A switch between two modes of synaptic transmission mediated by presynaptic inhibition

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Pre-synaptic inhibition reduces chemical synaptic transmission in the larval ventral nerve cord (VNC) of the crustacean stomatogastric ganglion. But its role(s) in shaping the multisynaptic interactions underlying neural network activity are not well studied. We therefore used the crustacean stomatogastric nervous system to study how presynaptic inhibition of the identified projection neuron, commissural modulatory neuron 1 (MCN1), influences the MCN1 synaptic effects on the gastric mill neural network. Tonic MCN1 discharge elicits gastric mill network neurons and activates the gastric mill rhythm. Each network neuron, the lateral gastric (LG) neuron, presynaptically inhibits MCN1 and is electrically coupled to its terminals. We show here that this presynaptic inhibition selectively reduces or eliminates transmitter-mediated excitation from MCN1 without reducing its electrically mediated excitatory effects, thereby switching the network neurons excited by MCN1. By switching the type of synaptic output from MCN1 and, hence, the activated network neurons, this presynaptic inhibition is pivotal to motor pattern generation.

The gastric mill rhythm is generated by a neural network located in the stomatogastric ganglion (STG) [2]. In the crab, Cancer borealis, this network is influenced by presynaptic inhibition, including MCN1, that innervate the STG from the commissural ganglia (CoGs: Fig. 1a) [3, 4]. MCN1 excites the gastric mill neurons LG, interneuron 1 (Int1) and the dorsal gastric (DG) neuron, but tonic MCN1 activation also excites a gastric mill neuron in which LG impulse bursts alternate with those in LG and DG (Fig. 1b). LG and Int1 burst in alternation, in part because they synaptically inhibit each other [5] (Fig. 1c). The alternation between LG and DG results from the LG-mediated presynaptic inhibition of the MCN1 terminals within the STG [6]. We documented the presence of this presynaptic inhibition by recording from the stomatogastric nerve axon (SNAx) of MCN1 (MCN1SNAx: Fig. 1a) [7]. However, MCN1SNAx-initiated action potentials propagate past the MCN1SNAx recording site before they are affected by the presynaptic inhibition. Therefore, we use the DG neuron as a reporter of MCN1 chemical synaptic transmission in the STG. DG is an effective reporter because it is chemically excited by MCN1 and it is not synaptically influenced by any STG neurons.

A central aspect of the mechanism whereby MCN1 activity elicited the gastric mill rhythm that remained to be determined involved how each LG burst was generated, given that MCN1 was the only source of excitation to LG in these experiments and LG activity inhibited the STG terminals of MCN1. We found recently that LG in fact continues to receive excitatory postsynaptic potentials (e.p.s.ps) from MCN1 while LG is inhibiting this neuron (N = 33 preparations; Fig. 2a). This suggested that these e.p.s.ps resulted from the electrical connection between LG and MCN1. This possibility was supported by the fact that the MCN1-elicited e.p.s.p was initiated earlier in LG than in Int1 (N = 30 preparations; Fig. 2a). Moreover, when all chemical transmission was suppressed, the e.p.s.p was eliminated in Int1 but persisted with unchanged amplitude in LG (N = 6 preparations; Fig. 2b).

We also selectively eliminated chemical transmission from the STG terminals of MCN1 by impaling MCN1SNAx with a microelectrode filled with potassium acetate (KAcetate) instead of potassium chloride (KCl). KAcetate eliminates chemical transmission from individual SNAxs while leaving electrical...
coupling intact. Under this condition, the e.p.s.p. again persisted in LG, but not in Int1 (N = 5 preparations; Fig. 2c; see also Fig. 3). We can therefore conclude that the MCON1-mediated e.p.s.p. in LG results from electrical coupling. The persistence of electrical e.p.s.p.s indicates that presynaptic inhibition does not eliminate the MCON1 action potentials in the STG, although the effective membrane resistance underlying this inhibition remains to be elucidated.2,14-15

