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Selective Modulation of Spike Duration by Serotonin and the Neuropeptides, FMRFamide, SCP_B, Buccalin and Myomodulin in Different Classes of Mechanoafferent Neurons in the Cerebral Ganglion of *Aplysia*

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An examination of the cellular properties and synaptic outputs of mechanoafferent neurons found on the ventrocaudal surface of the cerebral ganglion of *Aplysia* indicated that the cerebral mechanoafferent (CM) neurons are a heterogeneous population of cells. Based on changes in action potential duration in response to bath applications of 5-HT in the presence of TEA, CM neurons could be divided into 2 broad classes: mechanoafferents whose spikes broaden in response to 5-HT (CM-S_B neurons) and mechanoafferents whose spikes narrow in response to 5-HT (CM-S_N neurons). Morphological and electrophysiological studies of the CM-S_N neurons indicated that they were comprised of previously identified interganglionic cerebral–buccal mechanoafferent (ICBM) neurons and a novel set of sensory neurons that send an axon into the LLAB cerebral nerve and have perioral zone receptive fields that are similar to those of ICBM neurons. Changes in spike width due to 5-HT were correlated with changes in synaptic output as indicated by the magnitudes of EPSPs evoked in postsynaptic neurons. Electrical stimulation of cerebral nerves and connectives also produced spike narrowing or broadening, and the sign of the effect was a function of the parameters of stimulation. Both heterosynaptic facilitation and heterosynaptic depression of EPSPs evoked in follower cells could be demonstrated. A variety of putative neuromodulators other than 5-HT were also found to affect the duration of action potentials in both classes of CM neurons. FMRFamide had effects opposite to that of 5-HT. SCP_B and a recently characterized *Aplysia* neuropeptide, buccalin, broadened the spikes of both CM classes. Another neuropeptide, myomodulin, decreased the duration of CM-S_B neuron spikes but had no effect on CM-S_N spikes. Since the CM neurons appear to mediate a variety of competing behaviors, including feeding, locomotion, and defensive withdrawal, the various neuromodulator actions may contribute to the mechanisms whereby behaviors are selected and modified.

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The cerebral ganglion of *Aplysia* contains populations of mechanoafferent neurons that transmit sensory information from structures of the head of the animal (Rosen et al., 1979, 1982). These neurons provide a vantage point for studying the modulation of behavior at the primary sensory level. In many respects, the cerebral mechanoafferent (CM) neurons are similar to the *Aplysia* mechanosensory cells described in the pleural and abdominal ganglia (Byrne et al., 1974; Byrne, 1980; Walters et al., 1983a). For example, EPSPs evoked in the follower (postsynaptic) neurons of the primary mechanoafferents of all 3 ganglia exhibit profound low-frequency depression (Castellucci et al., 1970; Castellucci and Kandel, 1974; Rosen et al., 1979; Byrne, 1980; Walters et al., 1983a) and posttetanic potentiation (Walters and Byrne, 1984). Whereas the pleural and abdominal mechanosensory neurons appear to function largely in mediating defensive responses (e.g., withdrawal, inking), the available data suggest that the CM neurons constitute a heterogeneous population that may be involved in various behavioral functions. Two groups of CM neurons were previously described (Rosen et al., 1982). One group of CM neurons sends axons to the buccal ganglia, where they monosynaptically excite motoneurons to the buccal muscles that produce feeding movements (Rosen et al., 1982). Intracellular stimulation of this type of interganglionic cerebral–buccal mechanoafferent (ICBM) neuron can induce patterned activity in the buccal ganglion. The second group of CM neurons does not send axons to the buccal ganglia. It, along with the first group of CM neurons, excites cerebral motoneurons that innervate the lips or tentacles (Fredman and Jahan-Parwar, 1977; Rosen et al., 1979).

Considerable evidence indicates that the synaptic efficacy of the mechanoreceptor neurons in the abdominal and pleural ganglia of *Aplysia* can be modified by serotonin (5-HT) (Brunelli et al., 1976; Klein and Kandel, 1978; Siegelbaum et al., 1982; Walters et al., 1983b; Abrams et al., 1984; Pollock et al., 1985). 5-HT appears to modulate transmitter release, at least in part, by affecting the duration of the presynaptic spike (Klein and Kandel, 1980; Klein et al., 1982). This effect is reflected in recordings from the soma of the neuron (Klein and Kandel, 1978), particularly if the spike is already broadened by pharmacologically blocking K^+ channels with tetraethylammonium (TEA). Therefore, as a first step in characterizing the cellular properties of the CM neurons and to determine whether their synaptic output can be modulated, we investigated changes in spike duration in response to bath application of 5-HT while

the cerebral ganglion was superfused with artificial seawater (ASW) containing TEA.

In the second part of the study, we examined the effects of nerve stimulation on the spike width and the synaptic output of the CM neurons. Our results, together with recent findings on abdominal mechanoafferents (Abrams et al., 1984; Belardetti et al., 1987; Brezina et al., 1987; Goldberg et al., 1987; Mackey et al., 1987; Piomelli et al., 1987), suggested that more than one neuromodulator may affect the synaptic efficacy of the CM neurons. Therefore, in the last part of this study we examined the effects of a variety of putative neuromodulators, including the 4 neuropeptides: FMRFamide, SCP_B, buccalin, and myomodulin. All of these substances are present in muscles and neurons involved in feeding behavior in *Aplysia* (Lloyd et al., 1984, 1985a, b, 1987; Richmond et al., 1984; Brown et al., 1985; Weiss et al., 1986c; Cropper et al., 1987a–c). FMRFamide (Price and Greenberg, 1977; Lehman et al., 1984; Schaefer et al., 1985) and SCP_B (Morris et al., 1982) are known to affect pleural and abdominal neurons (Abrams et al., 1984; Belardetti et al., 1987; Brezina et al., 1987; Piomelli et al., 1987), whereas the effects of the recently sequenced peptides myomodulin and buccalin (Cropper et al., 1987a–c) on pleural and abdominal mechanoafferents are not known (see, however, the recent reports of Alevizos et al., 1987, and Cleary et al., 1987). The results indicate that the CM neurons constitute a heterogeneous group of mechanosensory cells that show unique patterns of responses to a broad spectrum of neuromodulators. A variety of behaviors involving CM neurons may be integrated and modulated by synaptic mechanisms operating at the level of the sensory neurons themselves.

Materials and Methods

Subjects. The experimental subjects were *Aplysia californica* weighing 250–350 gm. The cerebral and buccal ganglia were removed together with extended segments of their intact nerves and connectives. To reduce contractions of the ganglionic sheath, the ganglia were immersed for 45 sec in 0.5% glutaraldehyde in ASW. The ganglia were pinned, ventral side up, to the Sylgard base of a 2.5 ml recording chamber that contained ASW. The sheath overlying the mechanoafferent cell clusters was removed. Polyethylene suction electrodes were attached to selected nerves and connectives for extracellular nerve stimulation.

Recording techniques. Neurons were impaled with double-barrel microelectrodes. Conventional electrophysiological techniques were used for both intracellular recording and stimulation (Rosen et al., 1979). Data were displayed on a storage oscilloscope and stored on magnetic tape. In order to obtain high-resolution ink writeouts of brief action potentials, the data were stored at 15 or 3.75 ips (Hewlett Packard Tape Recorder) and played back at 15/16 ips. A gravity-driven perfusion system (1–12 ml/min) permitted continuous superfusion of the ganglia with different solutions. The standard perfusion medium used in most experiments consisted of ASW with 50 mM TEA (Kodak), 10 mM Tris (Trizma) buffer (pH = 7.6), and a high concentration of divalent cations (Mg^{2+}, 100 mM, 2 × normal; Ca^{2+}, 50 mM, 5 × normal). Calcium blocking agents were not used in our experiments since these agents can interfere with spike broadening produced by the TEA and may, in addition, interfere with agonist-induced spike broadening. In some experiments, sodium action potentials were eliminated by the addition of TTX (60 μM) to the ganglion bath prior to the administration of neuromodulators. Neuromodulators were generally applied by means of brief (0.5–1.0 sec) micropipette injection (10–50 μl) in the vicinity of the neuron. The neuromodulators were dissolved in the same solution as the perfusion medium. Frequently, it was possible to assess the response of an individual cell to 3 or more substances applied in serial fashion. A total of 107 CM neurons from 84 animals (generally 1–3/animal) were studied.

Procedure. A typical experiment began with the recording of action potentials from a CM neuron while the ganglion remained in normal ASW. The cell was stimulated by a 3 msec intracellular current pulse slightly above spike threshold. The repetition rate of the stimulus was generally 0.2 Hz. The ganglion was then continually superfused with the high divalent cation ASW containing TEA. Following the establishment of a stable "TEA spike," various nerves and connectives attached to extracellular electrodes were individually stimulated with brief (4 msec) current pulses. This procedure was used to determine if nerve stimulation produced either orthodromic responses in the sensory neuron, indicative of the presence of an axon of that neuron in the stimulated nerve, or changes in spike duration, indicative of a modulatory input via that nerve.

