Neuromodulation independently determines correlated channel expression and conductance levels in motor neurons of the stomatogastric ganglion

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First published October 12, 2011; doi:10.1152/jn.00622.2011.—Neuronal identity depends on the regulation of numerous molecular components, especially ionic channels, which determine the electrical signature of a neuron. Such regulation depends on at least two key factors, activity itself and neuromodulatory input. Neuronal electrical activity can modify the expression of ionic currents in homeostatic or nonhomeostatic fashion. Neuromodulators typically modify activity by regulating the properties or expression levels of subsets of ionic channels. In the stomatogastric system of crustaceans, both types of regulation have been demonstrated. Furthermore, the regulation of the coordinated expression of ionic currents and the channels that carry these currents has been recently reported in diverse neuronal systems, with neuromodulators not only controlling the absolute levels of ionic current expression but also, over long periods of time, appearing to modify their correlated expression. We hypothesize that neuromodulators may regulate the correlated expression of ion channels at multiple levels and in a cell-type-dependent fashion. We report that in two identified neuronal types, three ionic currents are linearly correlated in a pairwise manner, suggesting their coexpression or direct interactions, under normal neuromodulatory conditions. In each cell, some currents remain correlated after neuromodulatory input is removed, whereas the correlations between the other pairs are either lost or altered. Interestingly, in each cell, a different suite of currents change their correlation. At the transcript level we observe distinct alterations in correlations between channel mRNA amounts, including one of the cell types lacking a transcript level we observe distinct alterations in correlations between the other pairs are either lost or altered. Interestingly, in each cell, a different suite of currents change their correlation. At the transcript level we observe distinct alterations in correlations between channel mRNA amounts, including one of the cell types lacking a.
dependent on the release of neuromodulators from neurons located in adjacent ganglia that project into the STG (Luther et al. 2003; Thoby-Brisson and Simmers 2002). These substances have paracrine actions on pyloric neurons and are thought to regulate activity via short- and long-term actions (Khorkova and Golowasch 2007; Swensen and Marder 2001).

Individual neurons of the STG CPGs have distinct biophysical properties as well as gene expression profiles. For example, individually identified neurons of the pyloric network have highly variable kinetics and conductance density levels of the hyperpolarization-activated current, \( I_H \) (Peck et al. 2006), and the transient \( K^+ \) current, \( I_A \) (Goldman et al. 2001; Golowasch et al. 1999; Khorkova and Golowasch 2007; Peck et al. 2001).

Particular pyloric neurons also differ in the quantitative relationships of expression of voltage-gated channel genes (Baro et al. 1997; Schulz et al. 2006, 2007), with different cell types expressing different relative amounts of voltage-gated channel mRNA and distinct correlations of channel mRNA levels (Schulz et al. 2007). These results suggest a differential regulation (and coregulation) of ion channel gene transcription in distinct STG neuron types. Together, the overall regulation of ion channel gene expression and posttranscriptional processing must play a substantial role in generating the unique output of each cell type.

In this study, we characterized the correlations of ionic currents expressed by two key neuronal subtypes of the pyloric network, the PD and lateral pyloric (LP) neurons, under different neuromodulatory conditions using a combination of electrophysiological and molecular analyses. We also examined the role of synaptic interactions and altered activity in the modification of these correlations.

**METHODS**

Adult male Jonah crabs (C. borealis) were purchased from local fishermen (Newark, NJ) and kept in cooled seawater aquaria at \( \sim 12^\circ \text{C} \). Animals were anesthetized by cooling for 15–30 min on ice. The foregut was removed, and the STG, with a portion of the nerves attached, were isolated as previously described (Harris-Warrick 1992; Silverston et al. 1976).

The removal of neuromodulatory input (i.e., decentralization) was accomplished by either transection of the stomatogastric nerve (stn) or by blocking action potential transmission along the stn, which carries all known neuromodulatory inputs from adjacent ganglia (esophageal and commissural ganglia) to the STG (Fig. 1A). To block transmission, a Vaseline well was built around the desheathed nerve, and either the well was filled with isotonic (750 mM) sucrose plus \( 10^{-6} \) M tetrodotoxin (Biotium), or the stn was cut as close to the STG as possible. Preparations in which experiments lasted more than 12 h (such as decentralization experiments) were maintained in organotypic culture at 6°C between recording sessions during which the preparation was bathed in physiological saline.

