Synaptic Modulation Contributes to Firing Pattern Generation in Jaw Motor Neurons During Rejection of Seaweed in Aplysia kurodai

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Nagahama, Tatsumi, Kenji Narusuye, and Hidekazu Arai. Synaptic modulation contributes to firing pattern generation in jaw motor neurons during rejection of seaweed in Aplysia kurodai. J. Neurophysiol. 82: 2579–2589, 1999. Japanese species, Aplysia kurodai, feeds well on Ulva but rejects Gelidium (Geli.) or Pachydictyon (Pach.) with different rhythmic patterned movements of the jaws and radula. During ingestion the jaws open at the radula-protrusion phase and remain half open at the initial phase of the radula-retraction, whereas during rejection the jaws open similarly but start to close immediately after the onset of the radula-retraction. These can be induced not only by the natural seaweed but by the extract solutions. We previously showed that the change of the patterned jaw movements from the ingestion to the rejection may result from the decrease in the delay of the firing onset of the jaw-closing (JC) motor neurons during their depolarization. This diminished delay produces a phase advance relative to the radula-retraction phase. In that study, we showed that during ingestion the buccal multi-action (MA) neurons may generate the delay of firing onset of the JC motor neurons by producing monosynaptic inhibitory postsynaptic potentials (IPSPs) during the initial portion of their depolarization. In the present experiments, the firing patterns in the MA neurons induced by application of the Geli. or Pach. extract to the lips were explored in the semintact preparations. During the Pach. response the duration and the firing frequency of the MA firing at each depolarizing phase were decreased in comparison with the Ulva response. No decreases in the MA firing were observed during the Geli. response, suggesting that the similar patterned jaw movements during rejection of Geli. and Pach. may be generated by different neural mechanisms. Moreover, the size of the MA-induced IPSP in the JC motor neurons was largely decreased by application of the Geli. or Pach. extract to the lips in the reduced preparations consisting of the tentacle-lips and the cerebral-buccal ganglia. Voltage-clamp experiments on the JC motor neurons showed that the size of synaptic current induced by the MA spikes was decreased by application of these solutions to the lips. The decrease was induced when the buccal ganglia were bathed in a solution to block polysynaptic pathways. These results suggest that the advance of the onset of the JC firing at each depolarizing phase during the Geli. or Pach. response may be mainly or partly caused by the decrease in the size of the MA-induced IPSP in the motor neurons. Modulatory action of cerebral neurons or peripheral afferent neurons in the lip region may contribute to this synaptic plasticity.

INTRODUCTION

The food preferences of the animals are very important for sustenance of their life. Behaviors associated with food preference are complex, because they must include the ability of the animal to recognize the odor from a distance or the taste accompanied by touch, decide between liking and aversion often with the help of their memory, and sometimes change the preference by learning the odor or the taste. After this processing, the animals show multiple behaviors such as ingestion or rejection under command systems and often modulatory systems. Therefore the study of the neural mechanism for the food preference can potentially contribute to our understanding of an important general question in neurobiology: how a specific sensory modality can alter behavior by reconfiguring the properties of a well-defined neural network.

It has been well-known that the gastropod mollusks are very useful models for the study of the neuronal basis of such complex behaviors (Kandel 1979). For example, the behavior has been well studied for odor preference (Audesirk 1975; Croll and Chase 1977, 1980; Peschel et al. 1996; Teyke 1995; Teyke et al. 1992; Willows 1978; Yamada et al. 1992) and taste preference (Chang and Gelperin 1980; Culligan and Gelperin 1983; Kupfermann and Carew 1974). The processing neural network has been also well studied for the odor preference (Gelperin and Tank 1990; Gelperin et al. 1993; Schütz and Basar 1994). In contrast, the neuronal basis of taste preference has been little studied. Only the neural pathway for the chemosensory input of the standard food has been studied (Bicker et al. 1982; Delaney and Gelperin 1990; Rosen et al. 1991).

