Maturation of Lobster Stomatogastric Ganglion Rhythmic Activity

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Richards, Kathryn S., William L. Miller, and Eve Marder. Maturation of lobster stomatogastric ganglion rhythmic activity. J. Neurophysiol. 82: 2006–2009, 1999. The stomatogastric ganglion of the adult lobster, Homarus americanus generates extremely regular pyloric rhythms with a characteristic period of 0.5–1.5 Hz. To study the changes in the pyloric rhythm during embryonic and larval development, we recorded excitatory junctional potentials evoked by lateral pyloric (LP) neuron activity. Early in development the motor discharge of the LP neuron was often irregular, preventing use of conventional analysis methods that rely on extracting burst times to calculate cycle frequency and its variability. Instead, cycle frequency was determined for the LP neuron from the peak of the power spectrum obtained from the occurrence times of excitatory junctional potentials in the pyloric (PY) neuron activity. Throughout embryonic and larval stages, the motor discharge of the LP neuron was often irregular, preventing use of conventional methods that rely on extracting burst times to calculate cycle frequency and its variability. Instead, cycle frequency for the pyloric rhythm was determined from the peak of the power spectrum of the action potential times (Miller and Sigvardt 1998; Rosenberg et al. 1989). For each trial, the corresponding list of event times was divided into three equal-time sections, and the final power spectrum was obtained as an average of the spectra calculated for each section. Each spectrum was normalized to the power at zero frequency to allow comparison of spectra. The full spectra show peaks at high-frequency (20–40 Hz) that correspond to the interspike intervals that occur when EJPs are tightly grouped, as in bursts, and peaks at lower frequency that roughly correspond to the frequency of bursts. In this paper we were concerned with the burst frequency but not with the frequency of firing within the burst, and therefore restricted our analyses to the lower frequency range of the spectra. An estimate of the variability in the activity was computed as the ratio of the area under the principal peak region of the power spectrum to the area under the spectrum from 0 to 3 Hz [including the peak region, and with the spectral value at frequency 0 (DC offset) set to 0]. The resulting “power ratio” can range between 0 and 1, is high (>0.5) for highly periodic activity (i.e., most of the power in the signal is found at the cycle frequency of bursting), and decreases with increasing variability in the motor rhythm (i.e., power is found at frequencies other than the cycle frequency. When bursting is highly regular so that the recorded signal approximates a square wave, “harmonic” peaks are seen in the power spectrum at multiples of the peak frequency. These harmonics are much less evident in the spectra of irregular activity. Although it is relatively straightforward to remove the harmonics from highly regular spectra, identification and removal of the harmonics from the cases of irregular activity is not possible. Therefore all of the power ratio calculations include the harmonics, and the relative power in the main spectral peak is underestimated because of the harmonic peaks.

METHODS

The eggs and larvae of H. americanus were obtained from the lobster-rearing facility located at the New England Aquarium. Embryos and larvae were staged as in Helluy and Beltz (1991). The larvae were either fed live or frozen brine shrimp, Artemia salina. Adult animals of both sexes were purchased from local fishermen. Adult stomatogastric nervous systems were dissected from the stomach, pinned in a dish, and superfused with chilled (9–13°C) saline. Saline composition was as follows (in mM): 479.12 NaCl, 12.74 KCl, 10 MgSO₄, 3.91 Na₂SO₄, 13.67 CaCl₂, and 5 HEPES, pH 7.45. Extracellular pin electrodes were used to record from the motor nerves (Fig. 1A). The entire stomachs of embryonic and larval animals were dissected and pinned in the dish. Intracellular electrodes were used to record from the p1 muscle, innervated by the LP neuron (Fig. 1A). The p1 excitatory junctional potentials (EJPs) were used as an assay for rhythmic motor pattern generation in the STG (Casasnovas and Meyrand 1995). The intracellular electrodes were filled with 0.6 M K₂SO₄ and had a resistance of 50–80 MΩ.