**Electrophysiology.** Neurons were identified and intracellularly recorded from exactly as described previously (Zhao et al. 2010). Extracellular recordings were performed with differential recordings using an AC amplifier (model 1700; A-M Systems) and two tungsten wires, one placed inside a Vaseline well built around a motor nerve (either the lateral ventricular nerve, lvn, or the pyloric dilator nerve, pdn, or both) and the other placed in the bath outside the well. Ionic currents were measured at 11–13°C in two-electrode voltage clamp, with current injection electrode resistances of 18–22 MΩ and voltage recording electrode resistances of 20–28 MΩ. K+ currents were measured in standard C. borealis physiological saline. The high-threshold, voltage-gated outward potassium currents, \( I_{\text{HTRK}} \), were activated from a holding voltage of \(-40 \) mV. The voltage-gated transient current, \( I_{\text{A}} \), was activated with depolarizing steps from a holding voltage of \(-80 \) mV. The high-threshold currents activated during the \( I_{\text{A}} \) activation protocol were removed by subtracting the currents previously measured from a holding potential of \(-40 \) mV. The hyperpolarization-activated current, \( I_{\text{H}} \), was measured using 8-s-long hyperpolarizing pulses from a holding potential of \(-40 \) mV.

Ionic conductance of \( I_{\text{HTRK}} \) and \( I_{\text{A}} \) was determined by dividing the current levels measured at \(+20 \) mV by the driving force, assuming a K+ reversal potential \( (E_{\text{K}}) \) of \(-80 \) mV, and ionic conductance of \( I_H \) similarly determined using a current measured at \(-110 \) mV and a reversal potential \( (E_{\text{H}}) \) of \(-10 \) mV (Haedo and Golowasch 2006; Khorkova and Golowasch 2007).

After ionic currents were measured under control conditions, the preparations were either treated with \( 10^{-5} \) M picROTOXIN or decentralized and placed at 6°C for 24 h. The currents were measured again using the same protocols, and the neurons were subsequently processed for RNA extraction (see below).

**mRNA quantification.** Quantitative PCR was performed as described by Schulz et al. (2006). Briefly, total RNA was isolated using the RNeasy microcolumn-based RNA extraction kit (Qiagen, Valencia, CA), reverse transcribed using SuperScript III reverse transcriptase (Invitrogen), and used as a template in real-time RT-PCR with program parameters previously measured from a holding potential of 40 mV.

Fig. 1. Stomatogastric nervous system and activity changes under different modulatory conditions. A: the stomatogastric nervous system. OG, esophageal ganglion; CoG, commissural ganglion; STG, stomatogastric ganglion; stn, stomatogastric nerve; lvn, lateral ventricular nerve; pdn, pyloric dilator nerve; mvm, medial ventricular nerve. The circle around stn represents a Vaseline well used to block action potential transmission (METHODS). B: effect of the removal of neuromodulatory input to the STG. Pyloric activity is temporarily lost after decentralization (4 h). Activity is slower but can be identified as pyloric 24 h after decentralization. C: effect of the removal of glutamatergic inhibitory synapses with picROTOXIN (PTX). The lateral pyloric (LP) neuron becomes, and remains, tonic over 24 h.
SYBR green (SA Biosciences, Frederick, MA). Primers specific for real-time PCR detection of Shal, BK-KCa, H, and 18S rRNA with SYBR green were developed and designed using Primer3 software and are the same as previously reported (Schulz et al. 2006, 2007).

**Statistics.** Comparisons of overall levels of mRNA and conductance density were made using a one-way ANOVA followed by post hoc Tukey’s t-test pairwise comparisons (Systat; Aspire Software International, Ashburn, VA). Correlation (parametric Pearson’s product moment and nonparametric Spearman’s rank correlation test) and linear regression analyses were carried out using Sigmastat (Systat). We compared correlation coefficients between experimental groups for a given pairwise correlation by performing a Fisher transformation of ρ using

$$
\rho' = (0.5) \log \left| \frac{1 + \rho}{1 - \rho} \right|
$$

and then calculating a Fisher’s z statistic as follows:

$$
z = \frac{\rho' - \rho''}{\sqrt{\frac{1}{n_1 - 3} + \frac{1}{n_2 - 3}}}
$$

Finally, slopes of relationships between conductances and between mRNA levels were compared between experimental groups with a modified ANCOVA (GraphPad Prism version 5; GraphPad Software, San Diego, CA).