The marine gastropod Aplysia has been reported to show clear food preference (Audesirk 1975; Carefoot 1967, 1970; Kupfermann and Carew 1974). Moreover, the neural mechanisms have been well studied for the food arousal state (Nagahama et al. 1993; Teyke et al. 1990; Weiss et al. 1982), the subsequent consummatory response consisting of rhythmic radula and jaw movements (Church and Lloyd 1994; Cohen et al. 1978; Evans et al. 1996; Nagahama and Takata 1988), and the central pattern generator (CPG) elements contributing to these patterned outputs (Hurwitz and Susswein 1996; Hurwitz et al. 1994, 1996, 1997; Kabotyanski et al. 1998; Kirk 1989; Nagahama and Takata 1989, 1990; Susswein and Byrne 1988). Some command-like neurons have also been found in the cerebral ganglia (Hurwitz et al. 1999; Rosen et al. 1991; Xin et al. 1999). However, little is known about the neural pathways for the chemosensory inputs (Fredman and Jahan-Parwar 1980; Jahan-Parwar 1972). Only the mechanoreceptor pathways for the tactile stimuli of the lip region have been studied (Rosen et al. 1982). The long range of our study is to reveal the neural mechanisms for the generation of the taste preference behavior of Aplysia.

In a preceding report we showed that the species of Aplysia that is indigenous to the coast of Japan, Aplysia kurodai, fed well on Ulva, but rejected a few species of seaweed such as Ulva
Gelidium (Geli.) or Pachydictyon (Pach.) with rhythmic patterned movements of the jaw and the radula (active rejection) (Nagahama and Shin 1998). It was also shown that these responses could be induced not only by the natural seaweed but by extract solutions, suggesting that the ingestion and rejection could be induced by the sense of taste alone. When the patterned jaw movements during the active rejection were compared with those during the ingestion, we found that during the Ulva ingestive response the jaws opened at the radula-protraction phase and remained half open at the initial phase of the radula-retraction, whereas during the Gelidium or Pachydictyon response the jaws similarly opened at the radula-protraction phase but started to close immediately after the onset of the radula-retraction. Moreover, we showed that during the ingestive response the three jaw-closing (JC) motor neurons (JC1–JC3) and one jaw-opening (JO) motor neuron (JO1) in the buccal ganglia underwent synchronous depolarization at the radula-retraction phase, but the JO1 always started firing before the JC motor neurons, suggesting that the JO1 may contribute to the jaw-opening (half open) during the initial part of the radula-retraction (Nagahama and Takata 1988). Recent experiments demonstrated that the firing onset of the JC motor neurons was advanced toward that of the JO1 at each depolarizing phase during the Gelidium or Pachydictyon active rejection responses (Nagahama and Shin 1998).

We previously identified the buccal multiaction (MA) neurons, probably equivalent to B4/B5 in Aplysia californica, which produced monosynaptic inhibitory postsynaptic potentials (IPSPs) in the ipsilateral JC motor neurons, and demonstrated that, during the rhythmic ingestive response, blockage of the spike activity of a MA neuron by passing a large hyperpolarizing current caused the effective advance of the firing onset of the ipsilateral JC motor neurons at each depolarizing phase (Nagahama and Takata 1989, 1990). These results suggest that the MA neurons may contribute to generation of a firing delay of the ipsilateral JC motor neurons at each depolarizing phase during the ingestive response. Therefore in the present experiments the contribution of the MA neurons to the firing pattern generation of the JC motor neurons during the active rejection was explored. Some of the findings in this article have been presented in a preliminary communication (Nagahama 1997).

**METHODS**

**Animals and seaweed**

Aplysia kurodai, weighing 50–450 g, were collected from the seashore of Sumoto and Yura in Awaji Island. Animals were maintained in aquaria filled with aerated and filtered artificial seawater (ASW) at 14°C. Three species of seaweed, Ulva pertusa (Ulva), Pachydictyon corticium (Pach.), and Gelidium amansii (Geli.) were also collected at a location with many animals. These were maintained in aquaria filled with ASW at room temperature or stocked in a freezer at below −20°C. Ulva was used for the animal food and was supplied every day, twice in the morning and evening.

**Recording from reduced preparations**

Two types of preparations, reduced and semi-intact, were used. The animals were dissected under Mg<sup>2+</sup> anesthesia at room temperature. The reduced preparation consisted of the tentacle-lip region, cerebral ganglia, buccal ganglia, and buccal muscles. The tentacle-lip region included the anterior tentacles, lips, perioral zone, and most anterior part of neck and anterior foot. The upper labial nerves, anterior tentacular nerves, lower labial nerves, and cerebral-buccal connectives arising from the cerebral ganglia were left intact, and the other cerebral nerves innervating the cut tissue edges were severed. The buccal mass was removed from the head region and then cut into halves along the midline to separate the paired symmetrical buccal musculature innervated by buccal nerves 2 and 3 (n<sub>2</sub> and n<sub>3</sub>). The peripheral buccal nerves except n<sub>3</sub> and n<sub>4</sub> were severed. A Lacite recording chamber was divided into five compartments (Fig. 1). The tentacle-lip region, cerebral ganglia, buccal ganglia, and paired buccal muscles were separately pinned to the silicone elastomer (Sylgard; Dow) surface of each compartment of the recording chamber, and petroleum jelly (Vaseline) was placed on the partitions between the compartments. The artery supplying the tentacle-lip region was perfused with fresh ASW. To avoid cutting the fine branches of the blood vessels, we initially visualized the arteries by perfusing a major branch of the aorta with ASW containing Fast Green dye (~5%). Then a branch of a peripheral artery directed toward the tentacle-lip region was cannulated with polyethylene tubing (0.5–1.0 mm diam). The seaweed extract stimuli were applied to the lip region with a Pasteur pipette.