Data were recorded continuously for 3–20 min. Action potential and EJP times of occurrence were extracted off-line from digitized electrode recordings, using in-house and commercial software (MiniAnalysis, Janelia). Due to the irregularity of the LP motor activity in most of the embryonic preparations, extraction and analysis of burst onsets and durations was not generally possible. Instead, cycle frequency of the pyloric rhythm was determined from the principal peak of the power spectrum of the action potential or EJP times (Miller and Sigvardt 1998; Rosenberg et al. 1989). For each trial, the corresponding list of event times was divided into three equal-time sections, and the final power spectrum was obtained as an average of the spectra calculated for each section. Each spectrum was normalized to the power at zero frequency to allow comparison of spectra. The full spectra show peaks at high-frequency (20–40 Hz) that correspond to the interspike intervals that occur when EJPs are tightly grouped, as in bursts, and peaks at lower frequency that roughly correspond to the frequency of bursts. In this paper we were concerned with the burst frequency but not with the frequency of firing within the burst, and therefore restricted our analyses to the lower frequency range of the spectra. An estimate of the variability in the activity was computed as the ratio of the area under the principal peak region of the power spectrum to the area under the spectrum from 0 to 3 Hz [including the peak region, and with the spectral value at frequency 0 (DC offset) set to 0]. The resulting “power ratio” can range between 0 and 1, is high (>0.5) for highly periodic activity (i.e., most of the power in the signal is found at the cycle frequency of bursting), and decreases with increasing variability in the motor rhythm (i.e., power is found at frequencies other than the cycle frequency). When bursting is highly regular so that the recorded signal approximates a square wave, “harmonic” peaks are seen in the power spectrum at multiples of the peak frequency. These harmonics are much less evident in the spectra of irregular activity. Although it is relatively straightforward to remove the harmonics from highly regular spectra, identification and removal of the harmonics from the cases of irregular activity is not possible. Therefore all of the power ratio calculations include the harmonics, and the relative power in the main spectral peak is underestimated because of the harmonic peaks.

RESULTS

Figure 1 shows a schematic of the recording configurations used in this work. Recordings of the adult motor patterns were made extracellularly from the motor nerves. Figure 1B shows the pyloric rhythm from an adult H. americanus. The traces labeled pdn and llvn show the alternate patterns of lateral pyloric (LP), pyloric (PY), and pyloric dilator (PD) neuron.

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activity. To measure the period and the regularity of the pyloric rhythm we recorded data and performed a Fourier analysis of the spike times extracted from the raw data (see METHODS). Figure 1, C and D, shows the power spectra of the recordings shown in Fig. 1B calculated from the activity of the PD (pdn recording) and the LP (largest spike on the llvn) neurons, respectively. Both spectra show peaks at the same frequency: 0.46 Hz. The activity of this preparation was highly regular (power ratio of 0.82 for calculated for the PD neuron activity and 0.71 for LP neuron activity). The average cycle frequency in six adult preparations was 0.64 ± 0.05 (SE) Hz as measured with both the LP and PD traces. The power ratio was 0.72 ± 0.064 measured from the PD activity and 0.61 ± 0.031 as measured from the LP neuron activity. These values are not statistically different (P > 0.07), although in all cases the regularity was slightly lower when measured with LP neuron activity than with PD neuron activity, possibly because there are two PD neurons contributing to the PD spectra, thus increasing the signal-to-noise ratio of the power spectrum.