In some experiments we investigated changes in the synaptic efficacy of CM neurons due to application of putative neuromodulators or heterosynaptic electrical stimulation of cerebral nerves. In these experiments we simultaneously recorded activity in a CM neuron and an excitatory follower neuron in the cerebral B cluster. Following heterosynaptic nerve stimulation, the input conductance of B neurons was estimated on the basis of the decay constant of the EPSP evoked by firing of the CM neuron. The constant was obtained by a log plot of the magnitude of the tail of the PSP over time, using pen recorder records digitized by means of a digitizer pad and Sigma Scan software.

Morphology. In order to further characterize neurons in the mechanoafferent clusters, which were initially classified by the nature of their response to 5-HT, Lucifer yellow iontophoresis (Stewart, 1978) was combined with conventional electrophysiological techniques. Following determination of the nature of the effect of 5-HT on spike duration, individual mechanoafferent cells were filled with Lucifer yellow dye (3%, in water) by passing hyperpolarizing currents (10 nA, 0.5 sec pulses, 1 Hz) for 15–30 min. In these experiments, the recording electrode was filled with 1 M lithium chloride, which appeared to diminish plugging of the Lucifer yellow electrode. Several hours were allowed for the dye to diffuse throughout the cell, and then the ganglion was fixed for 12 hr with a phosphate buffered solution (pH = 7.6) of 10% formaldehyde and 30% sucrose. The ganglion was then dehydrated, cleared in methyl salicylate, and viewed with a fluorescence microscope. Camera lucida drawings of the cells were made.

Results

Heterogeneity of responses to 5-HT among mechanoafferent neurons in the cerebral ganglion

Prior to control recording, the cerebral ganglion was superfused with TEA in high divalent cation ASW, and action potentials were elicited by intracellular current pulses. After 10 min of superfusion with the TEA solution, the duration of the action potentials (measured at half-amplitude) increased 4–10 times over that observed in normal ASW. Repeated low-frequency stimulation of the mechanosensory cells at 0.2 or 0.25 Hz produced stable spikes of constant amplitude (range, 80–100 mV), duration (range, 25–50 msec), and waveform. Following a 2 min period of control recordings, CM cells were tested for their responses to applications of 5-HT.

Brief application of 25 μl of 5-HT (pipette concentration, 10^{-3}–10^{-5} M) to the CM cell cluster during ongoing superfusion produced 1 of 2 responses. In a survey of 60 CM neurons in the anterolateral regions of the CM clusters, approximately half (28) exhibited spike narrowing to application of 5-HT. The remaining cells showed pronounced spike broadening. The finding that a substantial proportion of mechanosensory cells showed spike narrowing stands in sharp contrast to the spike-broadening response consistently reported for mechanoafferents in the pleural and abdominal ganglia of *Aplysia* (Klein and Kandel, 1978; Walters et al., 1983b). Figure 1*A* shows representative response characteristics of the population of mechanoafferents that showed spike narrowing to application of 5-HT (designated cerebral mechanoafferent, 5-HT narrowers, or CM-S_N neurons). The top trace (*A1*) is a control recording of a TEA-broadened spike (duration, 60 msec) prior to 5-HT exposure. Fifteen seconds after bath application of 25 μl 5-HT (10^{-4} M), the half-amplitude duration decreased to 20 msec (Fig. 1*A2*). Spike width recovered

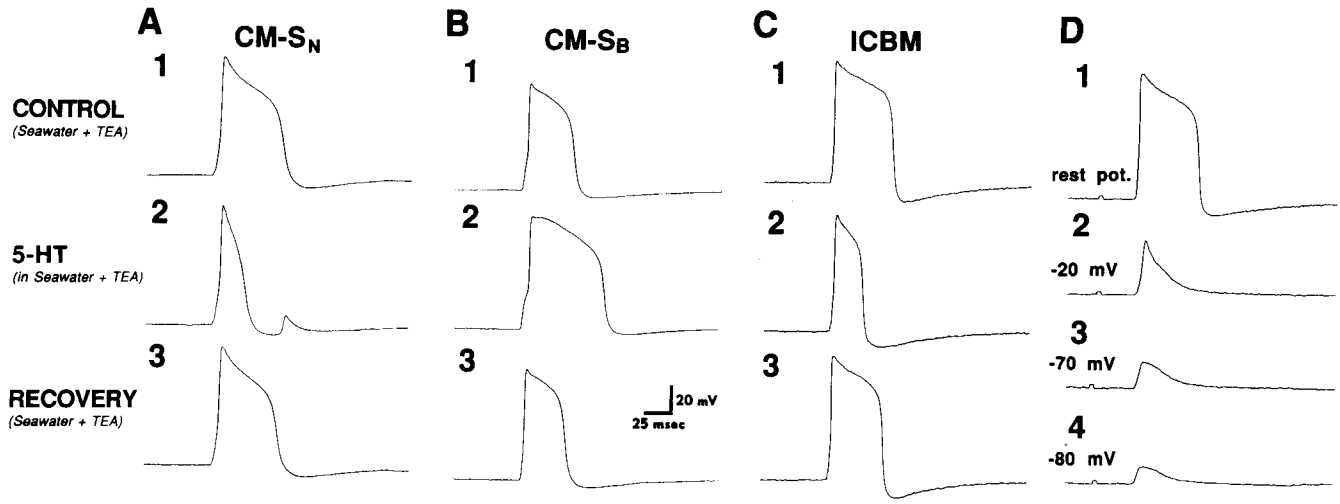


Figure 1. Effects of 5-HT on action potentials elicited in 2 classes of mechanoafferent neurons in the cerebral ganglion of *Aplysia.* Spikes were repeatedly evoked by intracellular current pulses (3 msec duration, 0.2 Hz) as the cerebral ganglion was superfused with TEA in high divalent cation ASW with 10 mM Tris buffer. *A,* Typical spike-narrowing response of a CM-S_N neuron before (*A1*), 15 sec after (*A2*), and 15 min after (*recovery, A3*) a small quantity (25 μl) of 10^{-4} M 5-HT was delivered to the ganglion bath by means of a micropipette. *B,* Typical spike-broadening response of a CM-S_B neuron before (*B1*), 15 sec after (*B2*), and 15 min after (*B3*) 10^{-4} M 5-HT was added to the ganglion bath. *C,* Spike-narrowing response of an ICBM neuron after 10^{-4} M 5-HT was added to the bath (*C2*). ICBM neurons are characterized by their having axons in the C-B connective and are considered as a subclass of the CM-S_N class of neurons. *D,* Identification of an ICBM neuron by the action potential (*D1*) that is elicited by a brief electrical stimulus (small artifact preceding the response) applied to the ipsilateral C-B connective. As expected for a directly elicited action potential, increasing hyperpolarization of the ICBM neuron progressively reduced the size of the spike (*traces D2–D4*).

to 90% of control 15 min after wash-out of the 5-HT (Fig. 1*A3*). The secondary response seen in trace *A2* was commonly observed in CM-S_N neurons and may possibly be related to the multipolar morphology of these cells (see Fig. 2).

Spike narrowing to 5-HT (10^{-4}–10^{-6} M) was also observed in the presence of the Na^+ channel blocker TTX (60 μM) added to the high divalent cation ASW (5 neurons in 4 preparations). The TTX was used to enhance the blockade of action potential conduction and thereby minimize the likelihood that the effects of the 5-HT were indirect, via other neurons with inputs to the sensory cells. However, the procedure does not rule out the possibility that indirect effects were mediated by neurons supporting Ca^{2+} spikes or neurons insensitive to the effects of TTX, although the use of a high-Mg^{2+}, high-Ca^{2+} solution, which raises the thresholds for action potentials, makes it unlikely that the effects were mediated by spiking interneurons.

Figure 1*B* shows a representative example of the population of mechanoafferent neurons that exhibited spike broadening instead of narrowing to bath applications of 5-HT (designated class CM-S_B). Fifteen seconds after exposure to 5-HT, the spike broadened from 35 msec (control duration) to 70 msec duration (Fig. 1*B2*). Spike duration returned to control levels after 15 min of wash-out (Fig. 1*B3*).

5-HT produces spike narrowing in an identified subclass of cerebral ganglion mechanoafferents with connections to the buccal ganglion

An analysis of the distribution of axons of the CM neurons, based on observation of direct action potentials elicited by extracellular nerve and connective stimulation, indicated that a previously identified subclass of mechanoafferent neurons (Rosen et al., 1982) were among those neurons that showed spike narrowing to 5-HT. These ICBM neurons had previously been shown to be the only sensory cells found in the cerebral ganglion mechanoafferent cell clusters that have axons in the cerebral–buccal (C-B) connectives. The ICBM neurons were further characterized by having 2 receptive fields, one in the tissue forming the inner border of the lips at the mouth opening (perioral zone) and a second in the muscle layers forming the inner surface of the buccal cavity. The ICBMs were also the only cells in the mechanoafferent clusters to make excitatory, monosynaptic connections to the giant, serotonergic, metacerebral cells in the cerebral ganglion and to identified interneurons B4 and B5 in the buccal ganglion (Rosen et al., 1982). Figure 1*D* shows the identification of an ICBM neuron by the direct spike that could be elicited by electrical stimulation of the C-B connective. 5-HT was found to effect a reversible decrease in the duration of the spike of 50% or more (Fig. 1*C*). This observation was found in 12 of 12 ICBMs tested.

