Neural Mechanisms Underlying Co-Activation of Functionally Antagonistic Motoneurons During a Clione Feeding Behavior

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Submitted 7 November 2005; accepted in final form 2 January 2006

Neural mechanisms underlying co-activation of functionally antagonistic motoneurons during a Clione feeding behavior. J Neurophysiol 95: 2560–2569, 2006. First published January 4, 2006; doi:10.1152/jn.01174.2005. The ability of some neural networks to produce multiple motor patterns required during different behaviors is a well-documented phenomenon. We describe here a dramatic transition from coordinated inhibition between two functionally antagonistic groups of motoneurons to their co-activation in the feeding neural network of the predatory mollusk Clione limacina. To seize its prey, Clione uses specialized oral appendages, called buccal cones, which are controlled by two groups of motoneurons: cerebral A (Cr-A) neurons controlling buccal cone protraction and cerebral B (Cr-B) neurons controlling buccal cone retraction. When Cr-A neurons are active, Cr-B neurons usually receive strong inhibitory inputs that terminate their firing, which leads to the full protraction and elongation of the buccal cones. We have found, however, that the Cr-A and Cr-B motoneurons sometimes burst simultaneously without any traces of inhibition in the Cr-B motoneurons. This transformation of the neural network activity from inhibitory interactions to co-activation presumably occurs during the late “extraction” period of the feeding behavior when buccal cones become partially retracted and rhythmically active. The transition from the inhibitory interaction to co-activation is controlled by the activity of a single pair of cerebral interneurons (Cr-Aint interneurons), which are electrically coupled to the Cr-A neurons and monosynaptically inhibit Cr-B neurons. Normally, the Cr-Aint interneurons are active along with Cr-A motoneurons and inhibit Cr-B motoneurons. During a period of co-activation, however, these interneurons do not produce spikes, thus allowing Cr-A motoneuron activation without inhibition of the Cr-B motoneurons.

INTRODUCTION

Some neural networks are capable of producing multiple activity patterns, which underlie variations of the behavioral output. A few systems have been thoroughly studied to describe the structure of such networks, their activity patterns, relevance to the behavior, and neuronal mechanisms underlying network reconfiguration. It includes, for example, crustacean stomatogastric neural network (Blitz and Nusbaum 1997; Blitz et al. 1999; Dickinson et al. 1990; Harris-Warrick et al. 1992; Marder and Calabrese 1996; Weimann et al. 1991; Wood et al. 2000). It also includes buccal neural network of gastropod mollusks (Jing and Weiss 2001, 2002; Kupfermann and Weiss 2001; Morton and Chiel 1993). We report here another example of a neural network with two distinct patterns of activity found in the pteropod mollusk Clione limacina. This network demonstrates a radical transformation of activity from the coordinated inhibition between functionally antagonistic motoneurons to their rhythmic co-activation, which presumably occurs during specific “extraction” period of the feeding behavior.

Clione is a highly specialized carnivore, which feeds only on the shelled pteropod mollusks of the genus Limacina and has adapted feeding structures for their capture and extraction (Lalli 1970; Lalli and Gilmer 1989; Wagner 1885). To capture the prey, Clione uses tentacle-like oral appendages, called buccal cones, which surround the Limacina shell and hold it during the subsequent stages of feeding. Protraction and elongation of the buccal cones is a hydraulic phenomenon and is accomplished by squeezing the hemocoel into the central cavities of the cones (Hermans and Satterlie 1992; Lalli and Gilmer 1989). After the prey capture, the buccal cones manipulate the Limacina shell so that its aperture is pressed against the mouth of Clione. Then two other feeding structures, chitinous hooks and the radula, are used to extract the soft body of Limacina from its shell to be swallowed whole (“extraction” period). Rhythmic protraction-retraction movements of the chitinous hooks and the radula are highly coordinated in a constant phase relation (Malyshev and Norekian 2002). This phase-dependent coordination is observed on the behavioral level and recorded on the motoneuronal level with hook protractor neurons always active in the same phase with radula retractor neurons and hook retractor neurons bursting in phase with the radula protractor neurons (Malyshev and Norekian 2002). A large group of electrically coupled cerebral A (Cr-A) motoneurons controls opening of the skin folds and protraction of the buccal cones (Norekian and Satterlie 1993a); “tentacle motoneurons” according to Arshavsky et al. (1993). Another group of motoneurons, cerebral B (Cr-B) cells, controls buccal cone retraction (Norekian and Satterlie 1993a). Because Cr-A and Cr-B neurons control functionally antagonistic movements of the buccal cones, it is not surprising that activation of the Cr-A neurons is usually accompanied by inhibition of the Cr-B neurons (Norekian 1995; Norekian and Satterlie 1993a).