Interestingly, the amplitude of these electrical e.p.s.p.s increases with the duration and decreases with hyperpolarization of LG (Fig. 2f). Consequently, their amplitude was roughly double at membrane potentials near the peak versus the trough of the gastric mill-timed LG oscillations (Fig. 2g). This voltage dependence persisted when all chemical transmission was suppressed (N = 11 preparations; Fig. 2e, c). Voltage-dependent electrical coupling has been documented previously15 but its functional role is not well understood. Here, one likely function is to increase electrical excitability to LG while LG is presynaptically inhibiting its chemical excitatory input from MCON1. Additionally, the reduced amplitude of these e.p.s.p.s at hyperpolarized levels will diminish their ability to oppose the synaptic input from Int1, which is responsible for the LG interburst interval.

In addition to eliciting electrical e.p.s.p.s in LG (Fig. 2b, d), MCON1 elicits a transmitter-mediated excitation of LG (Fig. 2f). To show this excitation in Fig. 2f without interference from Int1 inhibition of LG, we hyperpolarized Int1 to prevent its activation by MCON1 stimulation. This transmitter-mediated excitation of LG develops slowly and, during the gastric mill rhythm, it enables LG to escape from Int1 inhibition15. Following termination of MCON1 activity, the underlying depolarization of LG decays slowly (Fig. 2f). In contrast, the purely electrical excitation of LG does not outlast MCON1 activity (see Fig. 3c).

Next we examined whether the MCON1-mediated electrical e.p.s.p. and long-lasting excitation both contributed to each gastric mill rhythm-timed LG burst. Therefore, we mimicked the alternating chemical and electrical transmission from a tonically active MCON1 that is a consequence of the rhythmic, LG-mediated presynaptic inhibition. To accomplish this, we alternated rhythmic stimulation of an unlabeled MCON1rostralis with stimulation of MCON1anteriormedialis, whose chemical transmission was selectively suppressed with KAacetate. Rhythmic stimulation of MCON1anteriormedialis alone elicited bursts in LG and DGG that were comparable to their gastric mill rhythm-timed activity (Fig. 3d). Each Int1 burst produced the fast, rhythmical inhibition in LG. Int1 consistently is activated sooner than LG in response to MCON1 stimulation (N = 13/13 preparations). Following each MCON1anteriormedialis stimulation, LG fired a few action potentials (Fig. 3a). This LG activity is considerably weaker than its activity during a gastric mill rhythm, but stronger than that resulting...
FIG. 3. MCN1-mediated electrical excitation contributes significantly to generation of each LG burst: panel a, stimulation of ion_{inj} (chemical transmission intact) in gastric mill rhythm-like bursts (bars; burst duration: 3.5 s; interburst frequency: 15 Hz) enables MCN1_{SNX} to excite INT1 (reflected in the increased amplitude, rhythmic inhibitory input to LG) and DG (dotted) rhythmically. Each stimulus duration corresponded to the duration of the INT burst/LG interburst interval during the gastric mill rhythm elicited by tonic ion_{inj} stimulation. After each ion_{inj} stimulation, LG fires a few action potentials. After the fourth ion_{inj} stimulation, each stimulation was followed by ion_{inj} stimulation (3 kHz for 5 s), which activated the K+ gap-filled microelectrode. Stimulation of ion_{inj} (15 Hz) activated MCN1, eliciting e.p.s.ps and a weak activation of LG, but it did not activate DG. There was also no activation of INT1, shown by the absence of inhibitory synaptic input to LG. Small amplitude unit in d in artes is from ion stimulation, a and c are from the same preparation, d, Electrical transmission from MCN1 to LG supports a maximal LG burst duration comparable to that occurring during the gastric mill rhythm. Experimental procedure is the same as a, except that following the final rhythmic activation of the unaltered MCN1_{SNX} ion_{inj} stimulation, the K+ gap-filled microelectrode was extended to 10 s. Intraburst firing frequency: MCN1_{SNX}, 15 Hz; MCN1_{LJ}, 20 Hz. Membrane potential: MCN1_{SNX}, -63 mV.