**RESULTS**

As has been shown previously (Luther et al. 2003; Thoby-Brisson and Simmers 2002), decentralization of the STG leads to the rapid but reversible loss of rhythmic activity of the pyloric network (Fig. 1B). The rhythmic pyloric activity recovers to levels and characteristics similar to those observed under control conditions with a delay of hours to days (Luther et al. 2003). The removal of most chemical synapses in the ganglion, which are primarily inhibitory glutamatergic, was accomplished using 10^-5 M picrotoxin, which disrupts the triphasic pyloric rhythm (Bidaut 1980; Marder and Eisen 1984), making the follower neurons (LP and other neurons) fire tonically, but leaves the pacemaker’s activity (monitored by the PD neurons’ activity on both the lvn and pdn) intact over the course of 24 h (Fig. 1C). Picrotoxin blocks inhibitory chemical synapses, which produces drastic changes in activity of LP but not PD neurons. This allows us to decouple activity-dependent mechanisms from neuromodulator-dependent correlations of mRNA and conductance levels.

**Ion channel conductances are correlated across channel types.** We hypothesized that, as shown previously for PD neurons (Khorkova and Golowasch 2007), ionic conductances are expressed in other pyloric neurons in a correlated fashion. To test this, we measured three ionic currents, \(I_{HTK}, I_A,\) and \(I_H\) in PD and LP neurons and estimated the ionic conductances (\(g_{HTK}, g_A,\) and \(g_H\)). Control PD neurons expressed pairwise correlations (Fig. 2, top row) very similar to those described previously (Khorkova and Golowasch 2007). Table 1 lists all coefficients of determination (\(R^2\)) and Pearson product moment coefficients (\(ρ\)) for each pairwise conductance comparison. We performed the same measurements in another identified neuron, the LP neuron, and found that these three conductances were all similarly correlated in a pairwise manner (Fig. 3, top row, and Table 1). The nonparametric Spearman’s rank correlation test was also used and gave nearly identical results to the Pearson product moment correlation test; thus we report only the latter tests in this report.

To determine what factors may control these correlations, we treated these preparations in two different ways designed to disrupt the major inputs these cells receive within the STG: in one we used picrotoxin to block the synaptic input these two cells receive, and in the other we blocked all neuromodulatory input to the cells (decentralization). For the picrotoxin group, we treated the entire stomatogastric nervous system (Fig. 1A) with 10^-5 M picrotoxin.

In this process of removing synaptic inhibition to LP, we greatly alter its firing pattern (Fig. 1C). Despite these manipulations, we observed that all pairwise correlations of the three ionic currents were maintained with similar correlation coefficients in both PD (Fig. 2) and LP neurons (Fig. 3, Table 1). Even though the \(g_{HTK}g_H\) pair for the LP neurons shows a marginally higher \(P\) value (0.056) than the cutoff of 0.05, we accepted it as indicative of a significant correlation.

For the “decentralized” group, significant differences between the two cell types were observed after 24 h: in PD neurons, only the pairwise \(g_A\) vs. \(g_H\) correlation was maintained, whereas the \(g_{HTK}\) vs. \(g_A\) and the \(g_{HTK}\) vs. \(g_H\) correlations were lost (Fig. 2, Table 1); in contrast, in LP neurons, all of the correlations were maintained, but the correlation coefficient for \(g_A\) vs. \(g_H\) was significantly lower in decentralized relative to control groups (Fig. 3, Table 1).

In addition to the presence or absence of a significant correlation between conductances, we determined whether correlations that were maintained between conditions (e.g., the same correlation present in control and picrotoxin groups) differed in the slope of the relationship. Despite the fact that correlations were maintained among conductance pairs across experimental groups, there were significant changes in a subset of these relationships in both PD and LP cells (Table 2). There was a significant decrease in the slope of \(g_{HTK}\) vs. \(g_A\) between the control and picrotoxin-treated groups, as well as a significant increase in the \(g_A\) vs. \(g_H\) slope of the decentralized relative to the control group in PD cells. However, there was no change in the slope of the relationship between \(g_{HTK}\) and \(g_H\) in control and picrotoxin-treated PD cells. For LP cells, the slope of the \(g_{HTK}\) vs. \(g_A\) relationship was lower in picrotoxin-treated LP cells, whereas the slope of all three relationships was lower in decentralized LP cells relative to controls (Table 2).