**Recording from semi-intact preparations**

The semi-intact preparation consisted of the intact head and neck of the animal, in which the cerebral ganglia, pleural-pedal ganglia, buccal ganglia, buccal mass, pharynx, and esophagus were left intact as described previously (Nagahama and Takata 1988). The peripheral nerves of the pleural-pedal ganglia and the branches of the cerebral nerves innervating the cut tissue edges were severed. To avoid longitudinal movements of the whole buccal mass, the distal ends of the extrinsic muscles were usually cut free. The preparation was transferred into a 300-ml recording chamber. Intracellular recordings from the buccal neurons were made with the buccal ganglia supported from beneath by a wax-covered metal table inserted into the esophagus. Ganglia were secured to the table by micropins. Stimuli were presented by perfusion of test solutions via the open end of silicone tubing held close to the lips. In this procedure the stimulating flow was applied to the lips and partially to the buccal cavity.

All experiments were carried out at a room temperature between 20 and 22°C.

**FIG. 1.** Schematic drawing of the arrangement of the reduced preparation in the recording chamber. The recording chamber was divided into 5 compartments that permitted isolation of the tentacle-lip region, cerebral ganglia, buccal ganglia, and a paired buccal muscles, and petroleum jelly (Vaseline) was placed on the partitions between the compartments. The artery supplying the tentacle-lip region was perfused with fresh artificial seawater (ASW).
Electrophysiology

Intracellular recordings from individual neurons were performed using conventional electrophysiological techniques. Neurons were impaled with low-resistance electrodes filled with 2 M potassium acetate (5-7 MΩ). Extracellular muscle and nerve activities were recorded with flexible polyethylene suction electrodes (tip diameter 200–400 μm). Similar suction electrodes were used for electrical stimulation of nerve trunks. The JC motor neuron was also voltage clamped by a two-microelectrode method. Two electrodes were filled with 2 M potassium acetate (voltage recording electrode, 5–7 MΩ; current-passing electrode, 2–3 MΩ). Membrane currents were read as the voltage drop across a resister (1 MΩ) interposed in the feedback loop (chassis ground mode). In these experiments the reversal potential of the inhibitory postsynaptic currents (IPSCs) or the IPSPs produced in the JC motor neurons maintained a stable level throughout the experiment.

Bath solutions

The composition of ASW used in the present experiments was as follows (in mM): 470 NaCl, 11 KCl, 11 CaCl₂, 25 MgCl₂, 25 MgSO₄, and 10 Tris-HCl (pH 7.8–7.9). In the reduced preparation, Mg²⁺ solution, Mg²⁺ concentration was raised threefold by replacement of Na⁺ to maintain osmotic balance.

Preparation of seaweed extract solution

Seaweed extract solutions were prepared fresh for each experiment. A 20-g sample of fresh seaweed was ground in a mortar and soaked for 30 min in 30–40 ml of ASW at room temperature. The mixture was centrifuged (12,000 g, 20 min) and the supernatant was used for stimulation.

RESULTS

Firing patterns in MA neurons during the response induced by seaweed extract

The MA neurons were previously identified in each of the paired buccal ganglia, and it was demonstrated that during the ingestive response induced by the Ulva extract these neurons may generate the delay of firing of the JC motor neurons at each depolarizing phase by producing monosynaptic IPSPs in the motor neurons (Nagahama and Takata 1989, 1990). Therefore in the present experiments the firing patterns in the MA neurons during the response induced by the Geli. or Pach. extract were examined by the use of semi-intact preparations to determine how the MA neurons contribute to the generation of the firing patterns in the JC motor neurons during the active rejection of the seaweed.

