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# Modulatory Synaptic Actions of an Identified Histaminergic Neuron on the Serotonergic Metacerebral Cell of *Aplysia*

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**Possible sources of excitatory synaptic input to the serotonergic metacerebral cell (MCC) were determined by stimulating various neurons in the cerebral ganglion. Firing of the previously identified histaminergic neuron C2 was found to produce synaptic input to the MCC. The synaptic input consists of fast excitatory-inhibitory synaptic potentials on a background of a slow EPSP. The slow EPSP appears to be monosynaptic and chemically mediated since (1) it persists in a solution of high divalent cations; (2) broadening of the presynaptic spike enhances the EPSP; (3) the size of the EPSP is a function of the $Mg^{2+}$ and $Ca^{2+}$ concentrations of the bathing solution; and (4) the EPSP can be mimicked by application of histamine to the MCC. The slow EPSP, in addition to firing the MCC, can increase the excitability of the cell, even under conditions in which C2 is fired at a rate too slow to produce a measurable EPSP when the MCC is at rest potential. This property appears to be due to the fact that the slow EPSP results from an apparent decrease of membrane conductance so that the size of the EPSP increases markedly as the cell is depolarized, and the EPSP appears to be highly voltage-dependent so that it is small or absent close to the rest potential of the MCC. When the MCC is voltage-clamped, application of histamine to the bath results in an inward current that disappears when the MCC is hyperpolarized. The potential at which the histamine-induced current reverses or disappears is dependent on the concentration of external potassium, suggesting that, at least in part, the slow EPSP is due to a decrease of potassium conductance. The data on C2 are consistent with its being an element of the neuronal system that mediates a state of food arousal in *Aplysia*.**

In the study of the neural basis of behavior, a major question of theoretical as well as clinical interest is, What are the mechanisms that modulate overall behavioral responsiveness? What factors determine global modulatory phenomena such as sleep–wakefulness, arousal–dearousal and alteration of mood? Feeding behavior in *Aplysia* provides a convenient and tractable model system for the study of a variety of modulatory variables. One such variable we have studied is food arousal, a behavioral state that is elicited by briefly exposing animals to food stimuli (Weiss and Kupfermann, 1977). The food arousal state in *Aplysia* is reflected by specific alterations of feeding behavior (Kupfermann, 1974; Susswein et al., 1978), as well as by generalized changes in behavior such as locomotion (Kupfermann, 1974) and cardiovascular responses (Dieringer et al., 1978; Koch et al., 1984). Several serotonergic neurons have been shown to mediate different behavioral manifestations of the food arousal state. Considerable evidence (Kupfermann and Weiss, 1982; Rosen et al., 1983; Weiss et al., 1975, 1978a, 1979) indicates that alterations of both the speed and intensity of biting are mediated by the metacerebral cell (MCC; also sometimes referred to as C1 or GCN), a giant serotonergic neuron (Eisenstadt et al., 1973; Gerschenfeld and Paupardin-Tritsch, 1974; Gerschenfeld et al., 1978; Weinreich et al., 1973) located in the cerebral ganglion. The metacerebral cell is normally inactive, but is prompted into activity by food stimuli (Kupfermann and Weiss, 1982; Weiss et al., 1986a). Tactile stimuli and other sensory inputs that elicit the food arousal state also can excite the MCC. We have hypothesized (Kupfermann and Weiss, 1981; Weiss et al., 1982) that food and other stimuli may activate a "central arousal system" consisting of a group of interrelated neurons that provide the excitatory drive to various subsets of neurons that then execute the appropriate effects of the arousal state on different classes of behavior. The MCC specifically mediates arousal effects on the buccal musculature (Weiss et al., 1975, 1978a). Three observations support the hypothesis of a central arousal system: (1) Food arousal is associated with a set of coordinated changes in multiple effector systems; (2) spike activity of the MCC outlasts the initiating stimulus; and (3) spike activity can be reinitiated much more readily for a long period after the cell is first excited by a brief arousing stimulus.

In order to explore a possible central arousal system we have attempted to define neurons that excite the MCC. In a previous study, Rosen et al. (1982) identified a population of mechanosensory neurons that provide one source of excitatory input to the MCC. This input, however, is weak, rapidly decrements, and does not outlast the food stimulus. We therefore sought other neurons that provide excitatory drive to the MCC. In this paper we report that the previously identified histaminergic neuron, C2 (McCaman and McKenna, 1978; McCaman and Weinreich, 1982, 1985; Weinreich 1977, 1978; Weinreich and Yu, 1977; Weinreich et al., 1975), produces a relatively large, nondecrementing excitatory synaptic input to the MCC. In accompanying papers (Chiel et al., 1986; Weiss et al., 1986a, b), we describe evidence that the histamine cell is a proprioceptive afferent neuron that is active during feeding behavior and that, in addition to its excitatory effect on the MCC, it has multiple actions on neurons involved in the coordination and patterning of feeding behavior. We conclude that C2 has some of the features expected of an element of a central arousal system, but other features indicate that it is a unique modulatory sensory neuron that does not readily fit into existing functional categories.

Some of the results presented in this paper have appeared in a preliminary communication (Weiss et al., 1978b).

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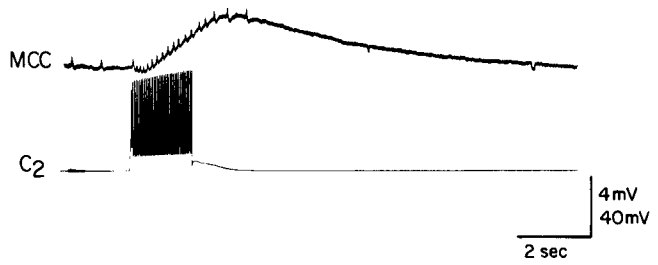


*Figure 1.* Response of the MCC to activation of the histaminergic neuron C2. Activation of cell C2 produces a series of fast potentials (excitatory–inhibitory) and a slow depolarization of long duration.

## Materials and Methods

Experiments were performed on *Aplysia californica* weighing 100–250 gm, supplied by Pacific Bio-Marine Co. (Venice, CA). They were maintained in 500 liter tanks containing aerated artificial seawater (ASW; Instant Ocean) at 15°C. For dissection, the animals were anesthetized by the injection of isotonic magnesium chloride (25% vol/wt). Isolated cerebral ganglia were then pinned, dorsal side up, to the Sylgard base of a 5 ml chamber. Connective tissue was dissected, while the ganglia were bathed in a solution of ASW containing 4× the normal content of $Mg^{2+}$ and 0.5× the normal content of $Ca^{2+}$. In order to reduce possible contractions of the ganglionic sheath when transmitters were to be bath-applied, the ganglion was immersed for 45 sec, prior to desheathing, in a solution of ASW containing 0.5% glutaraldehyde. Experiments were performed at room temperature.