Morphological characterization of mechanoafferent neurons exhibiting spike narrowing to applications of 5-HT

As indicated above, many of the CM neurons exhibiting spike narrowing to 5-HT were identified as ICBM neurons since they showed direct action potential responses to stimulation of the C-B connective. Other CM neurons also showed spike-narrowing responses to 5-HT, but did not exhibit action potential responses to connective stimulation. These neurons may have represented a separate subclass of CM-S_N neurons, or they may have been ICBM neurons that had their axons damaged in the desheathing of the cerebral ganglion. A third possibility is that these neurons represented ICBM neurons that only have axons in the contralateral C-B connective (Rosen et al., 1982). In order to distinguish between these possibilities, we used Lucifer yellow staining to examine the morphology of neurons that showed spike narrowing to applications of 5-HT. Two groups of morphologically distinct CM-S_N cells were identified. One appeared to consist of *bona fide* ICBM neurons that sent axons to the C-B connective. The dye fills revealed that 7 of the 9 ICBM neurons examined also sent axons to the cerebral–pleural

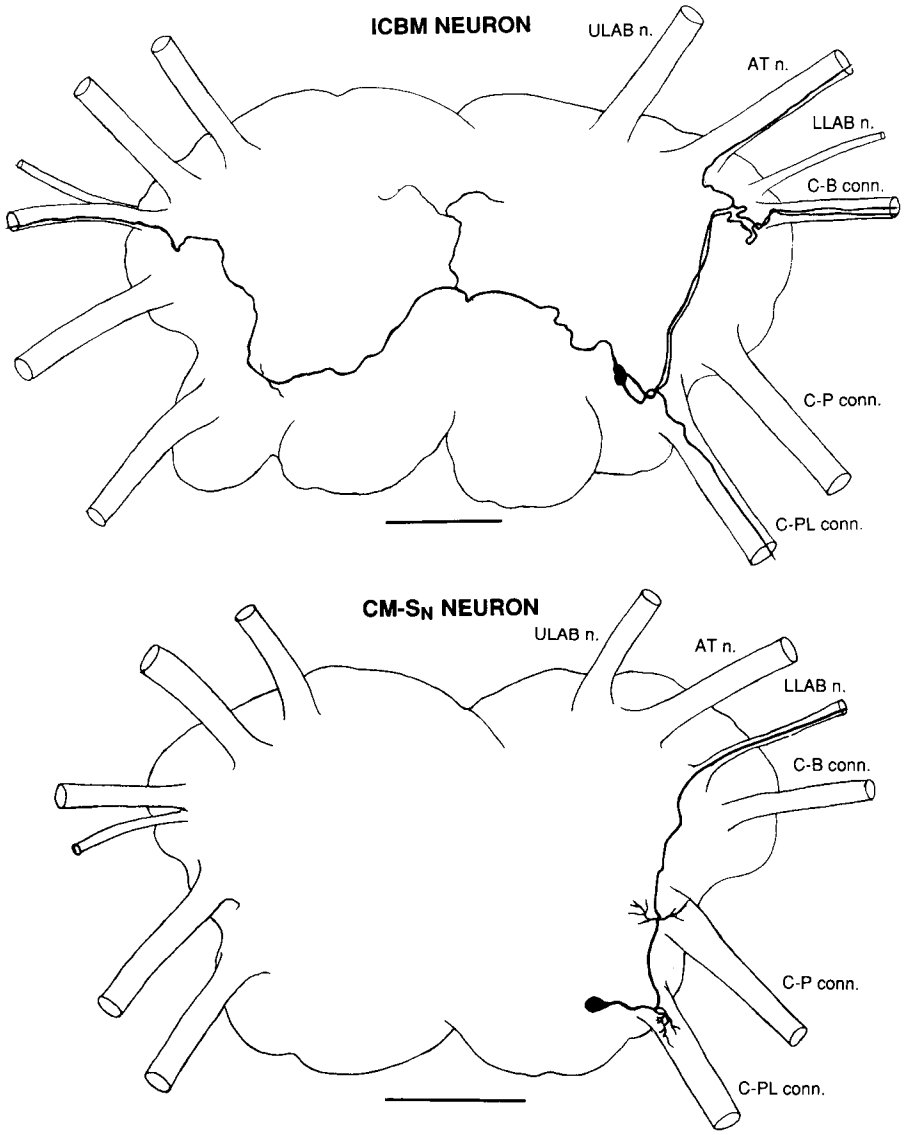


Figure 2. Camera lucida drawings of an ICBM neuron (*top*) and a non-ICBM CM-S_N neuron (*bottom*) that had intrasomatic iontophoretic injections of Lucifer yellow fluorescent dye. Calibration bar, 1 mm. Abbreviations: *ULAB,* upper labial; *AT,* anterior tentacular; LLAB, lower labial; *C-B,* cerebral–buccal; *C-P,* cerebral–pedal; *C-PL,* cerebral–pleural; *n,* nerve; *conn.,* connective.

(C-PL) connective (Fig. 2, top). The other group ($n = 3$) appeared to be a unique subclass of CM-S_N cells that had a single main axon in the lower labial (LLAB) nerve (Fig. 2, bottom). To further characterize this latter group of neurons, we conducted additional experiments to confirm that they were indeed sensory cells. We found that, as with ICBM neurons, bursts of action potentials could be evoked in the non-ICBM CM-S_N cells when mechanical, but not chemical, stimuli were delivered to receptive fields confined to the perioral zone, i.e., between the lips and the jaws (see Rosen et al., 1982). Moreover, similar to other CM neurons, these cells made monosynaptic connections to B cluster motoneurons. In several experiments, we found that an individual B cluster neuron received convergent input from each of the classes of mechanoafferents we described, i.e., CM-S_B, ICBM CM-S_N, and non-ICBM CM-S_N cells.

Modulation of spike duration and changes in postsynaptic potentials evoked in follower cells of the cerebral ganglion mechanoafferent neurons

Previous studies of mechanoreceptor neurons in the abdominal and pleural ganglia have shown that under certain conditions changes in spike width due to 5-HT (or other neuromodulators) are correlated with changes of synaptic output of the neuron (Brunelli et al., 1976; Klein and Kandel, 1978; Walters et al., 1983b). To study this issue in the CM neurons, we altered spike duration with 5-HT and examined the size of EPSPs evoked in follower cells, both in normal ASW and TEA-ASW in the presence of high divalent cations. These data indicate whether or not changes in spike duration recorded in the CM somata reflect alterations affecting transmitter release at the axon terminals of the neurons.

Figure 3 shows a typical result of experiments in which the effects of bath application of 5-HT on the action potential duration of a CM-S_B neuron and the change in magnitude of the EPSP evoked in a follower neuron were examined in the presence of TEA in high divalent cation ASW. The follower cell was a member of the identified B clusters of the cerebral ganglion (Jahan-Parwar and Fredman, 1976), many of which receive extensive monosynaptic inputs from cells in the mechanoafferent clusters (Rosen et al., 1979). A spike in the CM-S_B neuron was elicited once per minute. The EPSP evoked in the B neuron gradually decremented to 40% of its initial value, but reached a stable value of 5 mV after 10 min (Fig. 3*A1*). Following a brief pulse of 10^{-4} M 5-HT into the bath, the spike of the CM-

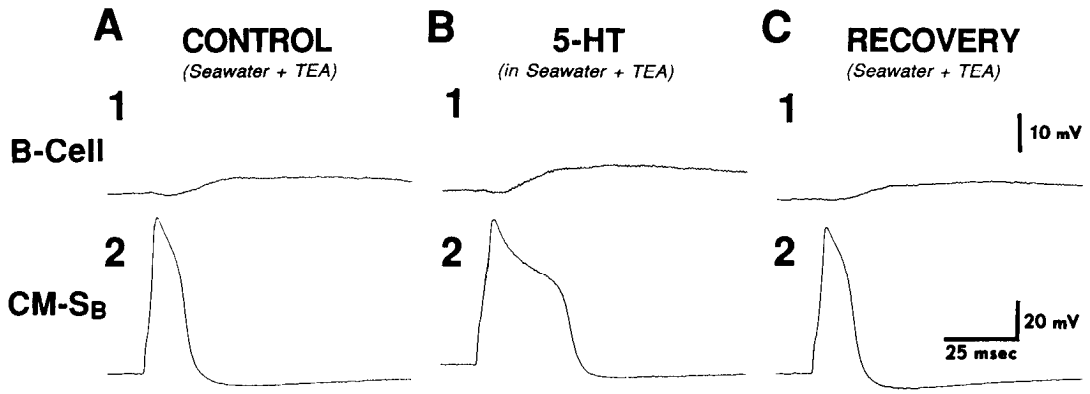


Figure 3. Effects of 5-HT on the action potential duration of a CM-S_B neuron (*bottom traces*) and the resulting EPSP evoked in a postsynaptic B-cell (*top traces*). Cerebral ganglion was superfused with TEA in high divalent cation ASW. The CM-S_B neuron was stimulated at the rate of 1/min. During control recording the amplitude of the EPSP was observed to decrement to 40% of its initial value, at which point it stabilized (*A1*). 5-HT was then applied by micropipette injection of 25 μl of a 10^{-4} M solution. CM-S_B spike broadening correlated with an increase in EPSP amplitude (*B*). During recovery the CM-S_B spike and the B-cell EPSP returned to control levels (*C*).