On application of seaweed extract to the lip region, rhythmic patterned activity was induced in the JC and the MA neurons. Simultaneous intracellular recordings of the rhythmic burst activity of these neurons during the ingestive response induced by the Ulva extract are shown in Figs. 2A and 3A. At each cycle the depolarization of the MA neurons always preceded that of the JC motor neurons. Then the MA neurons always started firing earlier than the JC motor neurons, and the JC motor neurons started firing accompanying the decrease in the firing frequency of the MA neurons. We previously demonstrated that when the spike activity of the MA neurons was suppressed by passing a large hyperpolarizing current during the rhythmic ingestive response, both MA and JC neurons were almost simultaneously depolarized (Nagahama and Takata 1990). Therefore the JC motor neurons may be additionally hyperpolarized by the firing of the MA neurons.

The rhythmic patterned activity of these neurons induced by the Geli. or Pach. extract is also shown in Figs. 2B and 3B, respectively. In both cases, the onset of the burst of spikes in the JC motor neurons advanced at each depolarizing phase, and the duration of the bursts of the JC spikes tended to elongate in comparison with the patterned activity of the same neuron during the Ulva response. These results were consistent with our previous report (Nagahama and Shin 1998). In that study, we showed that the normalized delay (Dn) obtained by dividing the delay time by the length of the JC depolarization was decreased on the average during the Geli. or Pach. response in comparison with the Ulva response. On the other hand, the MA neurons showed distinctive firing patterns during the Geli. and Pach. responses. During the Geli. response, the patterned activity of the MA firing scarcely changed in comparison with the Ulva response (Fig. 2B, n = 11). In contrast, during the Pach. response the duration of the burst of spikes in the MA neurons shortened (Fig. 3B1), or sometimes the MA neurons generated only a few spikes (Fig. 3B2) at each depolarizing phase in comparison with the Ulva response in the same neuron (n = 12). These results suggest that, although the firing patterns in the JC motor neurons during the Geli. and Pach. responses appear similar, they may be generated by distinct neural mechanisms, depending on the different tastes of the seaweeds.

Spike frequency of the MA bursts during the responses induced by seaweed extract

To compare the firing patterns in the MA neurons during the Ulva, the Pach., and the Geli. responses quantitatively,
the spike frequency of the MA burst at each depolarizing phase was measured every 200 ms for 7–10 consecutive cycles during stable rhythmic responses. Figure 4 shows the comparison of the time course of the mean spike frequency during the Ulva and Gelidium responses, or the Ulva and Pachydictyon responses in the same preparation. No differences in the MA spike frequency between the Ulva and Gelidium responses were detected (n = 11). In contrast, the burst duration of the MA neuron became shorter during the rhythmic bursts induced by the Pachydictyon application in comparison with the Ulva application (Fig. 4B, circles; n = 12). The maximum spike frequencies observed with these two stimuli were nearly identical. In those tests that elicited only a few spikes at each depolarizing phase of the MA neurons, the initial slope of the spike frequency within each train appeared to be similar to that of the Ulva responses (Fig. 4B, triangles). These results suggest that the MA neurons show different firing patterns during the Pachydictyon response in comparison with the Ulva response, and the pattern change in the MA neurons may not come from the decrease in the size of depolarization of the MA neurons but from the shortening of the depolarization. These results also suggest that the shortening of the burst duration of the MA neurons may contribute to the advance of the onset time of the JC firing at each depolarizing phase during the active rejection of Pachydictyon in comparison with the ingestion of Ulva, but that other mechanisms are likely to be involved in the rejection of Gelidium.

Modulation of MA-induced IPSPs in JC motor neurons during the Gelidium response

During the Gelidium response we observed no differences in the duration of the burst of spikes or the spike frequency of the MA neurons at each depolarizing phase in comparison with the Ulva response. Therefore other possible factors that would advance the onset of the JC firing at each depolarizing phase were explored. One possible factor may be a change in the size of the IPSPs produced by the MA spikes in the JC motor neurons. In such a case, we would expect the transient depression of the synaptic efficacy by some modulatory neurons without a change of the rhythmic activity. Application of the seaweed extract to the lip region in the semi-intact preparations usually produced the active rhythmic response of the membrane potential in both the JC and depolarizing phase (B1) or sometimes the MA neuron generated only a few spikes (B2) at each depolarizing phase in the same preparation.
the MA neurons, and it was very difficult to make an experiment for the synaptic efficacy. In these experiments therefore we used the reduced preparations in which the rhythmic response was not usually induced by application of the seaweed extract, but the synaptic modulation could be permitted. As a result, it was found that the size of the IPSPs in the JC motor neurons induced by the MA spikes was decreased after application of the Geli. extract to the lip region. Moreover, the decrease in the IPSP size occurred even when the external solution of the buccal ganglia was replaced by Mg\(^{2+}\), Ca\(^{2+}\)-rich ASW to suppress polysynaptic activity within the buccal ganglia.