**Ion channel correlations at the mRNA level: distinct from conductance correlations.** Pairwise comparison of channel mRNA levels under control conditions revealed a three-way correlation between \(BK-KCa, H,\) and \(Shal\) in PD neurons (Fig. 4, Table 3), consistent with the correlations observed at the ionic conductance level described above. Surprisingly, LP neurons showed no correlation of the \(BK-KCa–Shal\) pair, whereas the other two channel mRNA pairs showed strong and statistically significant correlation levels (Fig. 5, Table 3). Again, as before, to determine what factors may control these correlations, we treated one group of preparations with 10^-5 M picrotoxin for 24 h, decentralized a second group for 24 h, and measured channel mRNA levels in both. We observed that PD neurons treated with picrotoxin showed similarly strong and statistically significant correlations in all three pairs as in the control preparations (Fig. 4, Table 3). Consistent with the correlations observed under control conditions, in LP neurons treated with picrotoxin, the \(BK-KCa–Shal\) pair showed...
no correlation, whereas the other two channel mRNA pairs did (Fig. 5, Table 3).

Finally, we evaluated the pairwise channel mRNA correlation levels in the decentralized preparation group and found another surprise: in the LP neurons, the \( \text{BK-KCa–Shal} \) pair that had shown no statistically significant correlations in either the control or picrotoxin group now showed a moderate but statistically significant correlation. The other two channel mRNA pairs (\( \text{BK-KCa–H} \) and \( \text{Shal–H} \)) on the other hand, were mixed.

There was no significant change in \( \text{BK-KCa–H} \) correlation, but...
there was a significant decrease in the correlation coefficient of the Shal–H relationship (Fig. 5, Table 3).

We also determined whether the slopes of the relationships changed for mRNA correlations across treatment groups (Table 4). There was a significant decrease in both the BK-KCa vs. H and Shal vs. H relationships from control to the picrotoxin group, whereas the slope of the relationship between BK-KCa and Shal was maintained in PD cells. In decentralized PD cells, there was a significant decrease in the slope of the BK-KCa vs. Shal and BK-KCa vs. H relationships relative to control, and the Shal vs. H relationship was lost. For LP cells, there was a significant increase in the Shal vs. H relationship in picrotoxin, whereas the BK-KCa

<table>
<thead>
<tr>
<th>Table 2. Slopes for pairwise conductance-level relationships</th>
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<tbody>
<tr>
<td>$\frac{g_{\text{HTK}}}{g_A}$ vs. $\frac{g_{\text{HTK}}}{g_A}$</td>
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<tr>
<td>Slope</td>
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<tr>
<td>PD neuron conductances</td>
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<tr>
<td>Controls</td>
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<tr>
<td>Picrotoxin</td>
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<tr>
<td>Decentralized</td>
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<td>LP neuron conductances</td>
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<tr>
<td>Controls</td>
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<tr>
<td>Picrotoxin</td>
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<tr>
<td>Decentralized</td>
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Conductances were measured under control, picrotoxin, and decentralized conditions, and slopes for significantly correlated conductances were analyzed across groups via modified analysis of covariance (ANCOVA). Boldface indicates comparisons in which the conditions result in significantly different slopes from one another ($P < 0.05$). $^{a,b}$Different superscript letters for slope values indicate pairs for which the difference is significant (thus pairs with the same superscript are not different). NS, no significant pairwise correlation was found between the 2 conductance levels in a given treatment group.
vs. \( H \) relationship was unchanged. No significant changes were seen in the slopes of mRNA relationships relative to control in decentralized LP cells, although a novel \( BK-KCa \) vs. \( Shal \) relationship was detected.