We describe in this study that inhibitory inputs to the Cr-B neurons sometimes disappear, and both Cr-A and Cr-B neurons begin to burst at the same time. These periods of co-activation are frequently grouped into episodes of rhythmic activity that are linked in a constant phase relation to the rhythmic activity of the hooks and the radula. This transition from the usual
METHODS

Adult specimens of Clione limacina were collected at the Friday Harbor Laboratories, University of Washington (Friday Harbor, WA) and at the White Sea Marine Laboratory of the Zoological Institute (White Sea, Russia). Electrophysiological experiments were performed on reduced preparations consisting of the CNS, head, and wings. The animals were anesthetized in a 1:1 mixture of seawater and isotonic MgCl₂ and dissected in a silicone elastomer (Sylgard)-coated petri dish. All central nerves innervating the head remained intact. In the experiments that required simultaneous recordings from the Cr-B motoneurons and the Cr-Aint interneurons, a special adjustment had to be made in the preparations because these neurons were located on the opposite sides of the cerebral ganglia. To make both types of neurons available for recording, we cut the head of the preparation in half and twisted left or right cerebral ganglion around the cerebral commissure thus exposing ventral surface of one cerebral ganglion and dorsal surface of the contralateral ganglion. The Cr-B motoneurons and the Cr-Aint interneurons are bilaterally symmetrical cells and could be recorded in both left and right cerebral ganglia. Prior to electrophysiological recordings, the sheaths of the central ganglia were softened by bathing the preparation in a 1 mg/ml solution of protease (Sigma, type XIV) for 5 min, followed by 30-min wash. Intracellular recordings from individual neurons were made with glass microelectrodes (resistances: 10–30 MΩ) filled with 3 M potassium acetate. Electrophysiological signals were amplified, displayed, and recorded using conventional electrophysiological techniques (A-M Systems, intracellular amplifiers; Astro-Med and Gould, physiological recorders). Intracellular stimulation was achieved via an amplifier bridge circuit. Neurons were identified based on their position, activity patterns, interactions with other neurons, and behavioral responses of the head during their stimulation. To test for monosynaptic connectivity patterns, interactions with other neurons, and behavioral responses to extracellular stimulation, a high-divalent solution was used (110 mM MgCl₂ and 25 mM CaCl₂, pH = 7.4; prepared on filtered seawater).

In the behavioral experiments, animals were carefully pinned down to a Sylgard-coated petri dish. A Sony video camera was mounted on a dissecting microscope and used for recording feeding movements. Video records from a standard video tape recorder were digitized on the computer using a frame grabber from VideoVision and a Personal AVI Editor program (FlickerFree Multimedia Products). Digitized images were analyzed using video analysis software PhysVis. The anterior tips of hooks or skin folds were monitored, and their movements were plotted as a function of time.

RESULTS

Feeding behavior

Our investigation in intact animals revealed some new details about the operation of feeding structures during different stages of Chione feeding behavior. Opening of the skin folds and a complete protraction of the buccal cones was always observed during the initial periods of the feeding behavior—prey capture and subsequent manipulation of the Limacina shell to position the shell aperture against the mouth of Clione (Fig. 1, A and B). The primary role for the fully protracted buccal cones was to seize the actively swimming prey and hold it in a proper position. Then toothed radula and chitinous hooks became actively involved in the extraction of the Limacina body from its shell and were primarily responsible for holding the prey. During this extraction stage, buccal cones appeared to be not so crucial for holding the prey and became partially retracted, while skin folds were still widely open in all observed animals (n = 19 animals; Fig. 1C).