In a single preparation we could repeat application of the seaweed extract after washing with ASW about four to five times, and usually obtained at least two series of the Ulva responses and two series of the Geli. (or Pach.) response. Figure 5 shows a typical result for simultaneous recordings of the current-induced spikes in the MA neurons and the IPSPs produced in the ipsilateral JC motor neurons before and after application of the Ulva and the Geli. extract to the lip region with a Pasteur pipette in the same preparation (n = 13). The peak size of these IPSPs and the difference of the membrane potential from the resting potential in the JC motor neurons (Δ\(V_m\)) were measured every 10 s, and the time course of these values are shown in Fig. 6A (circles). The test immediately before the application of the seaweed extract was assigned as time 0, and the peak size of the IPSP and the Δ\(V_m\) of that test were plotted as 100% and 0 mV, respectively. Even in the absence of stimulation (control), the IPSP size tended to fluctuate and gradually decrease on the average (squares). After application of Ulva or Geli. extract, the relative size of the IPSPs decreased on the average, but the time course of the data observed after the Ulva application was very similar to that observed in control, suggesting that the gradual decrease may not be specifically related to the Ulva stimulus. In contrast, the extent of the decrease was much larger in the Geli. response than the Ulva response. The minimum level of the IPSP size was attained at ~110 s after the Geli. application in this case. The time course of the IPSP size after the seaweed extract application tended to vary in every trial. The triangles in Fig. 6A show the data obtained from the other trials of the Ulva and Geli. application in the same preparation. The relative size of the IPSPs also largely decreased after application of the Geli. extract, but the minimum level was attained at ~80 s after application. This tendency made us fall into trouble in estimation of the averaged time course. As we previously showed in the semi-intact preparations (Nagahama and Takata 1988), application of Ulva extract initially hyperpolarized and then depolarized the JC motor neurons. On the other hand, application of the Geli. extract initially tended to depolarize and then temporally hyperpolarized them. Although the membrane hyperpolarization would be expected to reduce the IPSP size, the large and long-lasting decrease in the IPSP size after application of the Geli. extract occurred after the return of \(V_m\) to basal levels, and is therefore not likely to be caused by the membrane potential change of the JC motor neurons. It is noted that there were differences in the duration and the amplitude of the synaptic potentials induced in the JC motor neurons after the Geli. application between two series of experiments. These factors may partly cause the different time course of the IPSP size.

The amplitude and duration of the MA spikes before and after application of the Geli. extract were also compared. The spike amplitude was obtained by measuring the voltage difference between the abrupt rising point and the peak of the action potential. The spike duration was measured as the width at the midpoint of the rising phase. The results for the relative values for these two parameters are shown in Fig. 6B. In both cases the values at time 0 were assigned to 100%. These values changed very little after the Geli. application, suggesting that the modulation of the IPSPs in the JC motor neurons during the Geli. response do not reflect changes in the spike shape of the MA neurons.

These results suggest that during the Geli. response the decrease in the IPSP size may be induced, and it may contribute to the advance of the onset of the JC firing at each depolarizing phase.
Modulation of MA-induced IPSPs in JC motor neurons during the Pach. response

During the rhythmic Pach. response, the firing patterns in the MA neurons were more brief than those observed during the Ulva response, a factor that may contribute to the advance of the onset of the JC firing at each depolarizing phase. To determine whether a change in the synaptic efficacy further contributes to the advance of the onset of the JC firing, the size of the MA-induced IPSPs in the JC motor neurons was also compared before and after application of the Pach. extract. A typical result for simultaneous recordings of the current-induced spikes in the MA neurons and the IPSPs produced in the ipsilateral JC motor neurons before and after application of the Ulva and Pach. extract to the lip region in the same preparation (n = 11) is shown in Fig. 7. The time course of the relative IPSP size and the $\Delta V_{\text{m}}$ of the JC motor neuron for two trials of each test seaweed is also shown in Fig. 8A. The IPSP size tended to fluctuate and gradually decrease on the average even in the absence of stimulation (squares). After application of Ulva extract, the relative size of the IPSPs gradually decreased in a similar manner to the control, although the sizes were slightly smaller in the beginning of the Ulva response than the control. In contrast, application of Pach. extract largely decreased the size of the IPSPs. The time course of the IPSP size after the stimuli tended to vary in every trial, and the minimum level of the IPSP size was attained at 30–50 s after the Pach. application. In the experiments showing, the JC motor neuron was hyperpolarized after the Pach. application in contrast with the transient depolarization observed after the Gelidium application (cf. Fig. 6A). The Pach. application did not induce the hyperpolarization in all preparations and sometimes induced the depolarization. The time course of the relative amplitude and duration of the MA spikes before and after application of the Pach. extract in the same experiments is also shown in Fig. 8B. These parameters scarcely changed after Pach. application, suggesting that the modulation of the IPSPs in the JC motor neurons during the Pach. response may not reflect changes in the form of the MA neuron action potential. It is noted that the constant magnitude of the depolarizing DC current to elicit the MA spike in each trial often failed to fire the MA neuron just after application of the Pach. extract.

