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Cholinergic suppression: A postsynaptic mechanism of long-term associative learning

(acetylcholine/food avoidance learning/muscarinic/gastropod)

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ABSTRACT **Food avoidance learning in the mollusc *Pleurobranchaea* entails reduction in the responsiveness of key brain interneurons in the feeding neural circuitry, the paracerebral feeding command interneurons (PCNs), to the neurotransmitter acetylcholine (AcCho). Food stimuli applied to the oral veil of an untrained animal depolarize the PCNs and induce the feeding motor program (FMP). Atropine (a muscarinic cholinergic antagonist) reversibly blocks the food-induced depolarization of the PCNs, implicating AcCho as the neurotransmitter mediating food detection. AcCho applied directly to PCN somata depolarizes them, indicating that the PCN soma membrane contains AcCho receptors and induces the FMP in the isolated central nervous system preparation. The AcCho response of the PCNs is mediated by muscarinic-like receptors, since comparable depolarization is induced by muscarinic agonists (acetyl-β-methylcholine, oxotremorine, pilocarpine), but not nicotine, and blocked by muscarinic antagonists (atropine, trifluoperazine). The nicotinic antagonist hexamethonium, however, blocked the AcCho response in four of six cases. When specimens are trained to suppress feeding behavior using a conventional food-avoidance learning paradigm (conditionally paired food and shock), AcCho applied to PCNs in the same concentration as in untrained animals causes little or no depolarization and does not initiate the FMP. Increasing the concentration of AcCho 10–100 times, however, induces weak PCN depolarization in trained specimens, indicating that learning diminishes but does not fully abolish AcCho responsiveness of the PCNs. This study proposes a cellular mechanism of long-term associative learning—namely, postsynaptic modulation of neurotransmitter responsiveness in central neurons that could apply also to mammalian species.**

The neurotransmitter acetylcholine (AcCho) has long been implicated indirectly in the mediation of learning and memory in invertebrates and vertebrates alike, including humans. In invertebrates, for example, drugs that affect AcCho-mediated transmission alter visual learning in *Drosophila* (1). In vertebrates, intravenous administration of muscarinic antagonists, such as scopolamine and atropine, interferes with acquisition of conventional avoidance learning tasks in rats (2, 3). In humans, normal and pathological age-related deficits in learning and memory are associated with deficits in AcCho metabolism (4).

Although cholinergic systems have thus been implicated indirectly in learning, their role has not been established or analyzed directly. Here we utilize a molluscan "model" system to investigate the involvement of AcCho in learning, at the level of single, identified brain neurons. We show that the feeding motor program (FMP) of the mollusc *Pleurobranchaea* is elicited by cholinergic activation of a population of feeding command interneurons in the brain, that the corresponding cholinergic response exhibits several characteristics of the vertebrate muscarinic response, and that this muscarinic-like response is strongly suppressed by associative training in a conventional food avoidance task. Such cholinergic suppression in the paracerebral feeding command interneurons (PCNs) would in turn directly cause the learned suppression of feeding behavior. In contrast to previous studies employing molluscan model systems, the present work indicates that postsynaptic modifications in neurotransmitter responsiveness in central neurons underlie long-term associative learning. Inasmuch as the locus of learning is postsynaptic to neural pathways mediating the conditioned stimulus (CS) and the unconditioned stimulus (US), our study suggests a unique mechanism of associative learning.

MATERIALS AND METHODS

Specimens of *Pleurobranchaea californica* were obtained by trawling in Monterey Bay. In the first series of experiments, we examined the role of AcCho in mediating food detection and initiating the FMP. The central nervous system (CNS) (brain and attached buccal ganglion) was dissected free along with a flap of anterior chemosensory tissue (oral veil and tentacles), to which the brain was left attached by the appropriate nerves, as detailed elsewhere (5).

The preparation was maintained in cold sea water (5–7°C) during experiments. The brain was desheathed with fine forceps and the somata of identified PCNs (6) were impaled under visual control with 3 M KCl-filled glass capillary microelectrodes (tip resistance of 10–25 MΩ, measured in sea water). These brain interneurons were shown previously to serve as command and pattern-generating neurons for the FMP (6–9). Simultaneous extracellular recordings were made from various nerves using glass capillary suction electrodes. Food stimuli, consisting of a homogenate of fresh, raw squid, mixed with an equal volume of sea water, were applied by pipette to the oral veil and tentacles while recording the intracellular responses of the PCNs. The CNS was contained in a chamber that was separated by a watertight partition from the chemosensory tissues, thus preventing direct contact of food stimuli with the CNS.