The video analysis of the feeding behavior in intact animals during this extraction period also revealed that skin folds and retracted buccal cones were rhythmically active. This rhythmic activity was coordinated in a constant phase relation with the rhythmic movements of the hooks, which were visible through the transparent shell (n = 4 animals; Fig. 1C, 2). When hooks grabbed the prey soft tissue and retracted pulling it from the shell, the skin folds were widely open. When hooks released the tissue and protracted to reach deeper inside the shell, skin folds with partially retracted buccal cones closed around the shell helping to hold it in place. In other words, hook protrusion always coincided with skin folds closing, and hook retraction coincided with skin folds opening (Fig. 2). Although radula was not visible in intact animals during their feeding, we know from the previous investigation that there is a strict phase-dependent coordination between rhythmic movements

FIG. 1. Schematic drawings of Clione and its prey Limacina. A: regular background swimming. The buccal cones are retracted and covered by skin folds. B: Clione captures the prey with the fully protracted buccal cones. Skin folds are widely open. C: during the “extraction” stage of feeding, when the radula and hooks become actively involved in the extraction of the prey from its shell, buccal cones become retracted while skin folds remain widely open. Hooks are seen through the transparent shell of the prey.

FIG. 2. Video analysis of the rhythmic movements of the hooks and the oral skin folds during the “extraction” phase of feeding. Note that when the hooks are protracting, the skin folds are closing and when the hooks are retracting, the skin folds are opening.
of the hooks and the radula (Malyshev and Norekian 2002). Thus all three major feeding structures demonstrated rhythmic movements during the extraction period of the feeding behavior, which were highly coordinated in a constant phase relation.

In summary, the buccal cones demonstrated two distinct patterns of activity during the course of the feeding behavior. During the initial feeding response the covering skin folds were widely open and buccal cones fully protracted and elongated. During the extraction stage of the feeding behavior, the buccal cones were partially retracted and together with the widely open skin folds were rhythmically active in coordination with the rhythmic movements of the hooks and the radula. These behavioral observations suggested that a neural network controlling buccal cone movements should also demonstrate two distinct patterns of activity responsible for different types of behavioral responses.

**Two patterns of neural activity**

We have previously described that spike activity in the normally silent Cr-A motoneurons, which control opening of the skin folds and protraction of the buccal cones, corresponded with the inhibition of the functionally antagonistic Cr-B motoneurons, which control buccal cone retraction (Fig. 3A) (Norekian and Satterlie 1993a). This correlation between the Cr-A and Cr-B neurons was observed during their spontaneous activity or during intracellular stimulation of the Cr-A motoneurons, which induced polysynaptic inhibitory inputs to the Cr-B neurons (Norekian and Satterlie 1993a). In this study, we have found that inhibitory inputs to the Cr-B neurons sometimes disappear, leading to the completely different pattern of activity that consists of the lasting periods of co-activation of the Cr-A and Cr-B neurons. Such periods of co-activation were sometimes observed during spontaneous activity of the Cr-A and Cr-B motoneurons when synchronous excitatory inputs from unidentified source appeared in both types of neurons (n = 12 preparations; Fig. 3B). However, spontaneous spike activity in the normally silent and high-threshold Cr-A neurons was a rare and unpredictable event. More reliably, spike activity in the Cr-A neurons was always triggered by direct stimulation of identified excitatory interneurons. We have found in this study that intracellular stimulation of the Cr-BM interneurons (cerebral neurons triggering Buccal Mass rhythm) induced both patterns of the Cr-A and Cr-B neuron activity. The GABAergic Cr-BM interneurons have been previously shown to produce strong excitatory inputs to the radula and hook controlling neural network and to the Cr-A neurons (Norekian and Malyshev 2005). Our investigation demonstrated that the Cr-BM interneurons activated not only Cr-A neurons but also produced excitatory inputs to the Cr-B neurons (n = 25 preparations; Fig. 4, A and B). In most preparations, stimulation of the Cr-BM interneuron induced a prominent activation of the Cr-A neurons as well as initial activation of the Cr-B neurons that was quickly overrun by inhibitory inputs (n = 16 preparations; Fig. 4A). However, in some preparations we also observed lasting periods of co-activation without any trace of inhibitory inputs in the Cr-B neurons (n = 9 preparations; Fig. 4B). The transition from inhibitory pattern to co-activation and then from co-activation again to the inhibitory interaction was observed several times during the same experiment in some of these preparations (n = 6 preparations; Fig. 4C). This dramatic change from the inhibitory pattern of activity to the co-activation of functionally antagonistic motoneurons was somewhat surprising and triggered our further investigation of its possible role in the feeding behavior and mechanisms of the network reconfiguration, which allowed such a transformation. But first we wanted to know more details about the synaptic inputs to the Cr-B motoneurons produced by the Cr-BM interneurons.