S_B neuron broadened (Fig. 3*B2*) and the EPSP increased in amplitude and duration (Fig. 3*A2*). After 5 min of continued superfusion, both the EPSP amplitude and the CM-S_B spike width returned to control levels (Fig. 3*C*). Opposite results were observed in a similar experiment in which the effects of 5-HT on the spike duration of a CM-S_N neuron and the EPSP it evoked in a B cluster neuron were examined. For example, in the experiment shown in Figure 4, during the initial period of repetitive intracellular stimulation of the CM-S_N neuron, the evoked B-cell EPSP gradually declined to 30% of its initial value and then stabilized (Fig. 4*A*). A brief pulse of 10^{-4} M 5-HT caused the CM-S_N neuron spike to narrow (Fig. 4*B2*) and the B-cell EPSP to decrease in magnitude (Fig. 4*B1*). The effects could also be reversed with continued superfusion with the TEA, high divalent cation ASW medium (Fig. 4*C1*). Four of four CM-S_N to B-cell connections studied in 4 animals showed 5-HT-induced synaptic facilitation. Five of five ICBM to B-cell connections in 4 other animals showed 5-HT-induced depression.

The effects of bath applications of 5-HT on the efficacy of synaptic connections of CM-S_B and CM-S_N neurons to B cluster neurons were reexamined under more normal physiological conditions when the ganglion was bathed in TEA-free ASW. ICBM neurons were selected as examples of 5-HT-narrower neurons, since these could be readily identified by their action potential response to C-B connective stimulation. In procedures similar to those described above, we found that, following exposure to 5-HT, we were unable to detect significant changes in spike duration. Nevertheless, 5-HT increased the magnitude of B-cell EPSPs evoked by CM-S_B neurons and decreased the size of EPSPs evoked by the ICBM neurons. Both of these effects could be reversed following wash-out of the 5-HT.

As well as evoking EPSPs in B cluster neurons, ICBM neurons evoke EPSPs in the serotonergic, metacerebral cells (MCCs) in the cerebral ganglion, and in neurons B4 and B5 in the buccal ganglion (Rosen et al., 1982). We therefore examined the effects of 5-HT on the EPSPs evoked by ICBM neurons in these cells. 5-HT abolished the EPSPs in B4 and B5 in 3 of 3 separate experiments. We could not demonstrate consistent and reversible effects of 5-HT on the EPSP in the MCC, perhaps because the connection is weak to begin with, and rapidly decrements upon repeated stimulation.

Effects of cerebral ganglion nerve and connective stimulation on mechanoafferent neuron spike duration

In order to determine whether the effects of bath applications of 5-HT might reflect processes that could occur during synaptic

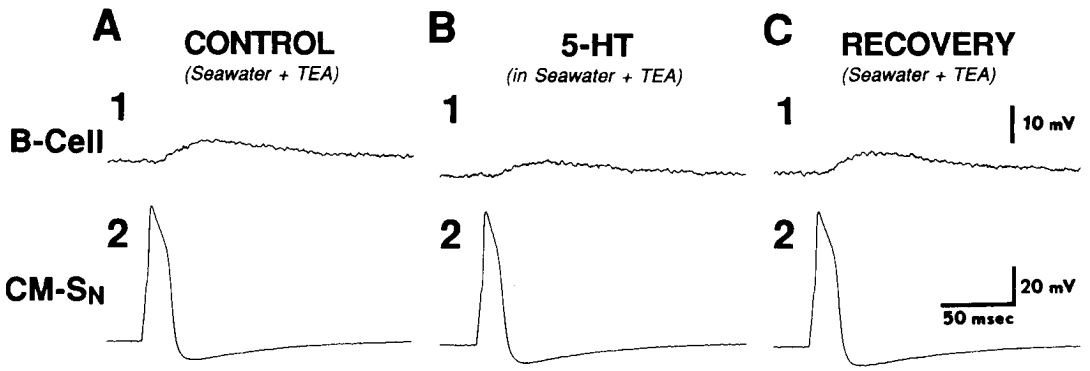


Figure 4. Effects of 5-HT on the action potential duration of a CM-S_N neuron (*bottom traces*) and the resulting EPSP evoked in a postsynaptic B-cell (*top traces*). The cerebral ganglion was superfused with TEA in high divalent cation ASW. The CM-S_N was intracellularly stimulated at the rate of 1/min. During control recording the amplitude of the EPSP was observed to decrement to 30% of its initial value, at which point it stabilized (*A1*). 5-HT was then applied by means of a micropipette injection of 25 μl of a 10^{-4} M solution. CM-S_N spike narrowing correlated with a reversible reduction in EPSP amplitude (*B*). During recovery the spike width and the EPSP amplitude returned to control levels (*C*).

activity in the ganglion, we investigated whether changes in spike width could be produced by synaptic input evoked by electrical stimulation of nerves and connectives of the cerebral ganglion. For nearly all of the mechanoafferent neurons tested, electrical stimulation of one or another nerve or connective modified the duration of TEA-broadened spikes by as much as 50%. In no case was a conventional synaptic potential recorded in the sensory neuron, although occasionally slow depolarizing or hyperpolarizing responses lasting many seconds were detected. The spike width of individual cells had the capacity to either broaden or narrow, depending upon which nerve was stimulated. Moreover, the same nerve or connective could, in certain instances, either broaden or narrow the spikes of an individual cell depending on the level of stimulating voltage applied, which we presume recruited different fibers. Figure 5 shows intracellularly evoked spikes recorded from a single CM-S_B neuron before (Fig. 5*A*) and after (Fig. 5*D*) anterior tentacular nerve stimulation. A single 2 V (4 msec) nerve shock produced spike narrowing lasting several minutes (Fig. 5*B*). Following recovery, a second, stronger (4 V) shock produced spike broadening lasting in excess of 10 min (Fig. 5*C*). The effect could be repeated, although the amount of narrowing and broadening was reduced. Indeed, a general feature of the modification of spike duration by nerve stimulation was that repeated stimulation resulted in progressively weaker responses, unless the preparation was allowed to rest for periods of an hour or more. This could be the result of the presence of TEA in the perfusion medium, which results in a large amount of transmitter release per spike and may produce transmitter depletion. Among the cerebral nerves and connectives tested, stimulation of the C-PL connective produced the largest modifications of spike duration. Stimulation of the C-B connective produced smaller effects, consisting chiefly of spike broadening.

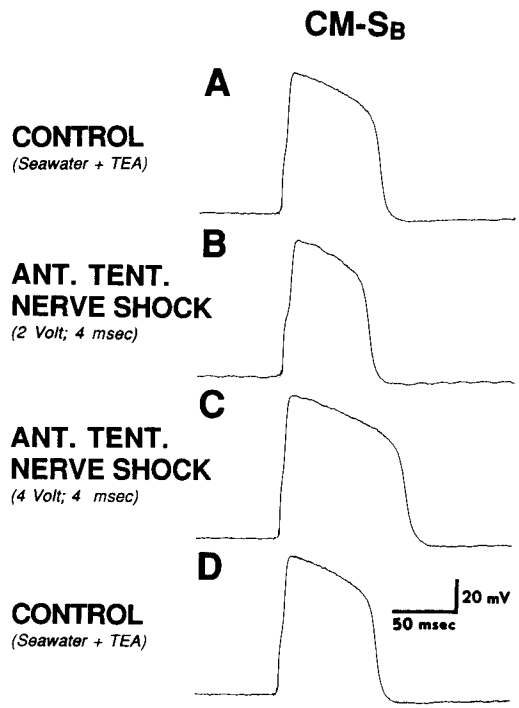


Figure 5. Effects of anterior tentacular (*Ant. Tent.*) nerve stimulation on the action potential duration of a CM-S_B neuron. For control recording (*A, D*), the neuron was stimulated intracellularly at the rate of 0.2 Hz while it was superfused with TEA in high divalent cation ASW, as previously described. A suction electrode was attached to the cut end of the ipsilateral anterior tentacular nerve. Stimulation of the nerve with a single 2 V, 4 msec current pulse produced spike narrowing (*B*) that lasted several minutes. Following recovery, a second 4 V, 4 msec pulse produced spike broadening (*C*) that lasted 10 min before the response recovered to control levels (*D*).