For intracellular stimulation and recording, neurons were impaled with double-barreled microelectrodes filled with 2 M potassium citrate and beveled with 0.05 μm grit (K. T. Brown type beveler). In experiments requiring accurate measurements of membrane potential, in addition to being impaled with a double-barreled electrode, the cells were impaled with a single-barrel electrode that was used to pass current. All preamplifiers were equipped with bridge circuits to nullify coupling potentials. The signals were led from the preamplifiers to an oscilloscope, an FM tape recorder, and a Gould pen-writer from which the hard copy of the data was obtained.

In voltage-clamp experiments, the voltage and current electrodes were filled, respectively, with 2 M potassium chloride and 2 M potassium acetate. The resistance of these electrodes varied from 2 to 4 MΩ. In order to reduce artifacts and oscillations, electrodes were shielded with aluminum foil wrapped around parafilm, which covered the electrodes. A Dagan voltage-clamp model 2500 preamplifier was used in these experiments. Unless otherwise noted, all experiments were repeated at least 3×.

## Results

*Histaminergic neuron C2 excites the metacerebral cell*

Exploration of the cerebral ganglion revealed that the left and right E clusters (Jahan-Parwar and Fredman, 1976) each contain a characteristic neuron that, when fired by intracellular stimulation, consistently produces excitatory synaptic input to the MCC. The position, appearance, and size of this neuron suggested (Weiss et al., 1978b) that it was the previously identified histaminergic neuron, C2 (McCaman and McKenna, 1978; McCaman and Weinreich, 1982; Weinreich, 1977, 1978; Weinreich and Yu, 1977; Weinreich et al., 1975). Although previous studies of C2 did not reveal that it made synaptic connections with the MCC, D. Weinreich (personal communication) and then Ono and McCaman (1980) confirmed our findings (Weiss et al., 1978b) that this connection was present. Consistent with the conclusion that the neuron was C2, we found that, as previously and subsequently reported (McCaman and McKenna, 1978; McCaman and Weinreich, 1982; Ono and McCaman, 1980; Weinreich, 1977), this cell made extensive synaptic connections with other cells in the E cluster. Furthermore, although the MCC showed only weak responses to histamine applied directly to its cell body, the application of histamine to the bath consistently produced a slow depolarization that had properties similar to the slow depolarization produced by the firing of C2 (Weiss et al., 1978b).

The synaptic potential evoked by C2 in the MCC is very slow in onset (Fig. 1) and is generally not seen unless a train of action potentials in C2 is elicited. The rising phase of the slow depolarization often exhibited small potentials that had the appearance of the joint excitatory–inhibitory potentials that occur spontaneously in the MCC (Weiss and Kupfermann, 1976). While the slow depolarization was consistently evoked in the same preparation, as well as between preparations, the fast potentials were not always seen, and when evoked they did not follow one-for-one the spikes in C2. We conducted a series of experiments to test the monosynapticity and the chemical nature of the synaptic potential evoked in the MCC by cell C2. Figure 2*A* shows that the synaptic potentials were blocked in a solution containing increased $Mg^{2+}$ and decreased $Ca^{2+}$ concentrations (3× Mg and 0.5× normal calcium). When the preparation was bathed in ASW containing increased $Ca^{2+}$ (3× normal), the slow synaptic potential increased in size, although a clear change in the fast synaptic potentials was not evident (Fig. 2*B*). These 2 results indicate that the slow synaptic potential is mediated chemically

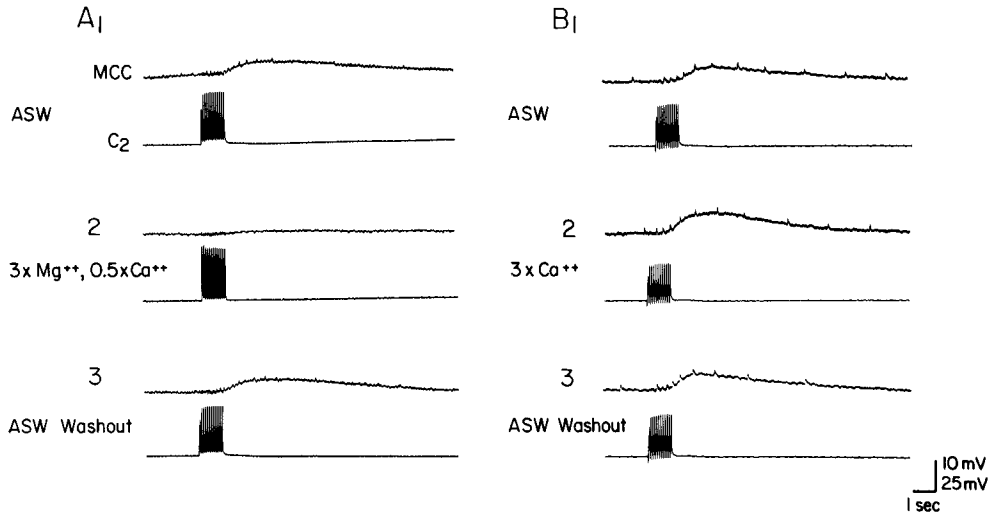


*Figure 2.* Chemical nature of the synaptic input from C2 to the MCC. *A*, Effects of bathing the preparation in a solution containing increased $Mg^{2+}$ (3× normal concentration) and decreased $Ca^{2+}$ (0.5× normal concentration): *1*, Synaptic response in a solution of normal artificial seawater (*ASW*); *2*, blockade of the synaptic response in a solution of high $Mg^{2+}$ and low $Ca^{2+}$; *3*, recovery of the synaptic response after washout with a solution of normal ASW. *B*, Effect of increasing $Ca^{2+}$ (3× normal concentration): *1*, Response in a solution of normal ASW; *2*, increased synaptic response in high $Ca^{2+}$ solution; *3*, the synaptic response returns to normal upon washout with ASW. Experiments in *A* and *B* were performed on the same preparation.

rather than electrically. Monosynapticity of the synaptic potentials was tested by 2 means: (1) injecting the presynaptic neuron with tetraethylammonium (TEA), which should broaden the presynaptic spike and increase the size of the monosynaptic connection; and (2) bathing the preparation in a solution of high divalent cations (3× $Mg^{2+}$ and 3× $Ca^{2+}$), which should raise the threshold of possible interneurons and therefore reduce or block polysynaptic responses. In Figure 3*A*, the injections of TEA increase the size of the slow potential. Figure $3B_2$ shows that when the ganglion was bathed in a solution of high divalent cations, stimulation of C2 no longer evoked the fast potentials, but the slow potential persisted (Fig. 3*A*). Ono and McCaman (1980) have provided evidence that a neuron termed C3 also makes monosynaptic excitatory connections to the MCC, and that C3 is electrically coupled to C2. Our experiment with high divalent cations suggests that the connection of C1 to the MCC is not likely to be mediated by C3, since a high divalent cation solution will raise the threshold of neurons, and all polysynaptic responses that involve spiking interneurons should be reduced or blocked. In addition, while in most preparations it was necessary to fire a burst of C2 action potentials to detect a postsynaptic response, in some preparations single spikes were sufficient to produce a discrete PSP with constant latency in the MCC (Fig. 3*C*). These experiments suggest that the slow excitatory potential is due to a monosynaptic connection to C2, whereas the fast potentials are evoked polysynaptically. Also consistent with this interpretation is the observation that the application of histamine evokes a similar EPSP in a preparation that is bathed in a solution that blocks synaptic release (see below).