**DISCUSSION**

Neurons have the ability to generate a functional output that is stable over long time scales. To ensure this, neurons express plasticity in their intrinsic properties and excitability (Daoudal and Debanne 2003; Davis 2006; Frick and Johnston 2005; Haedo and Golowasch 2006; Thoby-Brisson and Simmers 2002; Turrigiano and Nelson 2004; Zhang and Linden 2003), which manifests itself via changes in ionic channel properties (Zhang and Linden 2003), but the levels at which this is regulated, and the molecular mechanisms involved, remain to be understood. It has been shown previously that ionic chan-

Table 3. *Ion channel mRNA-level correlations*

<table>
<thead>
<tr>
<th></th>
<th>( BK-KCa ) vs. ( Shal )</th>
<th>( BK-KCa ) vs. ( H )</th>
<th>( Shal ) vs. ( H )</th>
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<tr>
<td><strong>R(^2)</strong></td>
<td>( \rho ) (( P ) value)</td>
<td>( \rho ) (( P ) value)</td>
<td>( \rho ) (( P ) value)</td>
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<tr>
<td><strong>PD neuron mRNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.848</td>
<td>0.921* (0.001)</td>
<td>0.869* (0.005)</td>
</tr>
<tr>
<td>Picrotoxin</td>
<td>0.886</td>
<td>0.941* (&lt;0.001)</td>
<td>0.899* (&lt;0.001)</td>
</tr>
<tr>
<td>Decentralized</td>
<td>0.238</td>
<td>0.487* (0.021)</td>
<td>0.550* (0.006)</td>
</tr>
<tr>
<td><strong>LP neuron mRNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.089</td>
<td>0.298 (0.216)</td>
<td>0.705* (0.001)</td>
</tr>
<tr>
<td>Picrotoxin</td>
<td>0.034</td>
<td>0.186 (0.585)</td>
<td>0.806* (0.005)</td>
</tr>
<tr>
<td>Decentralized</td>
<td>0.357</td>
<td>0.598 (0.015)</td>
<td>0.573* (0.020)</td>
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</table>

\( R^2 \) and \( \rho \) of PD and LP neurons were determined under control, picrotoxin, and decentralized conditions for pairs of ion channel mRNA levels. Boldface indicates a significant (\( P < 0.05 \)) correlation in the Pearson’s correlation analysis. \(^{ab}\)Different superscript letters between 2 groups indicate significant differences among correlation coefficients of these groups. Correlation coefficient analysis was not performed with groups in which a significant correlation was not found.

Fig. 4. Channel mRNA correlations in PD neurons. mRNA levels of \( BK-KCa \), \( Shal \), and \( H \) are graphed in all pairwise combinations. Each point represents a different cell. Regression lines are shown only for significant correlations (Pearson correlation analysis, \( P < 0.05 \)). Measurements were obtained under the 3 conditions indicated: control (top row), after 24 h in 10\(^{-3}\) M PTX (middle row), and 24 h after decentralization (bottom row). Numbers indicate mRNA copy numbers.
nels may be codependent at both the mRNA (Schulz et al. 2007) and the conductance levels (Khorkova and Golowasch 2007; MacLean et al. 2003) and that such codependence may be related to the homeostatic regulation of function in the pyloric network (Ball et al. 2010; Burdakov 2005; Franklin et al. 2010; Khorkova and Golowasch 2007; MacLean et al. 2005). The purpose of this study was twofold: first, to determine whether the apparent coregulation of ionic channel expression is cell-type specific, and second, to determine how neuromodulatory input and synaptic activity affects the correlated expression of ionic channels at both the ionic conductance and mRNA levels. We have shown that 1 neurons comodify ion channel mRNA (BK-KCa, Shal, and H) levels in a cell-specific manner: under control conditions (i.e., neuro-

![Fig. 5. Channel mRNA correlations in LP neurons. mRNA levels of BK-KCa, Shal, and H are graphed in all pairwise combinations. Each point represents a different cell. Regression lines are shown only for significant correlations (Pearson correlation analysis, P < 0.05). Measurements were obtained under the three conditions indicated: control (top row), after 24 h in 10^{-5} M PTX (middle row), and 24 h after decentralization (bottom row). Numbers indicate mRNA copy numbers.](http://jn.physiology.org/)