After establishing the baseline PCN depolarizing response to food stimuli applied to the chemosensory tissues, atropine was added to the chamber containing the CNS (final bath concentration of 0.1–1 mM) but not to the chamber containing the chemosensory tissues. The effect of atropine was

Abbreviations: AcMeCho, acetyl-β-methylcholine; AcCho, acetylcholine; CNS, central nervous system; CS, conditioned stimulus; FMP, feeding motor program; PCN, paracerebral feeding command interneuron; US, unconditioned stimulus.

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examined by applying food stimuli to the oral veil and tentacles every 20 min. Each application of food was preceded by a control application of sea water. The effect of atropine was reversed by flushing the bath containing the CNS with fresh sea water (4–5 liters in a period of 60–90 min).

In this same series of experiments the effect of bath-applied AcCho on the PCNs and on the FMP was examined by exposing the isolated CNS to AcCho. The preparation consisted of a brain and buccal ganglion, attached by the paired cerebrobuccal connectives, and was prepared for electrophysiological analysis as described above. Experiments were also performed on the isolated buccal ganglion.

In the second series of experiments, we studied the pharmacology of the AcCho response by direct application of AcCho and various cholinergic agonists and antagonists to the somata of the PCNs. The CNS was isolated in an experimental chamber and PCN somata were exposed and impaled as above. The somata were then stimulated chemically, either by bath application or by localized pressure or iontophoretic ejection directly onto the exposed somata. In the case of bath application and pressure ejection, the chemicals were dissolved in a carrier solution of sea water. In the case of iontophoresis, AcCho was dissolved (2 M) in distilled water and delivered through a high-resistance (100–150 MΩ) glass capillary pipette (10) using a WPI S-7061A iontophoresis module. The tip of the iontophoretic electrode was placed against the surface of the soma, as signified by a sudden and sustained increase in electrode resistance, and then withdrawn slightly but left in contact with the soma. AcCho was ejected using 1-sec pulses of 15–40 nA. A holding current of −15 nA was applied in order to prevent leakage of AcCho. All experiments entailed single applications of AcCho and, hence, desensitization to AcCho was not a factor. Chemicals applied to the PCNs and their concentrations are given in the *Results*.

In the third series of experiments, we assessed the effects of associative training on the responsiveness of PCNs to AcCho. Pairs of animals were matched for volume (±10%) and for feeding response thresholds (±1 logarithmic unit; ref. 11) and then divided at random into experimental and control categories. Experimental animals were then trained according to a food-avoidance conditioning paradigm that has been developed and tested extensively in previous studies (12–17). Briefly, experimental specimens received a CS (homogenized squid, prepared as described above) applied to the anterior chemosensory structures (oral veil, tentacles, and rhinophores) in hourly conditioning trials (13–15). If they exhibited the proboscis extension or bite-strike feeding response, or failed to withdraw in response to food stimuli, they received a 60-sec aversive electric shock (US) that was accompanied continuously by the CS. Each matched control animal received the CS and US in the same number and intensity as the corresponding experimental animal but separated by 0.5 hr (explicitly unpaired control protocol). Animals were tested before and after conditioning and control procedures by applying serially increasing logarithmic concentrations of squid homogenate to determine the threshold concentration necessary to elicit the feeding responses (11).

Experimental and control CNSs were removed 1–4 days following training and control procedures. Previous studies showed that the neurophysiological manifestations of behavioral learning survive the surgical removal of the CNS and, hence, can be studied in the isolated CNS (5). Trained specimens were used for electrophysiological analysis only if, on the day of use, they met the same stringent learning criteria used in earlier studies (5)—namely, a combined increase in proboscis extension and bite-strike thresholds of at least 4 logarithmic units. Control specimens were used only if they showed a combined increase of no more than 1 logarithmic unit. Approximately two-thirds of trained animals and four-fifths of control animals met these criteria. The CNS (brain and attached buccal ganglion) was isolated and PCN somata were exposed, as described above, while the preparation was maintained in cold sea water (11° ± 1°C). Subsequent electrophysiological analyses on the PCNs of trained and control specimens were carried out as described above.