*Modulation of the firing of the MCC by the activity of C2*

Despite the ostensibly weak synaptic effects of C2 on the metacerebral cell when C2 was not fired in high-frequency bursts, a relatively low rate of activity of C2 was capable of modulating the activity of the MCC when the latter was depolarized by a second input. Figure 4 shows an experiment in which cell C2 was fired at approximately 6 spikes/sec by means of injected current pulses that allowed a constant rate of C2 firing despite the synaptic input it received. At this rate of firing, C2 produced only a small synaptic potential that was not capable of triggering an action potential in the MCC (Fig. 4*A*). However, when a train of spikes was elicited in the MCC by means of a constant depolarizing current pulse (Fig. $4B_1$), the slow EPSP significantly increased the number of spikes elicited (Fig. $4B_2$). The capacity of the EPSP produced by C2 to increase the spiking of the MCC was a function of the size of the depolarizing current pulse injected into the MCC. Figure 5 shows the spiking of the MCC in response to the injection of current pulses of different sizes, both when C2 was fired and when it was inactive. A 10 sec pulse of 0.5 nA elicited 7 spikes in the MCC when C2 was inactive (Fig. 5*A*1), and elicited 10 spikes when C2 was fired, an increase of 43%. In contrast, a pulse of 1.0 nA elicited 13 spikes in the MCC when C2 was inactive (Fig. 5*B*1), but 28 spikes when C2 was fired (Fig. 5*B*2), an increase of 115%.

The ability of C2 to increase the excitability of the MCC was manifest even when C2 was fired at a rate that failed to produce a recordable synaptic potential in the MCC. Figure 6*A* shows a recording from the MCC in which no synaptic potential was evident when C2 was fired at 0.5 Hz. In Figure 6*B*, intracellular constant current pulses (monitored in the bottom trace) were injected into the MCC to simulate a second synaptic input. Prior to the activation of neuron C2, these constant current pulses were not able to produce an action potential in the MCC. However, when neuron C2 was activated, they became suprathreshold, despite the fact that activity of C2 produced no obvious synaptic potential in the MCC.

Our results on the effects of C2 on the excitability of the MCC

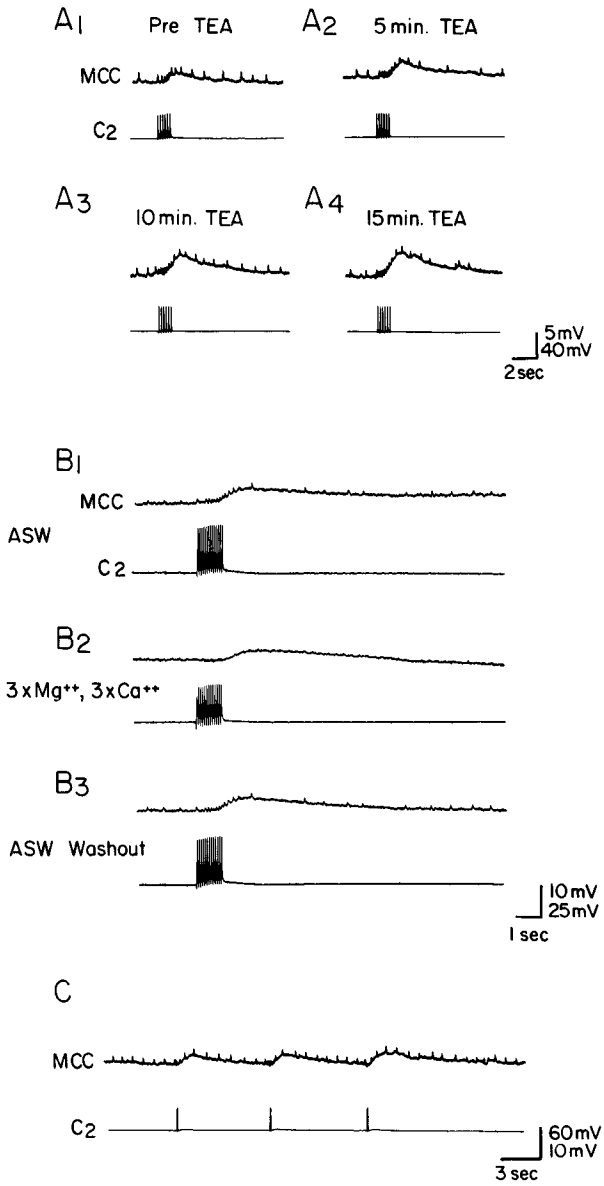


*Figure 3.* Evidence for the monosynapticity of connections of cell C2 to the MCC. *A*, Effects of the iontophoretic injection of TEA into C2: *1*, Response of the MCC to a train of 6 action potentials in neuron C2 prior to the beginning of TEA injections; *2–4*, synaptic potentials after TEA has been iontophoresed for 5, 10, and 15 min, respectively. *B*, Effect of increased concentration of divalent cations (3× normal concentration of $Mg^{2+}$ and 3× normal concentration of $Ca^{2+}$): *1*, The preparation was bathed in normal ASW and stimulation of C2 produced a synaptic potential composed of the fast potentials and the slow depolarization; *2*, when the preparation was bathed in a solution containing high divalent cations, the fast potentials disappeared but the slow depolarization persisted; *3*, upon return to normal ASW the fast potentials were reinstated. *C*, Example of a case where each action potential in C2 evoked a slow synaptic potential of constant latency in the MCC.

suggested that C2 may produce a conductance-decrease EPSP in the MCC. Since decreased conductance EPSPs increase in amplitude with depolarization, this could explain why depolarizing electrotonic pulses became effective in triggering action potentials in the MCC when cell C2 was active, even though the firing of C2 did not produce visible EPSPs at resting membrane potential. A conductance-decrease EPSP would also explain why, in the presence of the firing of C2, the excitability of the MCC was greater when the depolarizing pulses injected into

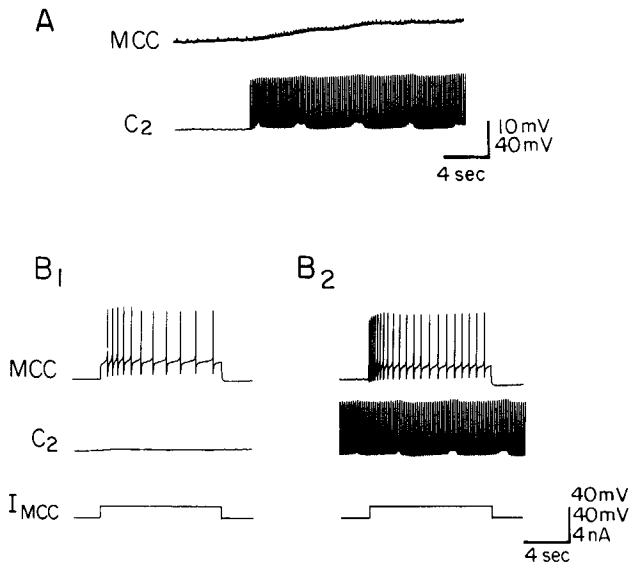


*Figure 4.* Firing of C2 at the same rate at which it failed to fire the MCC (*A*) produces a substantial enhancement of the number of spikes elicited in the MCC in response to a constant current depolarizing pulse. *B*, MCC responses (*1*) to depolarization in the absence of activity of C2, and (*2*) in the presence of the firing of C2.

it were bigger (for an analysis of a similar phenomenon see Carew and Kandel, 1977).