Table 4. Slopes for pairwise ion channel mRNA-level relationships

<table>
<thead>
<tr>
<th></th>
<th>BK-KCa vs. Shal</th>
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<th>BK-KCa vs. H</th>
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<th>Shal vs. H</th>
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<tr>
<td></td>
<td>Slope</td>
<td>SD</td>
<td>n</td>
<td>Slope</td>
<td>SD</td>
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<tr>
<td><strong>PD neuron mRNA</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.39a</td>
<td>0.07</td>
<td>8</td>
<td>0.43a</td>
<td>0.10</td>
</tr>
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<td>12</td>
<td>0.15a</td>
<td>0.03</td>
</tr>
<tr>
<td>Decentralized</td>
<td>0.14b</td>
<td>0.06</td>
<td>22</td>
<td>0.14a</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>LP neuron mRNA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Controls</td>
<td>NS</td>
<td>NS</td>
<td>19</td>
<td>0.35a</td>
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<td>Picrotoxin</td>
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<td>NS</td>
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<td>0.11</td>
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<td>0.05</td>
<td>16</td>
<td>0.21a</td>
<td>0.08</td>
</tr>
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</table>

mRNA levels were measured under control, picrotoxin, and decentralized conditions, and slopes for significantly correlated conductances were analyzed across groups via modified ANCOVA. Boldface indicates comparisons in which the conditions result in significantly different slopes from one another (P < 0.05).

Different superscript letters for slope values indicate pairs for which the difference is significant (thus pairs with the same superscript are not different). NS, no significant pairwise correlation was found between the 2 mRNA levels in a given treatment group.
modulatory and synaptic input present), PD neurons express these three mRNA species in a three-way correlated manner, whereas LP neurons appear to do so only for the pairs Shal vs. H and BK-KCa vs. H and not for the BK-KCa vs. Shal pair. 2) Removal of neuromodulatory input can destroy, induce, or alter the strength or slope of the correlated expression of ionic channel mRNA in a cell-specific manner: in PD neurons, all correlated pairs either lose or have significantly weakened correlations, whereas LP neurons show a gain in the correlation of BK-KCa vs. Shal but weakening of the correlations of the other two pairs examined. 3) At the conductance level, three-way pairwise correlations appear to be common to both cell types examined, but the response to the loss of neuromodulatory input is cell-type specific (i.e., different between PD and LP neurons) and is also distinct from that observed at the mRNA level. 4) Finally, chemical synaptic activity does not seem to play a role, at least for the two cell types examined, in controlling the correlated expression of the ionic currents we measured at either the mRNA or the conductance levels. Of these results, only the three-way pairwise correlations and the absence of activity-dependent regulation of ionic conductances in PD neurons have been shown previously (Khorkova and Golowasch 2007). Yet, it was previously unclear whether these effects acted solely at the level of membrane conductances or whether they extended to multiple levels of cellular organization such as mRNA for ion channels. Furthermore, it was not known whether these relationships would exist in multiple neurons in the network, including a “follower” neuron such as LP, or be limited to critical pacemaker cells such as PD.

There is a substantial overlap in correlations among conductances and those among mRNA levels; in PD cells, correlations were consistent in six of nine relationships between conductances and mRNA, whereas in LP cells, seven of nine were consistent. These results therefore implicate a functional link between regulation at the mRNA level and membrane conductance, which has previously been seen for LP but not PD neurons (Schulz et al. 2006). Yet, one of the more striking outcomes of our study is the correlations seen among channel mRNA types and those among ionic conductances, although both seemingly influenced strongly by neuromodulation, can themselves be distinct. These results indicate that neuromodulation plays a role, at least in part, in coordinating regulation across multiple levels of cellular organization. Nevertheless, our work suggests that modulators can have distinct and independent effects on, and perhaps independent pathways for, regulation of transcription and posttranslational mechanisms involved in generating membrane currents. This is consistent with recent studies demonstrating variation in similar kinds of relationships between mRNA and protein, presumably as a result of transcriptional and nontranscriptional-level variation (Foss et al. 2011). Although it is an obvious notion that mRNA abundance does not simply equate to mature protein or channel activity, this is, to our knowledge, the first demonstration of separate control by neuromodulators of membrane ion channel conductances and their mRNA. Given that our manipulations remove the input to the STG of a large number of neuromodulators [at last count, at least 27 (Marder and Bucher 2007)], it is conceivable that different modulators have distinct actions at different levels. However, it is interesting to note that a single peptidergic modulator (proctolin) has been shown to revert fully, in an activity-independent manner, all the effects of decentralization on conductance correlations in PD neurons (Khorkova and Golowasch 2007). Our results are significant in that they reveal important differences in how the correlated expression of ionic currents ultimately comes about, suggesting multiple levels of potential regulation: at the very least, at the levels of transcription, and also at some level between the beginning of translation and the full integration of the channel proteins into the plasma membrane.