Heterosynaptic facilitation and depression of EPSPs evoked in follower (postsynaptic) B-cells of cerebral ganglion mechanoafferent neurons

The data in the previous sections suggest that the synaptic output of the CM neurons may be modulated by mechanisms of heterosynaptic facilitation and depression evoked by electrical stimulation of nerves or connectives, and indeed this was found to be the case in approximately half of the neuron pairs that were studied. Figure 6*A* shows an example of heterosynaptic facilitation of the EPSPs evoked in a B-cell neuron (top traces) by single action potentials elicited in a CM-S_B neuron (bottom traces) when a facilitating stimulus, consisting of a brief train of electrical pulses, was delivered to the ipsilateral anterior tentacular (AT) nerve. Prior to nerve stimulation, the CM-S_B neuron was stimulated every 24 sec. The heterosynaptic stimulus was delivered following an initial 10 min period of homosynaptic depression, during which time the amplitude of the EPSP evoked in the B-cell declined and then stabilized at 40% of its initial value. The following EPSPs showed a 100% increase in amplitude, and the first poststimulus EPSP was sufficient to trigger a B-cell spike. The facilitated EPSPs declined to prestimulus baseline levels several minutes after the nerve shocks. The facilitating effect of AT nerve stimulation could be mimicked by bath application of 5-HT (10^{-4} M). Since nerve stimulation and bath applications of neurotransmitters frequently altered the membrane potential of CM neurons by evoking slow depolarizations or hyperpolarizations, we manipulated membrane potential to see if it would activate steady-state currents that might enhance transmitter release and account for the facilitation of the EPSP we observed (Klein and Kandel, 1978; Shimahara and Peretz, 1978; Shapiro et al., 1980; Coates and Bulloch, 1985; Weiss et al., 1986d). An examination of the synaptic connection between a CM neuron and a B-cell that exhibited heterosynaptic facilitation indicated that, although manipulation of membrane potential in the soma of the CM neurons by relatively large depolarizing steps could produce alteration of the spike amplitude and half-amplitude spike width, it did not affect the EPSPs recorded in the follower B-cell. These findings suggest either that changes in resting membrane potential at the synaptic terminals of these neurons has a minimal effect on the release of transmitter or, more likely, that the terminals are too electrically distant from the soma to be affected by currents injected into the soma.

Since 5-HT and nerve stimulation can produce spike narrowing in some CM neurons, we attempted to determine if heterosynaptic depression of synaptic connections between CM neurons and their follower cells could be demonstrated. Figure 6*B* is an example of a connection between a CM neuron and a B-cell that exhibited heterosynaptic depression following stimulation of the C-PL connective. Prior to nerve stimulation this connection exhibited homosynaptic depression of EPSP amplitude. After the EPSP amplitude stabilized at 60% of its initial value, nerve stimulation resulted in a subsequent reduction of EPSP amplitude, with recovery after several minutes. Heterosynaptic depression or facilitation was found to be dependent on the specific CM neuron tested. In a given preparation, a

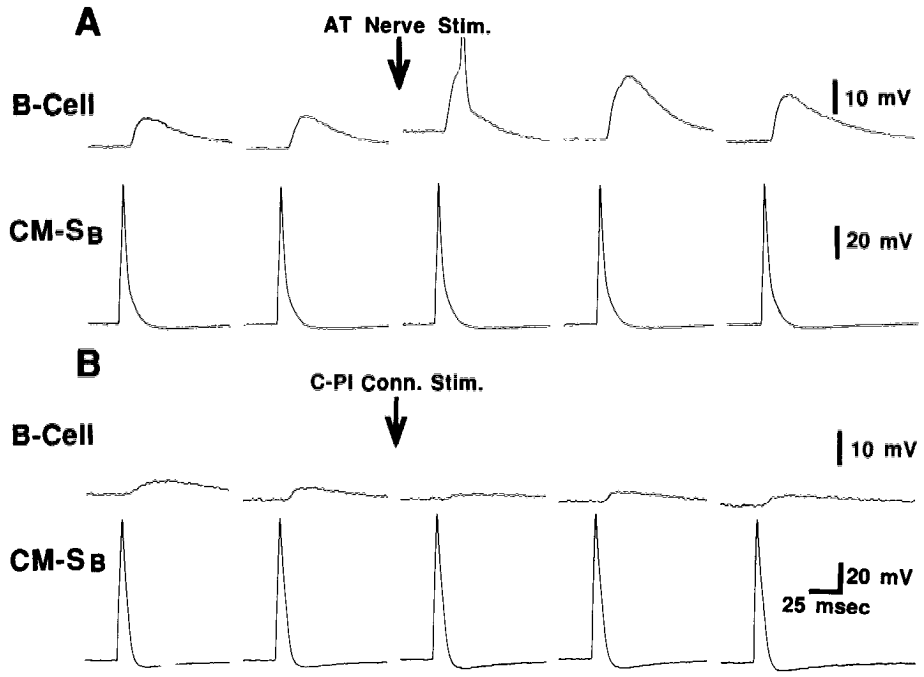
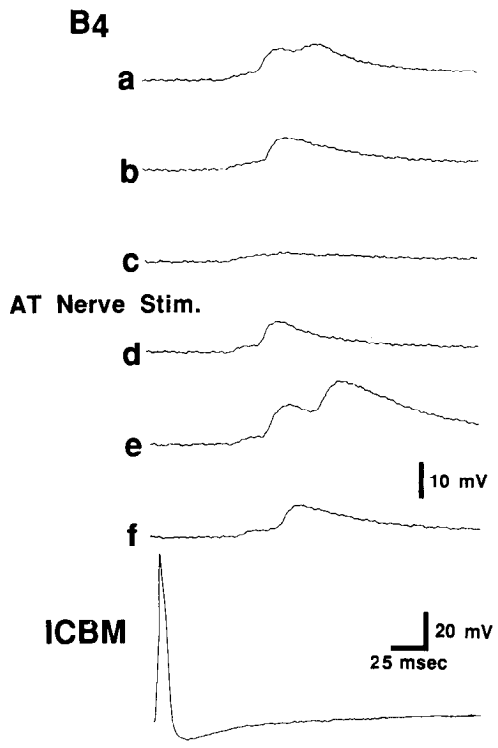


Figure 6. *A,* Heterosynaptic facilitation of the EPSPs evoked in a B-cell (*top traces*) by action potentials elicited in a CM-S_B neuron (*bottom traces*). The heterosynaptic stimulus was a 5 sec train of 2 Hz nerve shocks (4 msec, 4 V) delivered to the ipsilateral AT nerve (*arrow*). Single spikes were elicited in the CM-S_B neuron by 3 msec intracellular current pulses delivered every 24 sec. Following an initial period of homosynaptic depression, during which time the amplitude of the EPSP evoked in the B-cell declined to 40% of its initial value and then stabilized, the heterosynaptic stimulus was delivered. The following EPSPs showed a 100% increase in amplitude. The amplitude of the first postshock EPSP was sufficient to trigger a B-cell spike. The facilitated EPSPs declined to baseline levels several minutes after the nerve shocks. In this experiment the ganglion was bathed in high divalent cation ASW with 10 mM Tris buffer. *B,* Heterosynaptic depression of EPSPs evoked in a B cluster neuron (*B-cell, top traces*) by action potentials elicited in a CM-S_B neuron (*bottom traces*). The heterosynaptic stimulus was a 5 sec train of 2 Hz nerve shocks (4 msec, 4 V) delivered (*arrow*) to the ipsilateral C-PL connective. Single spikes were elicited in the CM-S_B neuron by 3 msec intracellular current pulses delivered at a rate of 1 per 35 sec while the ganglion was superfused in high divalent cation ASW. After an initial period of homosynaptic depression during which the EPSP amplitude stabilized, the nerve shocks were delivered. The amplitude of the following EPSPs was decremented further, but the effect was short-lived, and the EPSP amplitude returned to its preshock level within several minutes.



constant stimulus applied to the AT nerve could produce either heterosynaptic facilitation or heterosynaptic depression of the EPSP evoked in a given B-cell, depending upon which of 2 different CM neurons was stimulated to evoke the EPSP.

In order to determine whether the changes in EPSP amplitude resulting from nerve stimulation might be due to a postsynaptic

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Figure 7. Heterosynaptic facilitation of EPSPs evoked in identified buccal interneuron B4 (*B4, a–f*) by action potentials elicited in an ICBM neuron (*bottom trace*). The stimulus was a train of 5 nerve shocks (4 msec, 4 V) delivered to the ipsilateral AT nerve. Single action potentials were elicited in the ICBM neuron by 3 msec intracellular current pulses delivered every 30 sec. The ganglion was superfused with high divalent cation ASW. The 3 compound EPSPs before (*a–c*) and after (*d–f*) the heterosynaptic stimulus are shown. Prior to nerve stimulation the ICBM spike evoked a multiphasic compound EPSP in B4 (*a*). Repeated stimulation resulted in decrements in 3 discernible components of the EPSP (*b, c*). Moreover, 2 of the components dropped out of the response, leaving the shortest latency, presumably monosynaptic, component. Following nerve stimulation the 2 di- or polysynaptic components reappeared (*d, e*). The amplitudes of these responses were greater than in control recording. Furthermore, the second component gradually increased in amplitude following nerve shock (*e*), suggesting that its increase in amplitude was not simply due to recovery from repeated stimulation during the period in which the response was not present but rather to some other active process. The records fail to indicate any facilitation of the short-latency, presumably monosynaptic, component of the evoked EPSP.