*Slow EPSP is associated with an apparent decreased conductance*

In order to measure conductance changes underlying the slow synaptic potentials, we fired a long burst of action potentials in cell C2 and monitored conductance changes in the MCC by injecting repeated constant current pulses. In Figure 7*A*, we see that, during the slow EPSP, the voltage drop across the membrane caused by the injected current increased significantly, which

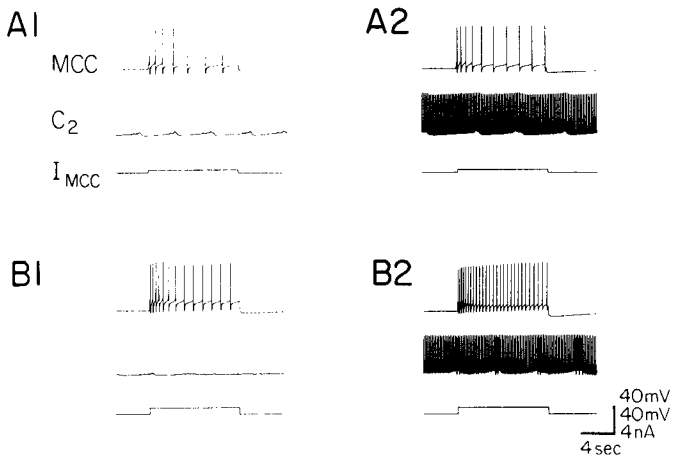


*Figure 5.* Effects of MCC depolarization on the efficacy of C2 potentiation of MCC firing. *A*, Intracellular injection of a 0.50 nA current pulse into the MCC in the absence (*1*) or presence (*2*) of activity of neuron C2. *B*, Injection of a 1.0 nA current pulse into the MCC in the absence (*1*) or presence (*2*) of C2 activity. When a small current pulse was injected into the MCC, stimulation of C2 added 3 action potentials to the firing of the MCC; whereas when a larger current pulse was injected, stimulation of the C2 added 15 action potentials to the burst in the MCC. Firing of C2 by itself at the rate used in this experiment depolarized the MCC by about 4 mV and never elicited spikes.

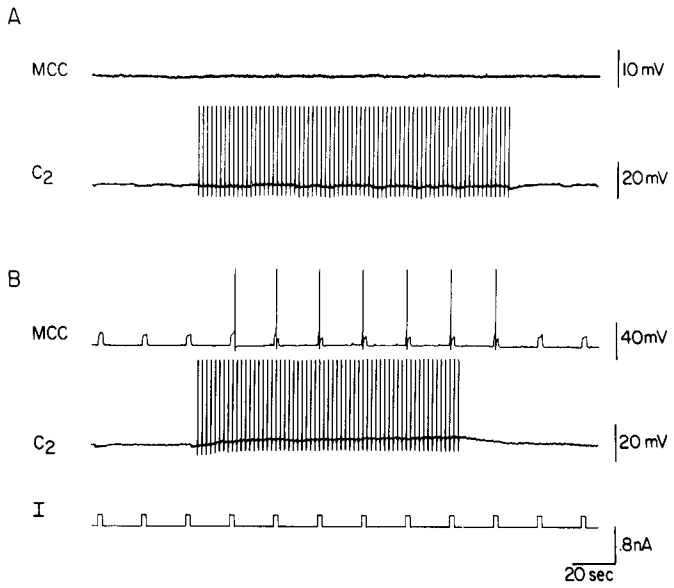


*Figure 6.* Effects of a very low rate of C2 activity on the threshold for initiation of action potentials by the MCC. *A*, When C2 was fired at a very low rate, it failed to produce any obvious synaptic potential in the MCC. *B*, A constant current pulse that did not produce an action potential in the MCC became suprathreshold when cell C2 was stimulated at the frequency that, in *A*, did not produce any measurable EPSP. Current injected into the MCC is indicated in *I*.

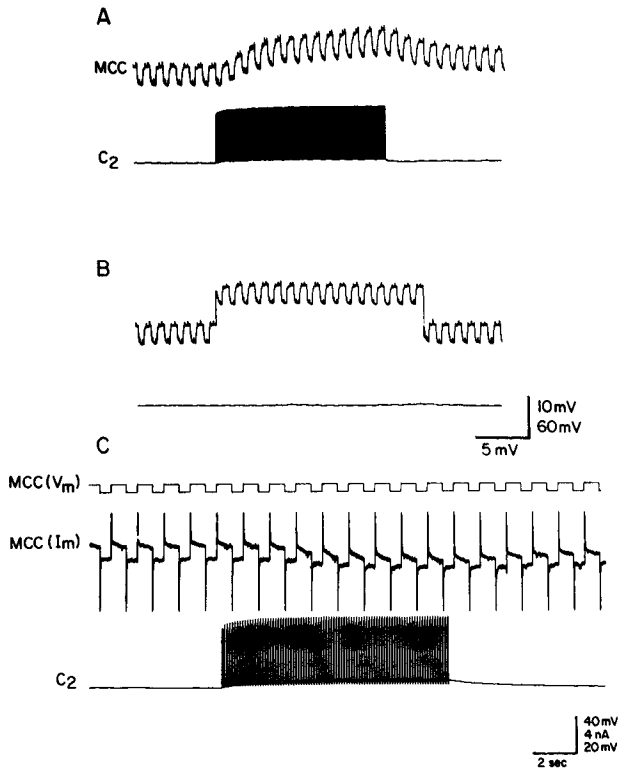


*Figure 7.* Conductance changes in the MCC during the slow EPSP evoked by stimulation of C2. *A*, Constant current pulses injected into the MCC increased in amplitude during the slow EPSP. *B*, The MCC was depolarized by an intracellular current injection to the same membrane potential recorded during the EPSP in *A*. Little or no change of the input resistance of the MCC was observed. *C*, Under voltage clamp, the current required to move the membrane potential of the MCC by 10 mV decreased during the slow EPSP produced by activation of C2.