Modulation of MA-induced IPSCs in JC motor neurons during the responses induced by seaweed extract

To remove the effects of the membrane potential changes during application of seaweed extract (Figs. 6A and 8A), the JC motor neurons were voltage-clamped in a series of experiments. The size of the IPSCs induced by the MA spikes was compared before and after application of the seaweed extract. In these experiments the buccal ganglia were bathed in Mg$^{2+}$, Ca$^{2+}$-rich ASW to suppress the polysynaptic activity within the buccal ganglia. The membrane potential of the JC motor neurons was clamped at −40 mV because the inhibitory effects of the MA neurons on the JC motor neurons during the rhythmic response usually appear near the firing threshold of the JC motor neurons.
Figures 9 and 10 show typical results for the effects of application of the seaweed extract to the lip region on the MA-induced IPSCs in the JC motor neurons (Ulva and Gel.), n = 11; Ulva and Pach., n = 10). The magnitude of the IPSCs evoked every 10 s was explored, and the results for two trials of each test seaweed in the same preparation are shown in Fig. 11. Both the Gel. and Pach. application produced long-lasting decreases in the IPSC amplitude in comparison with the control or the Ulva application. As contrasted with the change in the IPSC size, the IPSC size rapidly decreased after the Gel. application and attained <75% at 10 s after application, suggesting that events related to the depolarization of the JC motor neurons immediately following just after Gel. application (Fig. 6A) may contribute to the slow decrease in the IPSP size. On the other hand, after the Pach. application the IPSC size slowly decreased.

During the voltage-clamp experiments the size of the IPSCs was observed to fluctuate with time in a similar manner as the IPSP size (Figs. 6, 8, and 11), but the time course of these values was almost similar in all experiments. Therefore the mean value of the relative IPSC size at each measuring time for two series of experiments in each preparation was averaged over all preparations (Fig. 12). In the case of Ulva, the IPSC size gradually decreased, but the change in the IPSC size induced by the application was scarcely detectable in comparison with the control data. In contrast, application of the Gel. or the Pach. extract rapidly decreased the IPSC size. The averaged values at 30 s after application were 75.3 ± 4.6% for Gel. (mean ± SE, n = 11) and 79.7 ± 2.3% for Pach. (n = 10), and the difference from the value for the control (96.5 ± 4.5%, n = 15) or the Ulva (97.2 ± 4.2%, n = 15) was significant (P < 0.001, 2 sample t-test). These values for the Gel. or the Pach. application approached those for the control later, suggesting that the synaptic modulation induced by the Gel. and the Pach. application may be temporary. It is also noted that the effect induced by the Gel. application lasted longer than that induced by the Pach. application.

Discussion

The purpose of the present study is to show how the CPG network, which generates the firing patterns in the JC motor neurons, is modulated by the specific types of sensory inputs. The cellular mechanism for the modulation of neural circuits has been well studied in vertebrate and invertebrate CPG networks (Grillner et al. 1995; Marder and Calabrese 1996; Pearson 1993). Reconfiguration of the neural circuits is often due to the action of chemical modulators that modify the synaptic efficacy or the properties of individual neurons. In the detailed studies of the crustacean stomatogastric ganglion...
(STG), some biogenic amines, neuropeptides, or interneurons containing these substances have been found to drive specific patterns of STG programs (Bartos and Nusbaum 1997; Harris-Warrick and Marder 1991; Selverston 1995; Simmers et al. 1995). Our present results may propose the existence of some interneurons that modulate the buccal CPG network in *Aplysia kurodai*.