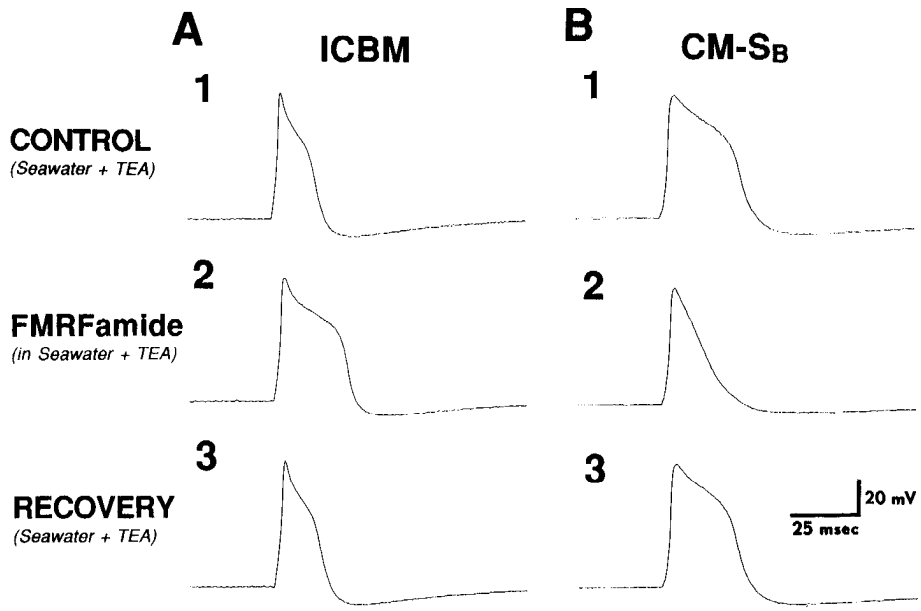


Figure 8. Effects of FMRFamide on the duration of action potentials elicited in a CM-S_N (ICBM) and a CM-S_B class of mechanoafferent neuron. Spikes were elicited by intracellular current pulses (3 msec duration, 0.2 Hz) while the ganglion was superfused with TEA in high divalent cation ASW (*A1, B1*). Following a brief 25 μl microinjection of 10^{-3} M FMRFamide into the perfusion chamber, the ICBM spike broadened to 150% of its control value (*A2*) and the CM-S_B spike narrowed to 40% of its control value (*B2*). Continued superfusion with TEA in high divalent cation ASW resulted in recovery of the broadened and narrowed spikes to their control levels of spike duration (*A3, B3*).

change of conductance, the input conductance was measured for 4 of the B-cells exhibiting heterosynaptic facilitation and for 2 B-cells exhibiting depression (see Materials and Methods). We found that changes in input conductance could not account for the changes in synaptic size following nerve stimulation. In fact, for reasons that are not clear, the conductance measurements indicated a change opposite that which could account for an alteration of the PSPs by a postsynaptic change of conductance.

Heterosynaptic facilitation of EPSPs evoked in ICBM neuron followers in the buccal ganglia

Since it is possible to demonstrate branch-specific facilitation of EPSPs evoked by mechanoafferent neurons in the abdominal ganglion of *Aplysia* (Clark and Kandel, 1984), we sought to determine if it was possible to obtain heterosynaptic facilitation of the connections of ICBM neurons in the cerebral ganglion to identified cells B4 and B5, which are located in the buccal ganglia. Figure 7, *a–f* shows a series of compound EPSPs evoked in cell B4 by single ICBM spikes before and after delivery of a train of electrical stimuli to the ipsilateral AT nerve. The initial EPSPs consisted of 3 distinct components that could be distinguished by their latencies and amplitudes (Fig. 7*a*). Each exhibited low-frequency depression with repeated activation of ICBM spikes. The late and middle components gradually disappeared (Fig. 7, *b, c*), leaving only the early component, which was always present. Previous studies (Rosen et al., 1982) indicated that the early component represents a monosynaptic connection of the ICBM neuron to B4. The later components reflect indirect inputs mediated by interneurons which have recently been identified in the cerebral ganglion (Rosen et al., 1987). Following a nerve stimulus, the late components of the EPSPs evoked in B4 reappear (Fig. 7, *d, e*). Moreover, the am-

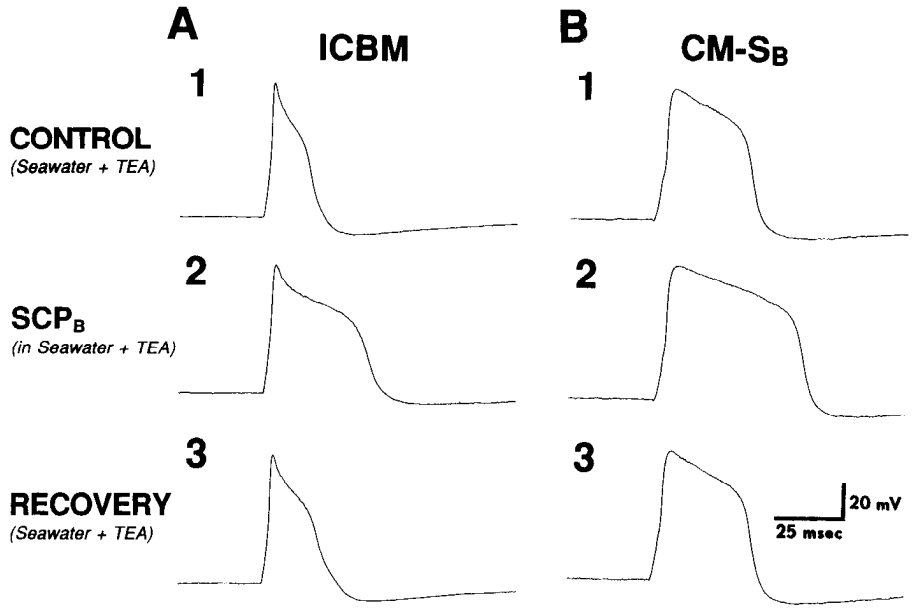


Figure 9. Effects of SCP_B on the duration of action potentials elicited in a CM-S_N (ICBM) and a CM-S_B class of mechanoafferent neuron. Spikes were elicited by intracellular current pulses (3 msec duration, 0.2 Hz) while the ganglion was superfused with TEA in high divalent cation ASW (*A1, B1*). Following a brief 25 μl microinjection of 10^{-5} M SCP_B into the perfusion chamber, both the ICBM and the CM-S_B spikes broadened to 200% (*A2*) and 175% (*B2*), respectively, of control values. Continued superfusion with TEA in high divalent cation ASW resulted in recovery of the broadened spikes to their control levels of spike duration (*A3, B3*).

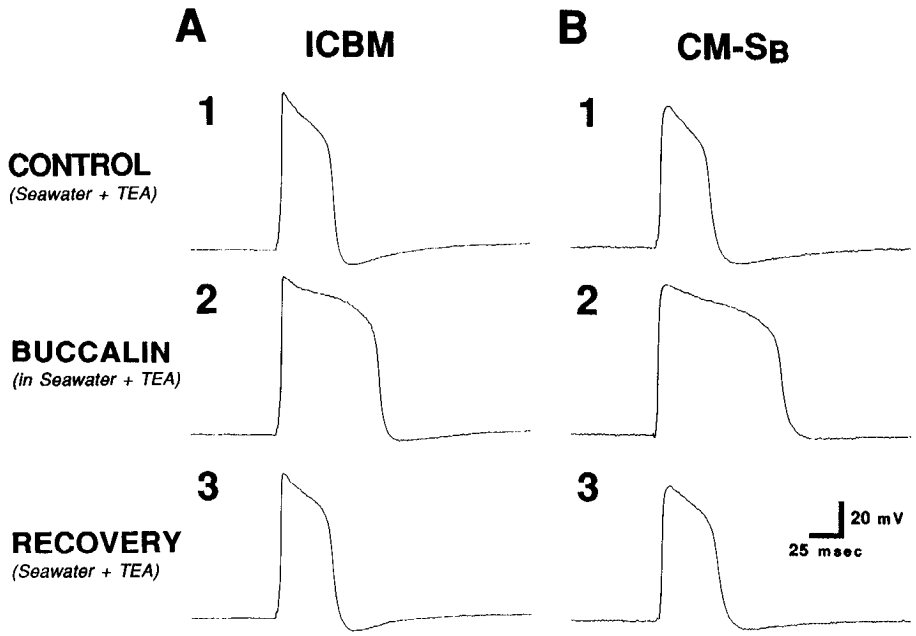


Figure 10. Effects of the neuropeptide buccalin on action potentials elicited in an ICBM (*A*) and a CM-S_B (*B*) type of neuron. With constant superfusion methods previously described, applications of buccalin broadened the spikes of both types of CM neurons. However, the effects were small (<10% change) and short-lived (1 min), even at high concentrations (10^{-3} M). The marked spike-broadening effects shown in *A2* and *B2* were obtained when buccalin application (25 μl at 10^{-4} M concentration) followed discontinuation of the superfusion. Several minutes of mixing and diffusion were required to obtain maximal responses. Continuation of superfusion had the immediate effect of decreasing spike width. Complete recovery to control levels was achieved in several minutes (*A3, B3*).

