity to the network, but are not maintained when the agonist is applied in the presence of TTX to eliminate activity, leaving only the metabotropic effects of the drug (Figure 3D; [46]).

CONCLUSIONS: WHAT IS THE RELATIONSHIP BETWEEN ACTIVITY-DEPENDENT PLASTICITY AND VARIABILITY IN CONDUCTANCES?

In parallel with our growth in understanding of feedback processes that give rise to cellular plasticity in the STG has come the realization that neurons of a given type are not uniform in the distribution of ionic conductances used to generate their output [49–51]. Indeed, some of the first evidence for activity-dependent plasticity in the STG was accompanied by a demonstration that ionic conductances vary substantially in populations of STG neurons, leading the authors to speculate that variability may itself arise from this plasticity [17]. We

are no closer today to having a direct answer to the origins of variability in circuit parameters in this system, despite numerous studies on both the phenomenon and the impacts of this variability [19,52–57]. On the one hand, changes in conductance ratios may arise over the lifetime of the animal as a result of constant homeostatic tuning, leading to variability across a population (Figure 4). This hypothesis suggests that STG neurons are “born” with somewhat constrained parameter sets that give rise to their activity, but accumulate changes over time that lead to variation in the population. Conversely, activity-dependent developmental tuning of STG neurons even in the embryonic stage [58–60] may give rise to STG networks that are already variable across individuals (Figure 4). If this is the case, then it must be determined as to whether conductance sets continue to diverge as the individuals age, or whether homeostatic mechanisms are initiated to maintain conductances and activity set-points that arise during development (Figure 4). Answering these questions has proven difficult in the STG, as study animals are often wild-caught as adults, difficult to rear in the lab, and juvenile animals difficult to come by – all of which make developmental studies in this system challenging. To make progress in this regard, new model systems will need to be developed or exploited to allow for investigations into cell-specific effects of activity-dependent plasticity and variability of ionic conductances in a developmental context.

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spike initiation in the axon affects activity and integration. This study looked at spike initiation sites in the axon of a sensory neuron in the stomatogastric system. Action potentials were consistently initiated at a specific region of the axon, and spike frequency was influenced by motor neuron activity, but only if spike initiation occurred at this location. Direct neuromodulation of the axon caused ectopic spike initiation, resulting in loss of signal integration from motor neuron inputs. This novel effect of acute neuromodulation therefore is an example of modification of axonal signal integration via changing the location of spike initiation. [PubMed: 27335403]

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HIGHLIGHTS

Plasticity of excitability is a key mechanism for homeostatic control of output.

Activity-dependent feedback is a key regulator of plasticity of excitability.

Neuromodulation can affect excitability over longer-term time scales.

Changes in activity and excitability are associated with correlated channel mRNAs.

The implications of plasticity for cell-to-cell variability are discussed.

Figure 1. Activity-dependent plasticity of intrinsic excitability in cardiac ganglion motor neurons of the crab, *Cancer borealis*

A. Effects on excitability of LC motor activity after exposure to 4AP, which blocks the A-type current in these cells. Pacemaker neurons of the network were silenced with TTX in this experiment, and activity elicited with DC current injections. 4AP shifts these cells from tonic spiking to a state of hyperexcitation, a burst potential. Within 60 minutes, intrinsic compensation occurs that restores excitability towards baseline concomitant with an increase in High-Threshold K^+ (HTK) currents made up of I_{BKKCa} and delayed rectifier currents. Representative recordings of the increase in I_A after TEA exposure are shown for control (dark green) and one hour after 4AP exposure (light green) [18]. **B.** A working model for homeostatic plasticity of intrinsic excitability via compensation between I_A and I_{BKKCa} [18]. Baseline excitability (1) is disrupted by the block of I_A with 4AP, causing an alteration in the excitability of the cell and its output in the network, in this case rapid initial firing frequency (2). This change results in the release of calcium from intracellular stores as well as influx through voltage-dependent calcium channels (3) that increases activity of the calcium-dependent phosphatase calcineurin (4). Calcineurin activity induces an increase in I_{KCa} (5), restoring the excitability of the cell and re-establishing motor neuron activity in the intact network (6).