**Synaptic connections**

The excitatory inputs from the Cr-BM interneurons to the Cr-B motoneurons were highly efficient. Nevertheless, they were apparently not monosynaptic—some of the induced Cr-BM neuron spikes failed in high-divalent solution to produce excitatory postsynaptic potentials (EPSPs) in these cells (n = 14 in 7 preparations). The Cr-BM interneurons as well as Cr-B neurons are located in the cerebral ganglia in a close proximity to each other (Norekian 1995; Norekian and Satterlie 1993a). However, in the reduced preparations, which had the buccal ganglia missing, the Cr-BM interneurons did not have any effect on the Cr-B neurons, suggesting that a polysynaptic connection between the Cr-BM interneurons and the Cr-B motoneurons occurred via the buccal ganglia (n = 5 preparations). To confirm this suggestion, we performed the following experiments. In the semi-intact preparations, we recorded several prominent excitatory inputs in the Cr-B neurons triggered by induced bursts of spikes in the Cr-BM interneuron and then cut the ipsilateral cerebro-buccal connective with fine scissors. After the cut, all Cr-BM neuron-induced responses in the Cr-B motoneurons completely disappeared confirming the "buccal..."
ganglia” pathway (n = 5 preparations; Fig. 5A). We have previously demonstrated that the Cr-BM interneurons activate Cr-A neurons via the Bc-PIN interneuron (Norekian and Malyshev 2005). The Bc-PIN (buccal Protractor Interneuron) is active during radula protraction phase as a part of the radula rhythm generator and sends an axon to the cerebral ganglia producing activation of the Cr-A neurons (Arshavsky et al. 1993). We have shown that the Cr-BM interneurons monosynaptically activate Bc-PIN interneurons, which in turn monosynaptically activate Cr-A neurons (Norekian and Malyshev 2005). It was logical to suggest that the transition of the excitatory inputs from the Cr-BM interneuron to the Cr-B neurons also occurred via the Bc-PIN interneurons. Simultaneous recordings from the Cr-BM neuron, Bc-PIN neuron and Cr-B neurons revealed that the Cr-PIN neuron was always spiking when the Cr-BM interneuron activated Cr-B neurons (n = 12 in 4 preparations). When the Bc-PIN interneurons were hyperpolarized and thus functionally removed from the network functioning, the Cr-B neuron response to the stimulation of the Cr-BM interneurons completely disappeared (n = 5 in 3 preparations; Fig. 5B). Moreover in all our experiments, the Cr-BM neuron-induced EPSPs in the Cr-B neurons appeared only when corresponding spikes were observed in the Bc-PIN interneurons (n = 8 presentations in 4 preparations; Fig. 5C). This provided strong evidence that Bc-PIN interneuron is indeed the element of the neural network that relays the excitatory inputs from the Cr-BM interneuron to the Cr-B neurons. The excitatory inputs from the Bc-PIN interneuron to the Cr-B neurons were apparently monosynaptic. Each induced Bc-PIN neuron spike produced an individual EPSP in the Cr-B neurons, which persisted without failure in high divalent solution (several presentations in each of the n = 6 preparations; Fig. 6A). The response in the Cr-B motoneurons was biphasic and consisted of the fast EPSPs and slow depolarizing potentials (n = 12 preparations; Fig. 6B). The time to peak for the fast EPSPs was 19.9 ± 1.1 ms (n = 11 from 3 preparations), whereas the time to peak for the slow depolarizing potentials was 1.38 ± 0.09 s (n = 7 from 3 preparations). The summation of slow potentials usually resulted in the lasting depolarization wave.