plitude of the later components is greatly enhanced compared with that seen in the prestimulus responses, whereas the amplitude of the early component remains unchanged (observed in 2 of 2 experiments). Additional ICBM activation again resulted in decrements in EPSP amplitude and the dropping out of the later component of the response (Fig. 7*f*). These data suggest that cerebral nerve stimulation may not modulate ICBM to buccal neuron connections but may facilitate an ICBM to cerebral interneuron connection through an, as yet unknown, pre- or postsynaptic mechanism.

Heterogeneity of responses to FMRFamide among mechanoafferent neurons opposite to that of serotonin

The varied effects of nerve stimulation on the CM neurons suggested that they may be modulated by substances other than 5-HT. Furthermore, previous studies have shown that, in addition to 5-HT, the activity of pleural and abdominal ganglia mechanoafferent neurons in *Aplysia* can be modified by the neuropeptide FMRFamide (Price and Greenberg, 1977) and other putative neurotransmitters (Abrams et al., 1984; Ocorr and Byrne, 1985) that have been localized to peptide containing cells in the abdominal, buccal, and circumesophageal ganglia (Lehman et al., 1984; Brown et al., 1985; Schaefer et al., 1985; Lloyd et al., 1987; Mackey et al., 1987). We therefore next tested the effects of bath applications of FMRFamide and various other putative peptide modulators on the classes of cerebral mechanoafferents that were differentiated by their responses to 5-HT. In these experiments, individual mechanoafferent neurons whose spikes were broadened with TEA in high divalent cation ASW were serially tested with bath applications of 5-HT and FMRFamide. The order of treatments was switched in successive experiments. When FMRFamide was applied to ICBM or non-ICBM CM-S_N neurons, spike broadening was observed (Fig. 8*A*). The effect was relatively short-lived (5–20 sec) and weak (spike widths 105–120% of control), even at 10^{-2}–10^{-4} M concentrations. In no case was spike narrowing observed. In additional control experiments, FMRFamide was applied to the ganglion bath without ongoing perfusion. Under this condition, spike broadening was enhanced but was still short-lived, indicating that the FMRFamide response in CM neurons rapidly desensitizes. By contrast, FMRFamide narrowed the "TEA spikes" of the CM-S_B class of mechanoafferent neurons, whose spikes broaden to 5-HT (Fig. 8*B*). These results indicate that like 5-HT, FMRFamide can distinguish among classes of cerebral mechanoafferents, although it produces opposite effects.

Uniformity of response to SCP_B among classes of mechanoafferent neurons in the cerebral ganglion

We next tested the effects of the neuropeptide SCP_B (Morris et al., 1982) on the duration of spikes of CM neurons. Of particular interest was the effect of SCP_B on the unusual class of neurons (CM-S_N, including ICBMs) that respond with spike narrowing to 5-HT. SCP_B in *Aplysia* had previously been shown to have effects that parallel those of 5-HT in several different tissues, including neurons, buccal muscle, and heart tissue (Abrams et al., 1984; Lloyd et al., 1984, 1985b), although some evidence suggests that SCP_B and 5-HT act on different receptors (Abrams et al., 1984; Lloyd et al., 1984, 1985b; Ocorr and Byrne, 1985). We found, however, that SCP_B had an effect opposite that of 5-HT on the ICBM neurons, i.e., it broadened the spikes (Fig. 9*A*). SCP_B also markedly broadened spikes of the CM-S_B neurons, i.e., on this class the neuropeptide had the same action as 5-HT (Fig. 9*B*). In every CM neuron tested, SCP_B broadened the "TEA spike."

Two new neuropeptides, buccalin and myomodulin, modulate action potential duration of cerebral mechanoafferent neurons

Buccalin and myomodulin are 2 recently characterized *Aplysia* neuropeptides (Cropper et al., 1987a–c) that are found in buccal muscle and in identified nerve cells (Alevizos et al., 1987; Cropper et al., 1987b, c). We have found that the actions of buccalin were generally similar to those of SCP_B, in that buccalin consistently broadened the spikes of neurons in both the CM-S_N and CM-S_B classes of neurons (Fig. 10). However, the modes of action of buccalin and SCP_B differed in that under conditions of constant superfusion, where the putative neuromodulator is

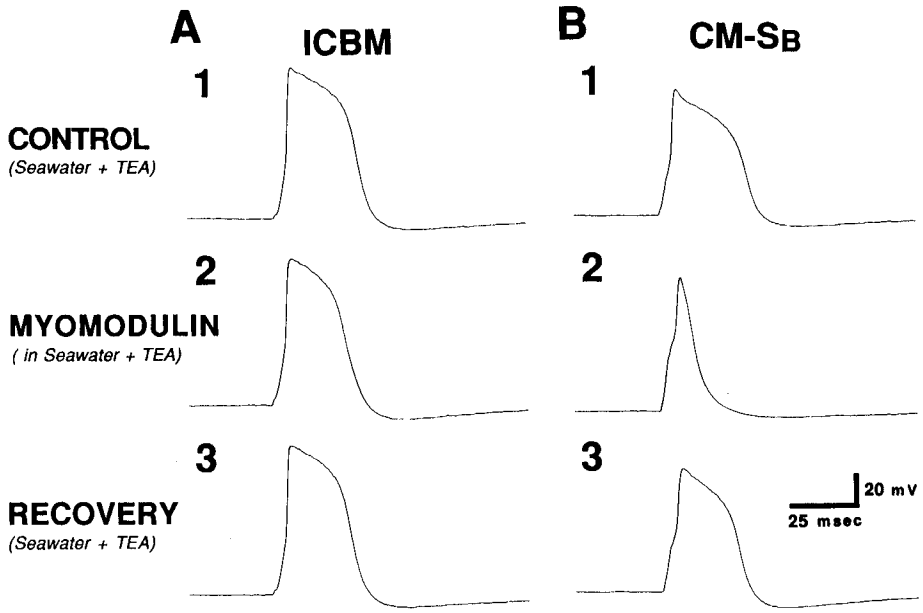


Figure 11. Effects of the neuropeptide myomodulin on action potentials elicited in an ICBM (*A*) and a CM-S_B (*B*) type of neuron. Myomodulin had no effect on the ICBM neuron (*A2*) regardless of concentration or rate of superfusion of the ganglion. Myomodulin produced marked spike narrowing in the CM-S_B neuron after a 25 μl (10^{-4} M conc.) quantity was applied to the ganglion bath during ongoing superfusion (*B2*).

applied and immediately washed away, buccalin typically produced only 10–20% spike broadening (at 10^{-3}–10^{-5} M conc.), whereas SCP_B produced broadening in excess of 200% of control. However, when buccalin was applied under stop flow conditions, spike width gradually increased until a dramatic effect, equivalent to that seen with SCP_B, was produced. When the wash-out was begun, spike width almost immediately began to return to control levels. By contrast, responses to SCP_B, as well as 5-HT, characteristically required several minutes before the beginning of recovery to control levels.

The neuropeptide myomodulin was most similar to FMRFamide in its actions. It produced marked spike narrowing in CM-S_B neurons but not in CM-S_N cells (Fig. 11). However, unlike FMRFamide, which produced a modest, short-lived spike broadening response in CM-S_N neurons, myomodulin had virtually no effect on these cells at concentrations ranging from 10^{-2} to 10^{-5} M.

Another agent we investigated that produced no effect on CM-S_N cells, or CM-S_B cells for that matter, was histamine. Histamine was of interest because it is the transmitter of identified neuron C2 of the cerebral ganglion (McCaman and Weinreich, 1985), which appears to be involved in food arousal in *Aplysia* (Chiel et al., 1986; Weiss et al., 1986a, b, d). Moreover, histamine produces presynaptic inhibition at terminals of buccal–cerebral interneurons (Chiel et al., 1988) and interneuron L10 in the abdominal ganglion (Kretz et al., 1986).