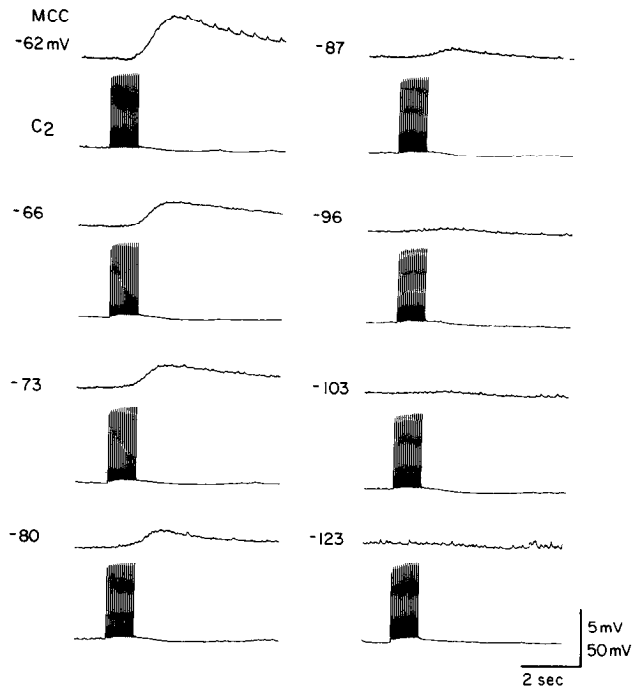


*Figure 8.* Effects of hyperpolarizing the MCC on the amplitude of the slow EPSP evoked by the stimulation of cell C2. Note that hyperpolarization of the MCC led to the abolition, but not reversal, of the EPSP.

suggests that the EPSP is associated with a decrease of membrane conductance. Since the MCC of *Aplysia* exhibits inward-going (anomalous) rectification (Weiss and Kupfermann, 1976; see also Fig. 12), we investigated whether the change in conductance of the MCC during the slow synaptic potential might have been due to the depolarization, which could have brought the MCC into a region where it showed a higher input resistance. Figure 7*B* shows an experiment performed on the same pair of cells as those in Figure 7*A*. Intracellular current injection was used to depolarize the MCC to the same membrane potential that was recorded during the EPSP evoked by C2. There was little or no change of input resistance over this range of depolarization. This suggested that the EPSP was indeed a decreased conductance EPSP, a conclusion further supported by voltage-clamp experiments in which membrane conductance was measured by the amount of current needed to produce a fixed voltage step. Under these conditions, membrane potential was controlled and therefore did not contribute an unknown variable to the measurement. As Figure 7*C* shows, during the slow EPSP evoked in the MCC by stimulating cell C2, the current pulses required to move the membrane potential of the MCC by 10 mV became 50% smaller, i.e., the membrane conductance decreased during the EPSP.

*Slow EPSP is voltage-dependent*

In order to understand the functional properties of the EPSP, we investigated its characteristics as a function of membrane potential. As expected for a decreased conductance EPSP, its amplitude became smaller when the MCC was hyperpolarized (Fig. 8). However, even when the cell was hyperpolarized to below −100 mV, the EPSP was still present; with further hyperpolarization it was abolished, but it never reversed. In order to ascertain whether the disappearance of the synaptic potential when the MCC was strongly hyperpolarized was accompanied by abolition of the change in membrane conductance, we measured changes of conductance during the EPSP while the MCC was voltage-clamped over a wide range of membrane potentials

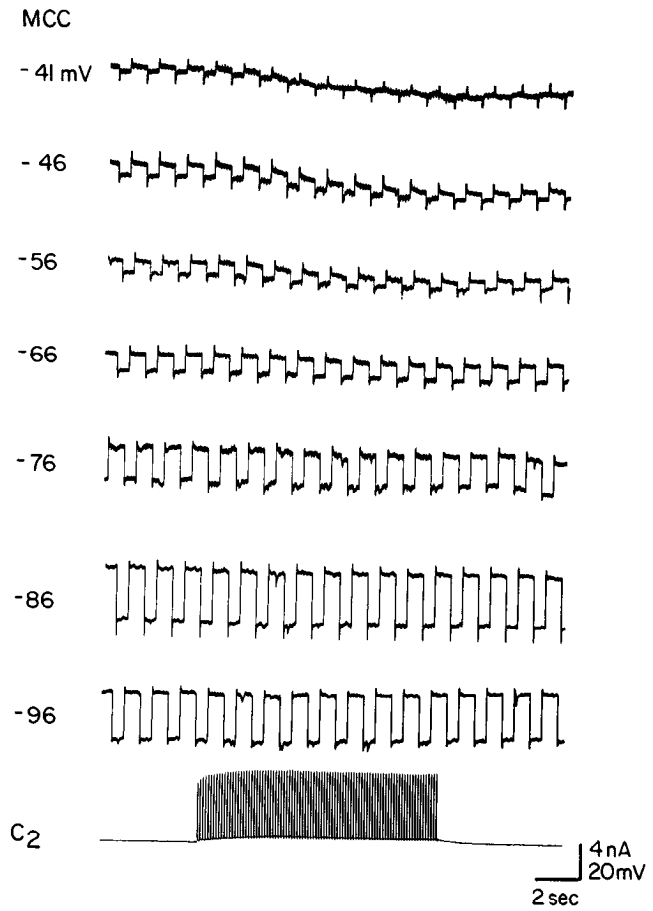


*Figure 9.* Measurements of conductance changes in voltage-clamped MCC during the slow EPSP produced by stimulation of C2. The MCC was held clamped at various potentials (ranging from −41 to −96 mV). At each potential, the cell was repeatedly stepped 10 mV. At hyperpolarized levels, both the slow inward current and the change in the current required to step the cell by 10 mV disappeared. Note that as the cell was held at more hyperpolarized potentials, the current pulses required to step the MCC by 10 mV increased in size, reflecting the increasing membrane conductance at hyperpolarized levels (anomalous or inward rectification). *Bottom trace* shows the firing of C2 for each of the membrane potentials of the MCC in the traces above.

(Fig. 9). In the depolarized range, firing of C2 produced an inward current, accompanied by a decrease in the size of current pulses needed to produce a constant voltage step. When the MCC was strongly hyperpolarized, no inward current was evoked by stimulation of cell C2, nor was there a change in the number of current pulses required for constant voltage steps. In other words, when the MCC was hyperpolarized, both the EPSP and the conductance change disappeared.