Mechanisms of co-activation

Based on the results of our investigation, the following synaptic connections have been established in the Clione feeding neural network that controls buccal cone movements (Fig. 7). The Cr-BM interneurons, which are located in the cerebral ganglia, produce monosynaptic excitatory inputs to the Bc-PIN interneurons located in the buccal ganglia. The Bc-PIN interneurons in turn monosynaptically activate both the Cr-A mo-
tuneurons, which control opening of the skin folds and pro-
traction of the buccal cones, and the Cr-B motoneurons, which
control buccal cone retraction. There is one additional impor-
tant element in the network—a bilaterally symmetrical cerebral
Cr-Aint interneuron, which is electrically coupled to the Cr-A
motoneurons and coordinates activity of the contralateral Cr-A
motoneurons (Arshavsky et al. 1993; Norekian and Satterlie
1993a). We have previously shown that these GABAergic
Cr-Aint interneurons monosynaptically inhibit the Cr-B mo-
toneurons, thus being responsible for the inhibitory influence
from the Cr-A motoneurons to the Cr-B motoneurons
(Norekian 1999). Induced burst of spikes in the Cr-Aint inter-
neuron always produced a prominent inhibition of the Cr-B motoneurons as well as activation of the Cr-A motoneurons
(n = 32 preparations; Fig. 8A). The Cr-Aint interneurons also
received monosynaptic excitatory inputs from the Bc-PIN
interneurons (n = 9 preparations; Fig. 8B) (Norekian and Maliyev 2005). This position of the Cr-Aint interneurons in the network suggested that they could be the key elements in the network reconfiguration during its transition from the inhibitory pattern to the co-activation pattern. Normally, the Bc-PIN interneurons activated both the Cr-A motoneurons and the Cr-Aint interneurons (n = 34 in 12 preparations; Fig. 8C). The Cr-Aint interneuron activation in turn resulted in the inhibition of the Cr-B motoneurons. However, in the preparations with the stable co-activation pattern, we have found that although the Cr-Aint interneurons received excitatory inputs from the Bc-PIN interneurons, they did not generate action potentials, while Cr-A motoneurons were sufficiently activated (n = 22 in 9 preparations; Fig. 9A). Because there was no spike activity in the Cr-Aint interneurons, the Cr-B motoneurons received only excitatory inputs from the PIN interneurons and did not receive inhibitory inputs from the Cr-Aint interneurons, thus resulting in co-activation of the Cr-A and Cr-B motoneurons. In a few preparations that showed a stable co-activation pattern and therefore no spike activity in the Cr-Aint interneurons after stimulation of the Bc-PIN interneurons, we depolarized Cr-Aint interneurons during PIN interneuron stimulation (n = 7 in 4 preparations). Such a depolarization allowed Bc-PIN neuron-induced EPSPs in the Cr-Aint interneurons to reach spike threshold and trigger a burst of action potentials (Fig. 9B). Although the Cr-B motoneurons received excitatory inputs from the Bc-PIN interneurons, their activation was terminated by much stronger inhibition from the Bc-PIN neuron-induced activity of the Cr-Aint interneurons (Fig. 9B). Thus the artificial increase in the excitability of the Cr-Aint interneurons resulted in the restoration of the inhibitory pattern of the Cr-A and Cr-B motoneuron activity.

Functional role of co-activation

Stimulation of the Cr-BM or Bc-PIN interneurons frequently
induced not only the initial co-activation of the Cr-A and Cr-B
neurons but also their lasting, in-phase rhythmic activity (n =
with the radula retractor and hook protractor neurons (protractor and hook retractor neurons and in the opposite phase motoneurons were active in the same phase with the radula coincided with the radula protraction. The Cr-A and Cr-B coincided with the radula retraction, whereas hook retraction (Malyshev and Norekian 2002). Hook protraction always co- the radula are highly coordinated in a constant phase relation previously shown that rhythmic movements of the hooks and the radula are strictly correlated with the rhythmic activity of two other feeding neural networks, which control movements of the hooks and the radula (n = 16 in 7 preparations). We have previously shown that rhythmic movements of the hooks and the radula are highly coordinated in a constant phase relation (Malyshev and Norekian 2002). Hook protraction always coincided with the radula retraction, whereas hook retraction coincided with the radula protraction. The Cr-A and Cr-B motoneurons were active in the same phase with the radula protractor and hook retractor neurons and in the opposite phase with the radula retractor and hook protractor neurons (n = 16 preparations; Fig. 11). Whereas the Cr-A motoneurons control opening of the skin folds and protraction of the buccal cones, two buccal motoneurons previously identified as Bc-L neurons (buccal neurons controlling Lips) control closing of the skin folds over the mouth of Clione (Norekian and Malyshev 2005). The Bc-L neurons were found to be also rhythmically active with their rhythm locked in phase with all other rhythmic neural networks of the feeding system. For example, they were always active in the same phase with the radula retractor neurons and in the opposite phase with the radula protractor neurons, such as Bc-PIN interneuron (n = 6 preparations; Fig. 12A). A lasting stimulation of the Cr-BM or Bc-PIN interneurons, which was capable of driving the feeding rhythm in the CNS, induced rhythmic activity in the Bc-L neurons as well as in the Cr-A and Cr-B neurons (n = 6 preparations; Fig. 12B). The Bc-L motoneurons were always active in the opposite phase with the Cr-A and Cr-B motoneurons.