In the present experiments the firing patterns in the MA neurons during the *Geli.* or *Pach.* response were explored in the semi-intact preparations to know the contribution of the MA neurons to the generation of the firing pattern in the JC motor neurons during the active rejection of the seaweed. When we compared these responses with the food-induced (*Ulva*) response, it was found that during the *Pach.* response the MA burst duration became shorter, or sometimes only a few spikes were generated at each depolarizing phase, whereas no change in the MA neuron firing pattern occurred during the *Geli.* response. We also found that the IPSP (or IPSC) size induced in the JC motor neurons by the MA spikes was decreased after application of the *Geli.* extract in the reduced preparations. A similar decrease in the IPSP size was observed after the *Pach.* application. Two factors, the decrease in the MA spike activity and the decrease in the IPSP size may contribute to the advance of the onset of the JC firing at each depolarizing phase during the active rejection in comparison with the ingestion. Our present results suggest that the active rejection of two species of seaweed, *Geli.* and *Pach.*, may be generated by different neural mechanisms, although the final behavioral outputs are very similar. As demonstrated previously (Nagahama and Shin 1998), these responses could be induced by the seaweed extracts. Therefore the most likely factors to induce these two types of the neural responses may be specific types of chemosensory inputs or taste. The observation that the *Pach.* application induced both types of neural function may be the result of stimuli of the taste mixture of the crude extract. The further study for the origin of the chemosensory inputs of two species of seaweed will be required.

In the present experiments we used the reduced preparation in which the upper labial nerves, anterior tentacular nerves, and lower labial nerves connecting the tentacle-lip region were left intact while the peripheral buccal nerves except n2 and n3 were severed. This preparation could permit the application of the seaweed extract to the tentacle-lip region to induce the synaptic modulation depending on the species of seaweed. We observed that the size of the MA-induced IPSP in the JC motor neurons was decreased by the *Geli.* or *Pach.* application to the lip region, whereas no specific decreases occurred after the *Ulva* application. These results suggest that the different taste of the seaweeds *Ulva*, *Geli.*, and *Pach.* may be recognized in the peripheral lip region or the cerebral ganglia, and that a decision between ingestion and rejection of the seaweed may be induced within this system. Thus this system may be very useful for the study of the generation of taste preference behavior. In contrast, Morton and Chiel (1993) reported in *Aplysia californica* that the animals rejected polyethylene tubing pushed into the

**FIG. 10.** Modulation of the MA-induced IPSCs in the JC motor neuron by application of the *Pachydictyon* extract to the lips in a reduced preparation. A: *Ulva* application. B: *Pachydictyon* application. The measuring conditions were the same as shown in Fig. 9.

**FIG. 11.** Time course of the size of the MA-induced IPSC in the JC motor neurons before and after application of the seaweed extract in the reduced preparations. The peak size of the IPSC was measured every 10 s for 2 trials of each test seaweed in the same preparations as shown in Figs. 9 and 10. The time just before application of the seaweed extract was assigned as 0, and the peak size of the IPSC at that time was chosen as 100%. A: *Ulva* (filled symbols) and *Gelidium* (open symbols, *Geli.*) application. B: *Ulva* (filled symbols) and *Pachydictyon* (open symbols, *Pach.*) application. Small open squares show the data for the absence of stimulation (Control).
many CBI neurons that send their axons directly to the CBC have been identified, and some of them are considered to be command-like neurons or modulatory neurons for the consummatory feeding motor program (Hurwitz et al. 1999; Rosen et al. 1991; Xin et al. 1999). Therefore the modulatory neurons presumed in the present experiments may be one or some neurons equivalent to the CBIs in *Aplysia californica*. On the other hand, direct pathways from the peripheral lip region to the buccal ganglia have been recently reported (Xin et al. 1995). Fredman and Jahan-Parwar (1980) also showed that the first-order synapses may be located in the central ganglia in the chemosensory and mechanosensory pathways from the anterior tentacles in *Aplysia californica*. In the other case therefore the MA-JC synapse may be directly modulated by the peripheral chemosensory cells in the lip region.