One possible explanation of why the EPSP could not be inverted is that the presynaptic terminals of C2 are electrically coupled to the MCC. Therefore, strong hyperpolarization of the MCC could conceivably hyperpolarize the terminals of C2 to the point that transmitter release would be suppressed. In a number of preparations, hyperpolarization of presynaptic cells has been shown to reduce transmitter release (Klein et al., 1980; Nicholls and Wallace, 1978; Shapiro et al., 1980; Shimahara and Peretz, 1978; Shimahara and Tauc, 1975), and we have found that, when C2 was hyperpolarized, the synaptic potential produced in the MCC was reduced or abolished (Fig. 10). To investigate whether hyperpolarization of the MCC affected the release of transmitter from C2, we took advantage of the long duration of the EPSP that it produces. Figure 11*A* shows that,

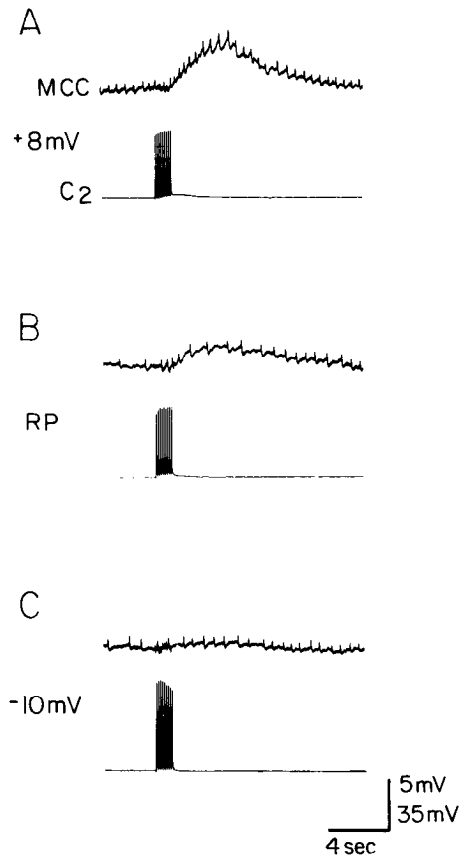


*Figure 10.* Effect of varying the holding membrane potential of C2 on the EPSP evoked by the activity of C2 in the MCC. Neuron C2 was fired using a train of square current pulses of fixed duration and frequency. The amplitude of the current pulses was adjusted so that at each holding level one pulse produced one action potential. The amplitude of the slow EPSP was biggest when C2 was depolarized 8 mV above the resting potential (*A*), intermediate when C2 was held at resting membrane potential (*RP*) (*B*), and almost abolished when C2 was hyperpolarized by 10 mV (*C*).

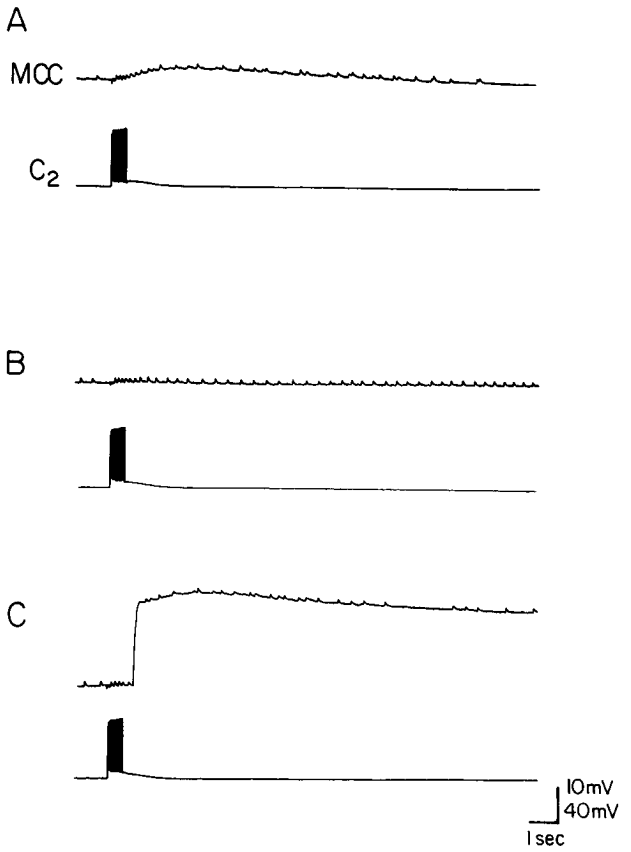


*Figure 11.* Effect of hyperpolarizing the MCC during transmitter release from C2. *A,* When the MCC was held at resting membrane potential, firing of C2 produced both the fast polysynaptic and the slow monosynaptic EPSPs. *B,* When the MCC was hyperpolarized 35 mV below resting potential, firing of C2 evoked only the fast polysynaptic component. *C,* When the MCC was hyperpolarized to the same extent as in *B,* but the hyperpolarization was released at the end of the burst of action potentials in C2, both the fast and slow components of the EPSP were seen.

when the metacerebral cell is at resting potential, a burst of spikes in C2 produces the characteristic slow EPSP, together with the fast EPSPs that appear to be polysynaptic (see above). When the MCC was hyperpolarized by 35 mV, the slow monosynaptic EPSP was abolished without a concomitant abolition of the polysynaptic response (Fig. 11*B*). This indicated that, if hyperpolarization of the MCC had an effect on the synaptic terminals of C2, the effect would have to be selective to the terminals on the MCC. That this is not the case can be seen in Figure 11*C*. The MCC was hyperpolarized by 35 mV before and during a burst of action potentials in C2, but was released from hyperpolarization at the end of the C2 burst. When the MCC was released from hyperpolarization, the slow EPSP (similar to the one that occurred at resting membrane potential) was seen, which indicates that transmitter release must have occurred from C2 when the MCC was hyperpolarized. In control experiments in which we released the MCC from similar hyperpolarization in the absence of firing of C2, no significant anodal break from hyperpolarization was observed. This experiment indicates that our inability to reverse the slow EPSP when the MCC was hyperpolarized was not due to an artifact of electrical coupling between the terminals of the MCC and C2.

An alternative explanation of the failure of hyperpolarization to reverse the EPSP is that the current injected into the soma of the MCC failed to effectively hyperpolarize the synaptic region of the cell. This could be due to the pronounced inward rectification of the MCC (Weiss and Kupfermann, 1976). As the MCC is hyperpolarized, its input resistance markedly decreases, creating a current shunt through the soma and proximal processes of the cell. Therefore, to obtain an improved space-clamp of the neuron, we performed a series of voltage-clamp studies in which the MCC was axotomized within 300 $\mu$m of the cell body. The current–voltage relation was determined by stepping from a holding membrane potential of −60 mV to various other potentials, and measuring the current at the end of a 5 sec pulse when the current had achieved a steady state. Current–voltage relations were determined in normal ASW and in the presence of bath-applied histamine ($10^{-4}$ M). The difference between the current–voltage curves in the presence and the absence of histamine was taken to represent the histamine-induced current. Under these conditions, the histamine-induced current again did not reverse. It disappeared at approximately −80 mV (Fig. 12). This suggested that we might be dealing with a decreased potassium-conductance EPSP that does not reverse because it has some rectifying or voltage-dependent properties. Alternatively, these results could be due to an increase of conductance to calcium or sodium if these conductances are highly voltage-dependent. We found, however, that under voltage clamp, histamine-induced inward current persisted in sodium-