**DISCUSSION**

**Two patterns of activity**

The buccal cones become fully protracted during the initial prey capture stage of the feeding behavior, when they grab and hold the prey, and the following “manipulation” period, when they turn the shell of the prey to bring its aperture against the mouth of Clione (Lalli 1970; Lalli and Gilmer 1989). Then rhythmically active hooks and the radula begin the extraction of the prey from its shell. Clione does not bite small pieces from the prey during feeding but pulls the entire body of the prey from its shell (Lalli 1970; Lalli and Gilmer 1989; Wagner 1885). In a previous study, we described the phase-locked coordination between rhythmic activity of the radula and hooks, which take turns in pulling the prey out of the shell keeping a constant extracting pressure (Malyshev and Norekian 2002). When the radula retracts pulling the prey out of the shell, the hooks become protracted. Then the hooks retract pulling the prey out of the shell, while the radula protracts. We have found in our current investigation that during this extraction period of the feeding behavior, the buccal cones are not fully elongated but partially retracted. Moreover, the skin folds with partially retracted buccal cones become rhythmically active, and their rhythm activity is locked in phase with the rhythmic movements of the radula and hooks. When the hooks grab the prey tissue and retract to pull it inside the buccal cavity, the skin folds are widely open. When the hooks release the prey and protract, the skin folds with the retracted buccal cones close around the Limacina shell to hold it in place. Thus all three feeding structures become rhythmically active in coordinated manner and participate in the extraction of the prey from its shell.

These behavioral observations demonstrated that there are two distinct patterns of the buccal cone activity during feeding behavior—fully elongated buccal cones during initial period of the feeding behavior and partially retracted rhythmically active buccal cones.
buccal cones during the extraction period. On the neuronal level, we have found that the buccal cone controlling neural network is capable of producing two dramatically distinct patterns of activity: inhibitory pattern and co-activation pattern.

A coordinated inhibition of the neural centers underlying incompatible behavioral responses has been described in different animals (Beall et al. 1990; Kovac and Davis 1980; Krasne and Lee 1988; Norekian and Satterlie 1996). Such inhibitory pattern of activity between functionally antagonistic motoneurons was also observed in the Clione feeding neural network (Norekian and Satterlie 1993a). When the Cr-A motoneurons that control opening of the skin folds and buccal cone protraction are active, the Cr-B motoneurons that control buccal cone retraction usually receive strong inhibitory inputs terminating their firing. We have found in this study that

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**FIG. 8.** A: induced burst of spikes in the Cr-A1nt interneuron produced strong inhibitory inputs in the Cr-B1 motoneuron and excitatory inputs in the Cr-A3 motoneuron. B: each induced spike in the Bc-PIN interneuron produced individual EPSP in the Cr-A1nt interneuron and Cr-A11 motoneuron that persisted in high divalent solution. C: induced burst of spikes in the Bc-PIN interneuron produced excitatory inputs in the Cr-A11 motoneuron and Cr-A1nt interneuron. Both cells are equally excited and generate sufficiently powerful bursts of spikes.