What are the possible mechanisms underlying this phenomenon? Cotranscription is a process by which the close proximity of molecular elements and genes involved in transcription increases the likelihood of them being simultaneously expressed (Sutherland and Bickmore 2009). This would result in ion channels appearing to be “tracking” each other’s expression levels. If transcription is the point at which this process of coexpression occurs, the many modifications and variations that can occur along the way as the ion channel transcripts are processed, transported, and translated, and the expressed channel proteins are then modified and exported into the plasma membrane, could explain the lack of perfect inter-ion channel correlation across preparations (Tables 1 and 3). Furthermore, this could provide an explanation for the lower level of correlation observed in most pairs at the conductance rather than at the mRNA level (when significant correlations exist). Nevertheless, it appears that correlations in expression may also be implemented downstream of transcription. For instance, in LP neurons (control conditions), the BK-KCa vs. Shal pair, and in PD neurons (decentralized conditions), the Shal vs. H pair, show no apparent correlation at the transcription level but a high level of correlation at the conductance level. Mechanisms to explain this could be the cotranslational interaction of ion channels similar to what occurs during ion channel subunit assembly (Shi et al. 1996), the cotrafficking into the plasma membrane (Vanoye et al. 2010), the coassembly of ion channels into macromolecular complexes (e.g., Arcangeli 2011; Frank 2011; Zhang et al. 2011), or the direct interactions between ion channels via nonconducting properties (Kaczmarek 2006). The results observed by MacLean et al. (2003) in which the transfection of lobster PD neurons with Shal mRNA resulted in the activity-independent enhanced coexpression of the H current, suggest that the control of coexpression does occur at a translational or posttranslation level. We did not measure transcription per se, but only steady-state mRNA levels, and thus the correlations of mRNA types could be the result of posttranscriptional regulation of mRNA stability. Therefore, our results indicate that neuromodulators may regulate either transcription directly or the stability of mRNA in a concerted manner.

The loss of cotranscription observed in PD neurons after decentralization could be attributed to neuromodulators activating a transcription regulation pathway that affects the coexpression of ion channels at any of the steps mentioned above. Interestingly, our data suggest that in different neuronal cell types, this process is different (i.e., inverse, such as in LP neurons), suggesting that neuromodulators may also act to repress cotranscription. Although activity is known to influence the expression of ion channels in a wide variety of systems, including the pyloric network (Cudmore and Turrigiano 2004; Desai et al. 1999; Golowasch et al. 1999a; Haedo and Golowasch 2006; Li et al. 2004; Loebrich and Nedivi 2009; Turrigiano et al. 1994; Zhang and Linden 2003), the
coexpression of the ion channels examined in this work appears not to be influenced by synaptic activity. Our results do not rigorously rule out activity as a factor, but STG neurons are known to be sensitive to patterned activity (Golowasch et al. 1999; Haedo and Golowasch 2006; Turrigiano et al. 1994), and synaptic activity is known to dramatically influence these patterns of activity in certain neurons (LP neurons, for example, see Fig. 1). Thus our results are consistent with a lack of effect on the correlated expression of ion channels, which was also demonstrated previously for PD neurons (Khorkova and Golowasch 2007).

What functional role does coexpression of ion channels serve? Given the high level of variability in the conductances of distinct ionic currents between neurons of the same type (see range along the axes of any of our graphs herein), it appears that coexpression of ionic currents is one way to reduce the global variability of the system by linking multiple highly variable currents to each other. It has been shown theoretically that this can indeed stabilize specific activity features (Ball et al. 2010; Burdakov 2005; Franklin et al. 2010; MacLean et al. 2005). The loss of correlations between ionic conductances may be an evolutionary adaptation of the neurons in this system to permit the independent modification of their levels as the pyloric network recovers its lost activity. By modifying the process that keeps these conductances linked to each other, constraints on the system may be eased to make it better able to visit alternative activity states that may result in the restoration of rhythmic neuronal activity and behavioral output.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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REFERENCES