Discussion

Three main conclusions can be derived from this study. First, the small neurons comprising the contiguous lateral and medial mechanoafferent cell clusters found on the ventrocaudal surface of each cerebral hemiganglion of *Aplysia* (Rosen et al., 1979) are a heterogeneous population of cells that can be subdivided into 2 broad classes on the basis of their responses to the neuromodulator 5-HT. Second, the synaptic output of the CM neurons is modifiable and may be either facilitated or depressed. Thus, locomotor, defensive, and appetitive behaviors, which appear to be regulated by CM neurons, may be subject to extensive modulation operating at the level of the very initial processing of sensory information. Third, the CM neurons exhibit a remarkable diversity of responses to many putative neuromodulators, including 4 different neuroactive peptides and 5-HT.

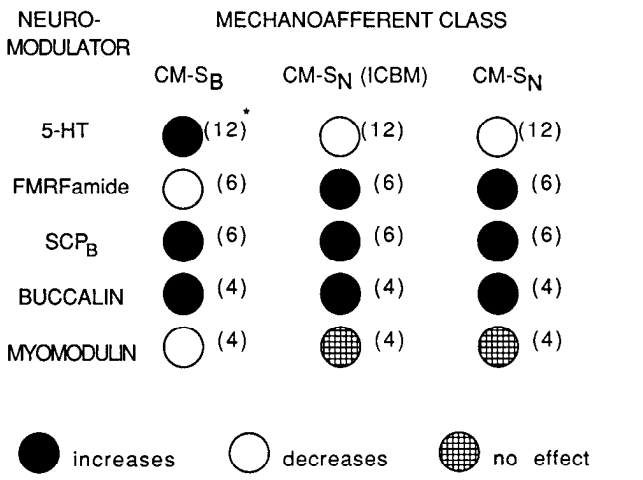
Heterogeneity of cerebral mechanoafferent neurons

Previous data indicated that the CM neurons could be divided into 2 groups, based on whether they sent an axon into the C-B connective (i.e., ICBM neurons) or failed to send such an axon into the connective (other CM neurons). In this study, we have found that CM neurons can also be distinguished on the basis of their opposite responses to 5-HT. In the presence of TEA, the duration of intracellularly evoked action potentials was increased by 5-HT application in one class of cells (CM-S_B) and decreased in another class (CM-S_N). Furthermore, the CM-S_N neurons can be subdivided. One type consists of the ICBM neurons (Rosen et al., 1982) and sends axons to the C-B connective. The other type does not send an axon into the connective, and dye fills support the idea that these are a morphologically distinct subclass of neurons whose spikes narrow to 5-HT. Like ICBM sensory cells, non-ICBM CM-S_N neurons respond to mechanical but not to chemical stimuli applied to a perioral zone receptive field. They also make excitatory monosynaptic connections to B cluster putative motoneurons (Fredman and Jahan-Parwar, 1977). Indeed, we have been able to demonstrate that individual B cluster neurons receive convergent input from ICBM neurons, non-ICBM CM-S_N neurons, and CM-S_B neurons. The present results suggest a new level of complexity in the classification of mechanoafferent neurons in the cerebral ganglion of *Aplysia*. They indicate that a biochemical (or pharmacological) dimension need be considered together with a morphological or physiological one in the functional classification of sensory cells in *Aplysia*.

Plasticity of the synaptic output of cerebral mechanoafferent neurons

A previous study of the CM neurons reported that stimulation of cerebral nerves could enhance the capacity of the neurons to evoke spikes in B-cells, but the effect could be accounted for by

Table 1. Effects of neuromodulators on action potential duration among classes of mechanoafferent neurons in the cerebral ganglion of *Aplysia*



* Numbers of individual neurons tested are in parentheses. The numbers do not reflect the proportion of each type that can be found in the cerebral mechanoafferent neuron clusters.

a postsynaptic action since the nerve stimulation evokes a tonic depolarization in the B-cells (Rosen et al., 1979). The present results suggest that by careful adjustment of the parameters of nerve stimulation, the CMs can be shown to exhibit presynaptic modulation of their output. Moreover, nerve and connective stimulation, as a function of the strength of the electrical stimulation, can produce both heterosynaptic facilitation and heterosynaptic depression of the EPSPs evoked by CM neurons in B cluster neurons. Thus, while in previous work, mechanical stimulation of the skin or electrical stimulation of the cerebral nerves always resulted in tonic depolarization of the B cluster neurons (Rosen et al., 1979), such stimulation may also produce simultaneous facilitatory and inhibitory effects on the terminals of the sensory neurons. These opposing effects may cancel one another, leaving the postsynaptic depolarization as the main determinant of the ability of the synaptic input to fire the cell.

Diversity of mechanoafferent responses to 5-HT and neuropeptides

The diverse effects of nerve stimulation on the output of the various CM neurons suggested that the CM neurons may be modulated by substances other than 5-HT, and, in fact, we have found that the CM neurons also respond to application of a number of peptide putative neuromodulators. Table 1 summarizes the responses of the different classes of CM neurons. Table 1 includes a subset of the total data, in which it was possible to observe the effects of 5-HT plus at least one other putative modulator. Although there was a great diversity of responses, the effects of a given substance on a given class of neuron were highly reproducible. Aside from the consistency of the effects within a class of CM neuron, the nature of the response to different neuromodulators failed to follow any simple logic or functional rules. Thus, whereas all neurons responded to FMRFamide in a manner opposite to 5-HT, the response to 5-HT failed to predict their response to the other neuromodulators we tested. The recently characterized peptide myomodulin (Cropper et al., 1987a, b) narrowed the spike of the 5-HT-broadener (CM-S$_B$) neurons, but had no effect on the 5-HT-narrower (CM-S$_N$) neurons. By contrast, buccalin (Cropper et al., 1987a, b) and SCP$_B$ always produced an increase in spike duration in both classes of neurons, although based on their time course of effects, their modes of action appear to be different.

The subclass of CMs that exhibit spike broadening to 5-HT (CM-S$_B$ neurons) appears similar in most regards to the mechanoafferent neurons that have been described in the abdominal and pleural ganglia of *Aplysia* (Byrne et al., 1974; Klein et al., 1982; Walters et al., 1983b; Pollock et al., 1985). The abdominal and pleural cells also show spike narrowing to FMRFamide and broadening to 5-HT and SCP$_B$ (Abrams et al., 1984; Belardetti et al., 1987; see also Ocorr and Byrne, 1985). Based on these similarities, we suggest that the CM-S$_B$ neurons are involved in defensive reflexes, as is the case for the abdominal and pleural mechanoafferents. In terms of their responses to the 5 different neuromodulators we studied, the CM-S$_N$ ICBM and CM-S$_N$ non-ICBM neurons are identical. These 2 subclasses of CM-S$_N$ neurons have similar receptive fields, and it is likely that they are involved in similar types of functions, most likely related to feeding behavior, since ICBM neurons can trigger motor output of the buccal ganglion and provide an excitatory input to the MCC (Rosen et al., 1982), a cell that contributes to the arousal of feeding (Weiss et al., 1975, 1978; Weiss and Kupfermann, 1977; Kupfermann and Weiss, 1982; Rosen et al., 1983).

The implications of the present findings are that neurons containing various neuromodulators which have been found in the central ganglia of *Aplysia* (Lehman et al., 1984; Lloyd et al., 1985a; Mahon et al., 1985; Cropper et al., 1987a–c; Mackey et al., 1987), or other similar acting agonists can enhance the output of one group of sensory cells and simultaneously depress the output of another group. This mechanism may provide a means of selecting for activation one of a number of neural circuits from a network (see Getting and Dekin, 1985; Marder and Hooper, 1985; Selverston and Moulins, 1985; Harris-Warrick, 1988) of which the mechanoafferent neurons are a part. The selection of a particular neural circuit may be the basis for the expression at a particular time of one of several possible behavioral responses mediated by mechanosensory inputs. It is clear from behavioral studies that mechanosensory input to the head of *Aplysia* has a powerful role in feeding, locomotion, and defensive behaviors. The present results raise the possibility that one of these behaviors may be selected to the exclusion of the others, at least in part, by modulation at the level of the sensory neurons (Davis et al., 1977; Weiss et al., 1986b; for a review of sensory gating during biting behavior in vertebrates, see Bushnell et al., 1987). It is not without interest that SCP$_B$ and buccalin broaden the spikes of all classes of mechanosensory neurons. These agonists may potentiate multiple neural circuits and as such may be neuromodulators that mediate a very general form of arousal in *Aplysia* (Kupfermann, 1974). Finally, there is evidence that feeding behavior in *Aplysia,* and in other gastropod molluscs, exhibits short- and long-term forms of learning involving either suppression of responses (Kupfermann and Pinsker, 1968; Mpitsos et al., 1978; Sahley et al., 1981; Walters et al., 1981; Susswein et al., 1986) or facilitation of responses (Colwill, 1985; Susswein et al., 1986). *Aplysia* also exhibit a combined time-dependent mixture of suppression or facilitation of feeding (Kupfermann and Weiss, 1981) and other responses

(Mackey et al., 1987; Marcus et al., 1987). It is possible that the neuromodulators described in this paper are involved in these phenomena.

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