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**FIG. 9.** A: in this preparation, which demonstrated a stable co-activation pattern of the Cr-A and Cr-B neuron activity, the induced burst of spikes in the Bc-PIN interneuron activated the Cr-A4 motoneuron but did not trigger action potentials in the Cr-A1nt interneuron. As a result, no inhibitory inputs were observed in the Cr-B1 motoneuron, which responded to the Bc-PIN neuron stimulation only with a strong excitation, thus resulting in co-activation of the Cr-A and Cr-B neurons. B: in the same preparation, the Cr-A1nt interneuron was depolarized, allowing Bc-PIN neuron-induced EPSPs to trigger action potentials in the Cr-A1nt interneuron. As a result, inhibitory inputs from the Cr-A1nt interneuron were produced in the Cr-B1 motoneuron terminating its activation by the Bc-PIN interneuron and resulting in the inhibitory pattern of the Cr-A and Cr-B neuron activity.
inhibitory pattern of activity is not the only motor pattern produced by these functionally antagonistic Cr-A and Cr-B motoneurons. The Cr-A and Cr-B motoneurons also demonstrate episodes of co-activation without any traces of inhibition in the Cr-B neurons.

We believe that during the initial prey capture and manipulation period of the feeding behavior, the Cr-A and Cr-B neural network produces the inhibitory pattern of activity. This activity pattern corresponds with the opening of the skin folds and the full protraction and elongation of the buccal cones facilitated by the inhibition of all retraction responses. The co-activation pattern of the Cr-A and Cr-B motoneurons and the behavioral activity it is expected to produce precisely corresponds to the extraction period of the feeding behavior. The Cr-A neuron activation produces opening of the skin folds, while simultaneous activity of the Cr-B neurons would prevent the hydraulic protraction of the buccal cones and would keep them in the retracted position. The initial Cr-A and Cr-B neuron co-activation is frequently followed by their in-phase rhythmic activity. This rhythmic activity lasts for several seconds and is strictly correlated with the rhythmic activity of two other feeding neural networks controlling movements of the hooks and the radula. This is precisely what we observe in our behavioral studies during the extraction period of the feeding behavior—rhythmic activity in all three feeding structures in a strict phase-dependent relation.

The best way to confirm the behavioral role of a specific motor output is to record it in the semi-intact preparation during a particular behavior. The specific features of the Clione preparation currently prevent us from performing such an

FIG. 10. The induced burst of spikes in the Bc-PIN interneuron triggered co-activation of the Cr-A1 and Cr-B1 neurons. This initial excitatory response then developed into a lasting rhythmic activity. Note that the bursts of activity in these 2 functionally antagonistic neurons always occurred in-phase with each other.

FIG. 11. Rhythmic activity in the Cr-A4, Cr-B1, and Bc-RR (buccal Radula Retractor) neurons. Note that the Cr-A4 and Cr-B1 neurons were active in the same phase, whereas Bc-RR neuron was bursting in the opposite phase. This rhythmic activity was triggered by a strong induced burst of spikes in the Cr-BM interneuron (not shown).

FIG. 12. A: rhythmic activity in the Bc-L motoneuron (skin folds closing) and the Bc-PIN interneuron (radula protraction). The cells were bursting strictly in anti-phase. This rhythmic activity was triggered by a strong induced burst of spikes in the Cr-BM interneuron (not shown). B: stimulation of the Cr-BM interneuron induced rhythmic activity in the otherwise silent Cr-B1 and Bc-L neurons. Note that rhythmic activity of the Cr-B1 neuron is locked with the rhythmic activity of the Bc-L neurons in anti-phase.
experiment. One of the main reasons is the fact that the buccal cone protraction, which is required for successful feeding behavior, is a hydraulic phenomenon (Hermans and Satterlie 1992; Lalli and Gilmer 1989). When we dissect the preparation to gain access to neurons for intracellular recording, we compromise the integrity of the central hemocoelic cavity, and the full protraction of the buccal cones becomes impossible along with the clear succession between feeding stages. However, we believe that the direct connection between the activity of individual motoneurons and the behavioral responses that they produce allow us to safely speculate about the possible behavioral role of different patterns of activity of the Cr-A and Cr-B neural network. In the future, we will investigate ways to induce clear and distinct feeding stages in semi-intact preparations designed for intracellular recording and to establish unequivocally the behavioral roles for different activity patterns in the feeding neural network.