Figure 2 shows raw data and power spectra from individual representative preparations at embryonic and at each larval stage, as monitored by an intracellular recording from the pl muscle. Unlike the data shown in Fig. 1, in all of these recordings there were episodes in which the LP neuron fired in relatively regular bursts, and other stretches in which it fired quite irregularly. The E75% recording shows an example of a preparation that had several single EJPs, making burst characterization difficult by conventional means. In the recordings shown from the larval times, bursts of EJPs were common, but the bursts were of considerably different durations and had largely variable interburst intervals. The recordings shown for the LIV preparation are considerably more regular than those seen at earlier times. Despite the irregularity of the early rhythms, all of the spectra showed one peak with more power than all the others (preferred peak frequency; Fig. 2). Table 1 presents pooled data for peak frequency and percent power in the peak for embryos, LI, LII, LIII, LIV, and adult animals. Both the frequency and regularity of the rhythm are low during embryonic and the early larval stages and increase as
derestimated in the more regular preparations (METHODS).

greater because the power ratio calculation is preferentially un-
doubled. The actual regularity of adult was most likely
twice between embryonic and adult times, whereas the power ratio

DISCUSSION

development proceeds. The mean frequency increased by \( \approx 2.5 \)-
fold between embryonic and adult times, whereas the power ratio
more than doubled. The actual regularity of adult was most likely
greater because the power ratio calculation is preferentially un-
derestimated in the more regular preparations (METHODS).

D I S C U S S I O N

Spontaneous rhythmic activity may function to tune both sensory and motor circuits during development (O’Donovan

<table>
<thead>
<tr>
<th>Stage</th>
<th>( n )</th>
<th>Peak Frequency, Hz</th>
<th>Power Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryo</td>
<td>26</td>
<td>( 0.30 \pm 0.02^* )</td>
<td>( 0.236 \pm 0.016^* )</td>
</tr>
<tr>
<td>LI</td>
<td>10</td>
<td>( 0.38 \pm 0.04^* )</td>
<td>( 0.211 \pm 0.02^* )</td>
</tr>
<tr>
<td>LII</td>
<td>7</td>
<td>( 0.38 \pm 0.08^* )</td>
<td>( 0.319 \pm 0.029 )</td>
</tr>
<tr>
<td>LIII</td>
<td>11</td>
<td>( 0.41 \pm 0.03^* )</td>
<td>( 0.318 \pm 0.033^* )</td>
</tr>
<tr>
<td>LIV</td>
<td>10</td>
<td>( 0.50 \pm 0.07 )</td>
<td>( 0.410 \pm 0.041 )</td>
</tr>
<tr>
<td>Adult</td>
<td>9</td>
<td>( 0.72 \pm 0.08 )</td>
<td>( 0.511 \pm 0.047 )</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \) is number of preparations. The embryonic and
larval stage measurements were made using intracellular recordings from the
lateral pyloric (LP)–innervated \( p1 \) muscle. The adult measurements were
made from extracellular recordings of the LP neuron on the \( ilv \). The means were
determined to be statistically different by a one-way ANOVA (\( P < 0.001 \)).
Pairwise multiple comparisons (modified Bonferroni test) showed that the
embryo, LI, LII, LIII were significantly different from the adult (\( P < 0.003 \))
and the embryo, LI, and LIII were different from the adult in power ratio
(\( P < 0.003 \)). Additionally, E and LI are significantly different from LIV in
power ratio (\( P < 0.003 \)). * Values significantly different from the adult.

In particular, irregular activity that precedes functional behavior could be important in the
formation of synaptic connections, and in modifying the
strengths of synapses and the intrinsic properties of the neurons
within a circuit (O’Donovan 1999). Casasnovas and Meyrand
(1995) showed that the embryonic STG is rhythmically active,
well before the stomach is processing food. Here we demonstra-
te that this activity is quite irregular, only slightly periodic,
and is slower than the adult pyloric rhythm. It is possible that
these early and irregular activity patterns may provide matura-
tional signals that allow the STG to coordinately tune its

In this study we used the activity in the muscle innervated by
the LP neuron as an assay of the rhythmic activity in the
pyloric region of the stomach. In our adult recordings, the
frequency of the rhythm obtained with the LP and PD neurons
was always identical, and the regularity of the rhythm seen
with the LP neuron activity was statistically indistinguishable
from that seen with the PD neurons. Figure 3 outlines a number
of possibilities that could account for the lower frequency and
more variable rhythms seen in the early developmental stages.