Figure 2. A working model for how tonic nM DA acts to enable activity-dependent regulation of I_H in LP neurons of the STG of the lobster, *Panulirus interruptus*

The graph represents I_H activity dependence in 5nM DA, with the line indicating the idealized fold change observed in response changes in LP duty cycle. The inset shows a working model for a mechanism regulated by tonic dopamine exposure. Dopamine receptor binding activates protein kinase A (PKA) which phosphorylates an unknown target (in blue) resulting in an increase in G_H . PKA also targets calcineurin, a calcium-dependent phosphatase. Calcineurin activation then works through an unknown substrate to reduce G_H in LP cells. The phosphorylation state of the unknown protein is superimposed upon the G_H activity-dependence curve in 5nM dopamine. Intracellular calcium concentration presumably is lowest at -100 and highest at +50% change in the LP duty cycle, and the gradient below the graph tracks subsequent calcineurin activation. PKA activity is constant because dopamine is constitutively present (red bar).

Figure 3. Activity-dependent feedback regulates correlated channel mRNA levels in pacemaker cells: modeling and experimental evidence

A. A simple biochemical scheme for activity-dependent channel expression. Channel mRNAs are produced at a rate α_m that depends on a Ca^{2+} -dependent factor, T , and degraded at rate β_m . Functional channel proteins are produced at a rate α_g from mRNAs and degraded at a rate β_g . This scheme is equivalent to an integral controller. Deviation from the target calcium concentration ($[\text{Ca}^{2+}]_{\text{tgt}}$) is accumulated in the mRNA (m) concentration (shaded region), which causes a change in ion channel expression (g). **B.** Membrane potential and conductance density evolution of a self-regulating neuron implementing integral control for its seven voltage-dependent conductances (fast sodium, g_{Na} ; slow Ca^{2+} , g_{CaS} ; transient Ca^{2+} , g_{CaT} ; A-type/transient potassium, g_{KA} ; Ca^{2+} -dependent potassium, g_{KCa} ; delayed-rectifier potassium, g_{Kd} ; hyperpolarization-activated mixed-cation, g_{H}). A total of 20 independent runs are shown with mean in bold. Voltage traces for an example neuron at the stages indicated are shown above the plot. **C.** Scatter plots of conductance distributions at steady state of the 20 neurons from the runs shown in panel B. **D.** Scatterplots for correlated mRNA levels for six ion channel mRNA pairs (*CbCaV1*, g_{CaS} ; *CbCaV2*, g_{CaT} ; *CbCaV3*, $g_{\text{Ca-LVA}}$ (low voltage activated); *shal*, g_{A} ; *shab*, g_{Kd} ; *Cb-IH*, g_{H}) from PD neurons from STGs of $N = 13$ crabs (*Cancer borealis*). The effects of activity on these correlated mRNAs

for a representative pair (*CbCaVI-shal*) is denoted with a dashed red box in the control PD neuron matrix, and the corresponding model conductances also noted with a dashed red box in panel C. These four experimental groups effectively decouple modulation from activity, as described in the text. In the two conditions where activity is preserved (Control and Decentralized+Pilocarpine), correlations are maintained. When activity is limited (Decentralized), even in the presence of a modulator agonist (Pilocarpine+TTX), the correlation is not maintained. These data indicate that activity-dependent feedback is at least partly responsible for correlated channel transcript levels in PD neurons.

Figure 4. A schematic of how variability could arise in STG neurons

Following neurogenesis and differentiation, developmental tuning processes result in conductance profiles in individual neurons that give rise to appropriate network output. These developmental tuning processes can result in *conserved patterns of conductance expression*, in which across individuals the networks converge on a common conductance profile, leading to minimal variability in the population. Alternatively, *developmental tuning creates conductance variability*, whereby different networks in different individuals achieve convergent output through unique tuning pathways – leading to variability in the population. Once appropriate output is achieved, then over the lifetime of the animal homeostatic compensation maintains the output. Because every individual experiences distinct environmental conditions, variability arises in the population as a result of homeostatic feedback. If after development these networks had very similar solutions, then this mature feedback compensation is the origin of variability in the population through *generation of conductance variability*. However, if developmental tuning had already resulted in variable solutions to network output, then variability measured in adult populations is either the result

of *maintenance of tuned conductances*, or *continued evolution of conductance variability* due to ongoing homeostatic compensation.

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