Neural mechanisms

The fact that the same neural circuit is capable of generating multiple motor patterns has been documented in a few other systems. For example, the stomatogastric ganglion of decapod crustaceans produces two motor patterns, the gastric mill rhythm and the pyloric rhythm, which can be separately elicited by different modulatory neurons (Blitz and Nusbaum 1997; Blitz et al. 1999; Dickinson et al. 1990; Harris-Warrick et al. 1992; Marder and Calabrese 1996; Meyrand et al. 1994; Tazaki and Tazaki 2000; Weimann et al. 1991; Wood et al. 2000). The buccal feeding neural network of the gastropod mollusks produces distinct ingestion and rejection motor patterns (Jing and Weiss 2001, 2002; Kupfermann and Weiss 2001; Morton and Chiel 1993). The respiratory neural networks in both mice and locust generate multiple breathing patterns (Lieske et al. 2000; Ramirez 1998). The basic mechanism of the transition between different activity patterns in the neural systems described above is a specific reconfiguration of a neural network due to the changes in the properties of individual neurons and synaptic connections between them.

According to our investigation, transition from the inhibitory pattern to co-activation pattern of activity of the functionally antagonistic Cr-A and Cr-B motoneurons in Clione was controlled by the activity of a single pair of the Cr-Aint interneurons. The Cr-Aint interneurons were instrumental in conveying inhibitory influence from the Cr-A motoneurons to the Cr-B motoneurons. They were electrically coupled to the Cr-A motoneurons and produced monosynaptic inhibitory inputs to the Cr-B motoneurons, thus inhibiting the Cr-B neuron spontaneous spike activity when the Cr-A neurons were active (Norekian 1999). However, the efficacy of electrical coupling between the Cr-A motoneurons and Cr-Aint interneurons was not so high as to produce strong spike synchrony in these neurons. In addition, the Cr-A and the Cr-B motoneurons did not have direct synaptic connections between them (Norekian and Satterlie 1993a). The indirect nature of the inhibition between the Cr-A and Cr-B motoneurons allowed for sufficient flexibility in the network. For example, we have previously described a brief period of activation of both Cr-A and Cr-B neurons followed by a prolonged inhibition of the Cr-B neurons, which was suggested as a mechanism for the high-speed hydraulic protraction of the buccal cones (Norekian and Satterlie 1993b).

Thus when synchronous excitatory inputs arrived to the Cr-A and Cr-B neurons, for example after stimulation of the Bc-PIN interneurons that produced monosynaptic EPSPs in both neuron types, the motor pattern of the network completely depended on the Cr-Aint interneuron activity. When the Cr-Aint interneurons responded with a burst of spikes, their strong inhibitory influence terminated any activity in the Cr-B motoneurons and therefore resulted in the inhibitory pattern of the Cr-A and Cr-B neuron activity. When excitatory inputs did not bring the Cr-Aint interneurons to their spike threshold but activated Cr-A motoneurons, inhibitory inputs from the Cr-Aint interneurons did not materialize resulting in co-activation of the functionally antagonistic Cr-A and Cr-B motoneurons. A somewhat similar mechanism has been proposed for the swim pattern-generating neural network in the mollusk Triton (Getting and Dekin 1985). The dorsal swim interneurons demonstrated reciprocal inhibition in a quiescent preparation but were also known to have a reciprocal excitatory connection during swimming. The authors hypothesized that transition from inhibition to excitation occurred due to the inhibition of some unidentified inhibitory interneuron in the network.

The main goal for our future investigation will be to identify specific modulatory processes that regulate the Cr-Aint interneuron excitability and therefore the resulting motor pattern of the buccal cone controlling neural network. We also plan to investigate the specific sensory pathways to the buccal cone neural network. The synchronous excitatory inputs to the Cr-A and Cr-B neurons were required to produce co-activation pattern. Occasionally, we saw spontaneous excitatory inputs. However, the most reliable source of such inputs for our experimental manipulations was Bc-PIN interneuron. We believe that the Cr-BM and Bc-PIN interneurons provide one (but certainly not the only) pathway for such synchronous inputs during natural feeding behavior. We also suggest that interneuronal pathway for the sensory inputs from the prey could be different during different stages of feeding. For example, synchronous excitatory inputs to the Cr-A and Cr-B neurons occur only during the extraction stage of the feeding behavior when co-activation pattern is presumably required.

GRANTS

This work was supported by National Science Foundation Grant IBN-0235107 and North Atlantic Treaty Organization Collaborative Linkage Grant 979205.

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