To remove the effects of the membrane potential change on the IPSP size, the JC motor neurons were also voltage-clamped and the IPSC size was compared before and after application of the Geli. or Pach. extract. We found that the IPSC size decreased with a similar time course in all experiments after application of each extract. When we explored the time course of the IPSC size averaged over all preparations, the decrease induced by the Geli. and the Pach. application appears to be temporary, and the effects by the Geli. application were larger and lasted longer than those by the Pach. application. The decrease in the IPSC size will reflect the real reduction in the IPSP sizes during the Geli. or Pach. response. For the Geli. application, the reduction in the IPSC size was <40% even in the maximum case (<25% on the average), and it appears that the extent is too small to induce the complete advance of the firing onset of the JC motor neurons at each depolarizing phase during the active rejection in comparison with the ingestion. There may be additional factors such as an increase in some excitatory inputs to the JC motor neurons at each depolarizing phase. The further study for the firing pattern generation in the JC motor neurons during the Geli. response will be required. On the other hand, during the Pach. response the decrease in the MA spike activity was induced in addition to the decrease in the IPSP size. We previously demonstrated in the semi-intact preparations that during the digestive response the blockade of the spike activity of a MA neuron by passing a large hyperpolarizing current effectively advanced the firing onset of the ipsilateral JC motor neurons at each depolarizing phase (Nagahama and Takata 1990). Therefore cooperation of these two factors may be sufficient to advance the firing onset of the JC motor neurons at each depolarizing phase during the Pach. rejection in comparison with the ingestion. In addition, it was also found in the voltage-clamp experiments that the size of the IPSC still tended to fluctuate with time. We previously defined the Dn values as the delay time of the JC firing normalized with the length of each depolarization and showed that these values were distributed but became smaller on the average during the Geli. or Pach. response in comparison with the Ulva response (Nagahama and Shin 1998). Specifically, our previous data showed the frequent overlap of the Dn value during the Geli. or Pach. response with that during the Ulva response. The overlap may be partly caused by the fluctuation of the IPSC size with time observed in the present experiments.

The synaptic modulation found in the present experiments may be caused by some neurons releasing modulatory transmitters from the axon terminals neighboring the MA-JC syn-
aptic regions. In *Aplysia californica* this type of heterosynaptic modulation has been well studied in the CNS and the neuromuscular synapses. Among them one type concerns learning, such as the sensitization of the gill withdrawal reflex (Castel-lucci and Kandel 1976; Cohen et al. 1997; Hawkins and Schacher 1989; Mackey et al. 1987; Stopfer and Carew 1996), and the other type concerns the modulatory control of the muscle movements at the neuromuscular synapses (Fox and Lloyd 1998; Nagahama et al. 1994; Weiss et al. 1978). In some cases, the modulatory neurons have been reported to act on presynaptic terminals and control transmitter release by affecting the duration of the presynaptic neuron spikes (Klein and Kandel 1980; Klein et al. 1982). Moreover, it has been reported that the modulatory chemicals such as serotonin and some neuropeptides can produce substantial changes in the spike duration of specific neurons (Rosen et al. 1989). Therefore we explored the effects of the *Geli* and *Pach*. application on the amplitude and duration of the MA spikes in the present experiments. It was found that these measures were not affected by application of the seaweed extract, suggesting that the possible synaptic modulation may be caused by postsynaptic mechanism. In the crustacean STG, exogenous application of dopamine can change the pyloric motor pattern (Anderson and Barker 1981; Eisen and Marder 1984; Flamm and Harris-Warrick 1986). It has been recently reported that dopamine causes a reduction in the IPSP size produced by the LP neuron in the PD neuron within the pyloric network, and the change may be caused by the postsynaptic mechanism (Ayali et al. 1998). A similar mechanism may also contribute to the reduction in the IPSP size in our system. On the other hand, immediately following the *Pach*. application the constant magnitude of the depolarizing DC current to elicit the MA spike often failed to fire the MA neuron. Such effects may reflect a change in the membrane conductance of the MA neurons by some synaptic inputs. The further study for the modulatory mechanism of a reduction in the MA-induced IPSP size will be required.

Our previous study showed that application of the *Pach*. extract often induced the advance of the onset of the JC firing at each depolarizing phase without elongation of the burst duration of the JC spikes (Nagahama and Shin 1998). Only a reduction in the inhibitory effects of the MA neuron on the JC firing will not be able to cause such a firing pattern change in the JC motor neurons. The *Pach*. application may also induce the shortening of the burst duration of the JC motor neurons in addition to that of the MA neurons by modulating the buccal CPG network. In *Aplysia californica*, it has been recently reported that the exogenous application of a neuropeptide, myomodulin or the firing activity in the CBI-8/9 containing this peptide modulates the feeding motor program, in which the duration of the radula retraction phase is reduced (Perrins and Weiss 1997; Xin et al. 1999). In our systems of *Aplysia kurodai*, some modulatory interneurons containing such a peptide may contribute to the change in the feeding motor program during the *Pach*. response.

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