Figure 3, top panel, illustrates the circuit that is responsible
for the adult pyloric rhythm in \( H. \ gammaurus \) and almost
certainly as well in \( H. \ americanus \) (Cardi and Nagy 1994;
Nagy and Cardi 1994; Nagy et al. 1994; Robertson and Moul-
lins 1981a,b). The commissural pyloric oscillator (CPO) net-
work is found in each commissural ganglion (CoG; Fig. 1) and
projects to the STG where it rhythmically drives and entrains
the pyloric network (Cardi and Nagy 1994; Nagy and Cardi
1994; Nagy et al. 1994; Robertson and Moulins 1981a,b). The
rhythmically active PD and anterior burster (AB) neurons
inhibit the LP neuron, which is thus entrained to fire in tight
pyloric-timed bursts (Fig. 3, adult). Therefore in the adult,
the frequency (measured either from the LP or the PD neurons)
will be a consequence of the frequency of the entraining CPO.

The three cases shown in Fig. 3 illustrate that the lower
frequencies and lower regularity seen early in development can
result from variability occurring at a number of different sites
in the circuit. In Case 1, the CPO and the pacemaker ensemble
may or may not be regular, but the LP neuron fails to follow its
rhythmic drive, either because of its intrinsic properties or

FIG. 2. LP neuron evoked excitatory junction potentials (EJPs) recorded in
the \( p1 \) muscle during embryonic and larval times. The left side of each panel
shows an intracellular recording from a muscle fiber in the \( p1 \) muscle from an
animal of the stage indicated. The right side of each panel shows the power
spectrum of the event times of the experiment represented to the left. F and PR
are as in Fig. 1. Vertical bars, 10 mV. Horizontal bars, 5 s. The resting
membrane potentials were \(-66, -56, -70, -66, \) and \(-69 \) mV, respectively.

FIG. 3. Possible causes for irregular motor patterns seen early in develop-
ment. Top panel: left shows the circuit responsible for regular motor patterns
in the adult. The commissural pyloric oscillator (CPO) in each commissural
ganglion (CoG) drives the pacemaker ensemble (\( \circ \)), which inhibits the LP
neuron (\( \bullet \)). The LP therefore fires regularly in alternation with the PD neurons.
Bottom panel: shows 3 cases, each of which can produce irregular LP neuron
activity (see text). AB, anterior burster.
because the synapses from the PD and AB neurons to the LP neuron are not properly tuned. In this case, the frequency extracted from the LP neuron activity could be different from that of the CPO and the PD neurons. In Case 2, the CPO may be strongly rhythmic, but unable to drive reliably the PD/AB ensemble, either because the projection to the STG is not present, the synapses are not properly tuned or because the AB/PD neurons do not have the appropriate intrinsic properties. In this case, the PD neurons (and therefore the LP neuron) may have a lower frequency than that of the CPO and may appear irregular. In Case 3, the CPO itself may not be regular or may be absent. The slow and irregular patterns produced by the LP neuron would therefore be indicative of lack of strong rhythmicity in the entire circuit.

In addition to the CPO, there are a large number of other modulatory inputs to the STG, which may modify both the intrinsic properties of neurons and the synaptic connections among them (Harris-Warrick and Marder 1991; Marder and Calabrese 1996). Although some of the neuromodulatory inputs are already present by mid-embryonic development, others are not present until late in larval life (Fénelon et al. 1998, 1999; Kilman et al. 1999). Therefore the irregularity and low frequency of the early rhythms may be a consequence of the modulatory environment seen early in development. Although the stomach starts to process food at LI, its full mechanical and structural properties develop slowly between LI and LIV (Factor 1995), during which time the remaining modulatory inputs to the STG first become apparent (Fénelon et al. 1999; Kilman et al. 1999). The increase in frequency and regularity in late larval and postlarval animals may occur because this is the time at which the activating modulatory inputs are fully present and active. Alternatively, the major changes in both frequency and regularity reported here could reflect developmental processes that tune both the intrinsic properties of circuit neurons and the synaptic connections among them.

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