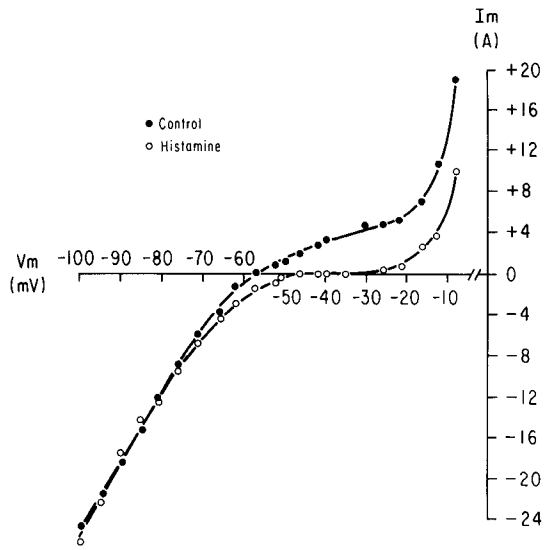


*Figure 12.* Effects of histamine on the I-V relations in the MCC. The MCC was held at −60 mV and the *I-V curve* was generated by stepping the cell to various membrane potentials for 5 sec. The current was measured at the end of the 5 sec step, when the currents attained a steady state.

free solutions (substituted for by Tris or sucrose). Experiments on the role of calcium gave inconsistent results. In 2 experiments, the histamine-induced current persisted in the presence of the calcium channel-blocker cobalt (30 mm), but in one experiment, cobalt appeared to block the histamine-induced current. In calcium-free solutions (supplemented with 10 mM EGTA), the histamine current persisted in one experiment and was blocked in another. Similar inconsistent sensitivity to external calcium has been observed for serotonin in decreasing a potassium current in sensory neurons of *Aplysia* (M. Klein, personal communication; see also Pollock et al., 1985; Walsh and Byrne, 1985).

*Histamine-induced current in the MCC is dependent on external potassium concentration*

To examine more directly the hypothesis that this EPSP is mediated by a decreased potassium conductance, we investigated the effect of varying the potassium concentration in the solution bathing the preparation (Fig. 13). In order to block possible polysynaptic actions of histamine, these experiments were performed in seawater supplemented with 100 μM TTX and $Mg^{2+}$ substituted, mole for mole, for $Na^+$, sufficient to bring the final $Mg^{2+}$ concentration to 150 mM. Potassium ion concentrations of 10, 60, and 100 mM were obtained by equimolar substitution of potassium for sodium. Three experiments were performed for each of these potassium concentrations. We found that in high concentrations of potassium we were able to reverse the synaptic current induced by histamine application. This reversal, similar to the disappearance of the synaptic current in normal (10 mM) potassium, corresponded to the predicted Nernst equilibrium potential for potassium (Fig. 13*B*). However, even when the synaptic current reversed in high potassium, it did not follow the expected ohmic relations; i.e., when the cell was stepped to hyperpolarized potentials greater than the reversal potential, the amplitude of the reversed current failed to increase. In fact, as the cell was stepped to more hyperpolarized potentials, the histamine-induced current got smaller and eventually returned to zero.

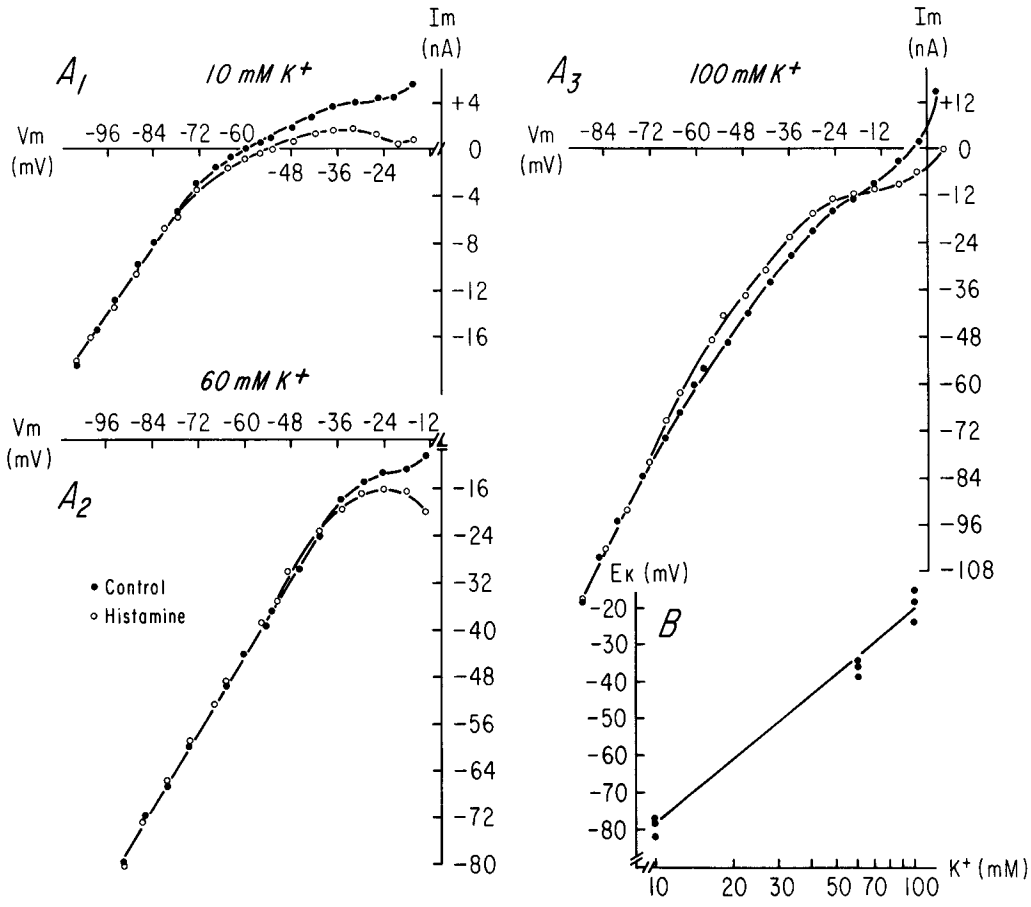


*Figure 13.* Effects of histamine and of varying external concentrations of potassium (10 mM in $A_1$, 60 mM in $A_2$, and 100 mM in $A_3$) on the I-V relations of the MCC. The MCC was held at −60 mV and the *I-V curve* was generated by stepping the cell to various membrane potentials for 5 sec, and measuring the current at the end of the step. *B, Solid line* represents the calculated equilibrium potential for $K^+$, based on the Nernst equation, assuming a $K_i$ of 232 mm (Sato et al., 1968). *Dots* represent the distribution of the measured reversal potentials of the histamine-induced current at 3 different external concentrations of potassium. Three experiments were performed for each of the following concentrations: 10, 60, and 100 mM.