FIG. 4. Presynaptic inhibition switches both the effective mode of MCN1 transmission and the targets activated by MCN1. When LG is not active (indicated by grey shading), MCN1 provides a fast chemical excitation to INT1 and a slow chemical excitation to DG and LG. The fast excitation of INT1 enables it to be activated in advance of LG. This further delays the LG burst onset because INT1 inhibits LG. When LG is relatively depolarized, the electrical e.p.s.ps that it receives from MCN1 are small. The inhibition of LG allows MCN1 to continue releasing transmitter and, as a result, DG is also activated. Eventually, the slow chemical excitation that LG receives from MCN1 enables it to escape from the INT1-mediated inhibition and LG begins to fire action potentials. b. When LG is activated, it inhibits INT1, and presynaptically inhibits MCN1. The presynaptic inhibition reduces or eliminates the MCN1-mediated excitation of INT1 and DG, and these neurons stop firing. The MCN1-mediated chemical excitation of LG is also terminated, but the LG membrane potential repolarizes slowly, remaining depolarized for several seconds. Its maintained depolarization during the first 3–5 s after LG begins firing increases the strength of the voltage-dependent electrical e.p.s.ps that it continues to receive from MCN1. This enables LG to continue firing action potentials. Eventually, the LG membrane potential repolarizes, reducing the effectiveness of the electrical e.p.s.ps and terminating the LG burst. This removes the presynaptic inhibition of MCN1, enabling MCN1 to again release transmitter and activate INT1 and DG. In a and b, black indicates active neurons, and grey indicates inactive neurons. Symbols are the same as in Fig. 1c.

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from isolated activation of MCNI\textsubscript{TOT} (Fig. 3b, c). To determine whether the MCNI electrical excitation of LG was sufficient to rescue the LG bursts, we then stimulated the K\textsuperscript{+}-Acetate-filled MCNI\textsubscript{TOT} after each MCNI\textsubscript{REY} stimulation (N = 7 preparations; Fig. 3d). This enhanced each LG burst sufficiently to make it equivalent to those occurring during the gastric mill rhythm (Fig. 3b).

The long-lasting depolarization of LG contributes to LG burst generation by increasing the effectiveness of the electrical c.p.s.p.s. The time course of this depolarization also influences the duration of each LG burst. As is evident in Fig. 3d, when the duration of the MCNI\textsubscript{TOT} activity is extended so that it outlasts the normal LG burst duration, the electrical c.p.s.p.s only activate LG while it remains sufficiently depolarized by the long-lasting excitation (N = 3). Also consistent with a diminishing excitation of LG is the fact that the mean intraburst firing frequency of LG during the gastric mill rhythm is significantly faster during the first second of each burst than during the final second (8.4 ± 1.1 Hz versus 4.9 ± 1.5 Hz; N = 6; P < 0.01, Wilcoxon Signed Rank Test).

These results indicate that the electronic excitation from MCNI to LG contributes significantly to LG neuron activity. Thus, by selectively inhibiting chemical transmission, the presynaptic inhibition from LG switches the effective mode of MCNI synaptic transmission. This enables MCNI to excite, alternately, different subsets of gastric mill neurons and thereby elicit the gastric mill rhythm (Fig. 4).

In many systems, tonic activation of projection neurons, such as MCNI, or application of their modulatory transmitters is sufficient to evoke rhythmic neural network activity\textsuperscript{11,21 24}. This has suggested that modulatory inputs often do not provide timing cues to the activated network. However, as shown here, in some systems there are local presynaptic events that alter the output from the distant terminals of such neurons, enabling them to have rhythmic influences on their network targets that are not evident from intra-somatic recordings. Recent studies have highlighted the presence of local properties in dendrites\textsuperscript{25,26} as well as presynaptic influences in other rhythmic neural networks\textsuperscript{27,28}. Therefore, similar presynaptic events are likely to shape neuronal interactions in all nervous systems.