## Discussion

The initial focus of this research was on locating neurons that might constitute elements of a central arousal system in *Aplysia*. The existence of a central arousal system was hypothesized on the basis of 2 observations. First, food stimuli potentiate a variety of responses, including feeding, locomotion, and cardiovascular responses. Second, the potentiating effects greatly outlast the initial presentation of the food. Potentiation of the different response systems is mediated by specialized neurons, each of which appears to be devoted to a single class of closely related behaviors. We term these neurons "arousal executor cells." One such neuron is the metacerebral cell, which potentiates consummatory aspects of feeding behavior by both peripheral actions on buccal muscles (Weiss et al., 1975, 1978a, 1979) and central actions on motor neurons and a putative central pattern generator that drives rhythmic feeding responses. Conceived of in the simplest terms, an element of a central arousal system should have the following characteristics:

1. It should excite arousal executor cells.
2. It should have widespread effects or be part of a system of interconnected neurons that drive a number of cells.
3. It should be activated by stimuli that evoke an arousal state.
4. It should produce effects that outlast the stimuli that excite it to action.

In this article, we have explored the first criterion and provide evidence that the MCC is excited by identified histaminergic neuron C2. The excitation is due to a slow EPSP, as well as a relatively weak input consisting of fast EPSPs that are blocked in the presence of high divalent cations and are clearly polysynaptic. The slow EPSP appears to be monosynaptic, on the basis of observations that it (1) persists in a high divalent cation solution, (2) is enhanced when the presynaptic spike is broadened by TEA, and (3) is mimicked by application of histamine, the likely transmitter of neuron C2 (McCaman and McKenna, 1978; McCaman and Weinreich, 1982; Ono and McCaman, 1980; Schwartz et al., 1986; Weinreich, 1977, 1978; Weinreich and Yu, 1977; Weinreich et al., 1975).

The slow EPSP that C2 produces in the MCC has all of the characteristics of a modulatory, as opposed to a mediating, synaptic potential (Kupfermann, 1979). First, the slow EPSP has a very slow onset and slow decay. Second, the slow EPSP appears to involve a nonconventional ionic conductance. Our evidence indicates that the EPSP is associated with a decreased conductance, which can function to amplify other synaptic inputs to the cell (Byrne, 1981; Carew and Kandel, 1977; Weight, 1974). Furthermore, the slow EPSP shows a strong voltage-dependence. Therefore, the synaptic current is small when the MCC is at resting potential, and totally disappears when the MCC is strongly hyperpolarized. This property can result in the enhancement of inhibitory inputs that hyperpolarize the MCC and drive it into the region of membrane potential where the slow EPSP is decreased (see Kandel and Tauc, 1966, for a similar property that arises as a consequence of anomalous rectification in the MCC of *Helix*). Conversely, in the presence of the slow EPSP, a decrease of a tonic IPSP will result in an enhanced depolarization because the EPSP will be amplified as the membrane potential depolarizes. The voltage-dependence of the slow EPSP could also explain the observation that, at low rates of firing of C2, the MCC at resting potential shows enhanced excitability to depolarizing inputs, even though C2 fires at a rate too low to produce any shift of membrane potential. With a more intense activity of C2, a substantial depolarization of the MCC occurs, even at resting potential, which is close to the potential at which no synaptic current is present. This may result from the fact that, because the EPSP is due to a decreased conductance, as the cell begins to depolarize it moves into a region in which an enhanced synaptic current exists. In other words, the synaptic potential has self-regenerative properties (Carew and Kandel, 1977).

Voltage-clamp experiments in which the external concentration of potassium ions was varied suggest that the histamine-induced slow synaptic potential is due, at least in large part, to a decreased conductance to potassium, and this conclusion is consistent with the evidence of McCaman and Weinreich (1985) on the very slow EPSPs produced by C2. Failure of the synaptic currents to clearly reverse at potentials hyperpolarized relative to the potassium equilibrium potential may be, to some extent at least, a consequence of constant field rectification (Goldman, 1943) which arises because of the uneven distribution of extracellular $K^+$ and intracellular $K^+$ (see, e.g., Siegelbaum et al., 1982). Consistent with this interpretation is the observation that a reversal of the histamine-induced current was observed when the external potassium ion concentration was raised. But even under those conditions, the inward current behaved anomalously and did not increase linearly with increasing hyperpolarization of the MCC. This suggests that, in addition to constant field rectification, the nonlinear properties of the slow EPSP may be due to voltage-gating of the relevant channels. It is also possible that the rectification is partly due to blockage of the potassium channel by sodium ions when the cell is strongly hyperpolarized (for a review of this mechanism, see Hille, 1984). It should be pointed out, however, that bath-applied histamine, under our experimental conditions, need not necessarily affect the same receptors that are activated by normal synaptic input.

In many respects the behavior of the histamine-modulated channels resembles that of the S channels that exist on sensory neurons of *Aplysia*, which are closed by serotonin (Klein and Kandel, 1978, 1980; Klein et al., 1982; Shuster et al., 1985; Siegelbaum et al., 1982), and the neuropeptides $SCP_A$ and $SCP_B$ (Abrams et al., 1984). In preliminary experiments we have found that serotonin also evokes a slow decreased conductance in the MCC, which raises the possibility that S channels also exist on the MCC or that the histamine-modulated channels are identical to S channels. Consistent with the interpretation that the MCC may contain S channels are the observations that the closing of S channels is mediated by a cAMP-dependent protein kinase (Klein and Kandel, 1978; Shuster et al., 1985; Siegelbaum et al., 1982) and that cAMP also may decrease membrane conductance in the MCC (Drake and Treistman, 1980; Treistman and Drake, 1979). In addition, similar to the conductance we have studied, the modulation of potassium conductance of mechanosensory neurons by serotonin can be affected by external calcium (Pollock et al., 1985; Walsh and Byrne, 1985). The histamine-induced current in the MCC also has some similarities to the slow depolarization induced by muscarine in sympathetic ganglion cells of the bullfrog. The muscarine-induced current is primarily due to a decrease of a calcium-independent potassium conductance (Adams et al., 1982) but may also have a component mediated by a decrease of a calcium-dependent potassium current (Pennefather et al., 1985).

In neurons of the vertebrate myenteric plexus, histamine (Grafe et al., 1980) and serotonin (Nemeth et al., 1984) also decrease potassium conductance. In myenteric neurons, however, the decreased potassium conductance is the calcium-dependent potassium conductance (see also Haas and Konnerth, 1983). For this reason, in the myenteric plexus, serotonin greatly reduces the spike-induced afterhyperpolarization that ordinarily severely limits the ability of the cells to exhibit repetitive spikes at high frequency. Judging by the absence of significant changes in the afterhyperpolarization of MCC spikes, the histamine-induced slow EPSP has little or no effect on the calcium-dependent potassium current. This is consistent with our finding that even in the presence of the slow EPSP, the MCC does not generally show high-frequency spike activity. Further, the MCC

normally fires at a low frequency (Kupfermann and Weiss, 1982) and is able to exert physiological effects even at rates of activity as low as 0.4 spikes/sec (Weiss et al., 1978a, b).

Regardless of the mechanism of the slow EPSP produced by C2, its existence in the MCC clearly makes C2 a candidate as an element of the system mediating food arousal in *Aplysia*. In subsequent articles, we examine other features of C2 and report that some, but not all, features of the neuron are consistent with its being an element of an integrated central arousal system.

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