RESULTS

Role of AcCho in the Feeding System. Application of food stimuli to the anterior chemosensory structures of naive specimens depolarized the PCNs (Fig. 1*A*), as reported (5). This food-induced depolarization was followed immediately by a cyclic motor rhythm recorded from buccal nerves and the salivary duct. This rhythm was identified as the FMP using quantitative criteria established earlier (18), including a protractor duty cycle of 33–50% (measured from buccal root 1 discharge), and cyclic discharge of the salivary duct in phase with protractor bursts.

After a baseline (control) level of food-induced depolarization was established as above, atropine was added to the bath containing the CNS (final concentration, 0.1–1 mM). Addition of atropine reduced the food-induced depolarization of PCNs progressively to near zero within 1–2 hr (Fig. 1*B*; *n*

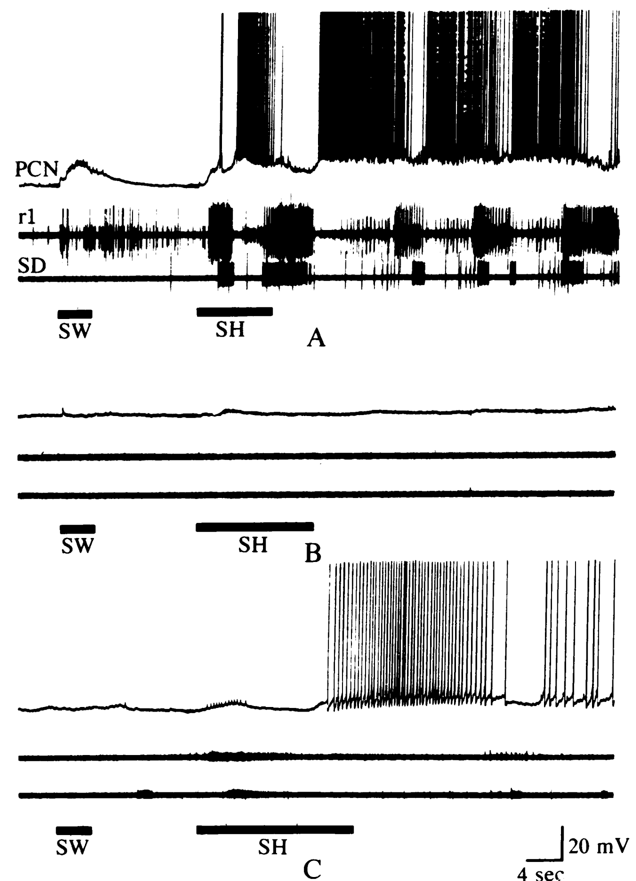

FIG. 1. Atropine blocks food-induced excitation of the PCNs and the FMP. (*A*) Application of sea water (bar, SW) and squid homogenate (bar, SH) to the oral veil and tentacles of a reduced preparation while recording intracellularly from a PCN (upper trace) and extracellularly from a buccal protractor nerve (root 1 or r1) and the salivary duct (SD). (*B*) The same sensory stimuli have no effect after bathing the nervous system in 1 mM atropine for 100 min. (*C*) Partial restoration of the response to sensory stimuli 80 min after the beginning of atropine washout.

= 6). The smaller depolarization caused by application of sea water to the oral veil and tentacles (Fig. 1A) was likewise abolished by atropine (Fig. 1B). Washout of atropine partially reversed these effects (Fig. 1C; n = 4), although in no case was the restoration of food-induced depolarization complete over the time course of these experiments (up to 4 hr).

Three control procedures were employed to establish that the response decrement described above was induced specifically by atropine acting on synaptic inputs. (*i*) In preparations not exposed previously to atropine, repetitive application of food stimuli at 20-min intervals caused depolarization of the PCNs with little or no decrement over periods up to 4 hr (n = 3). Therefore, the suppression of the response following atropine exposure cannot be ascribed to "habituation" or "fatigue." (*ii*) Action potential discharge was induced periodically during atropine exposure by injecting current into the PCN soma. Neither the spike amplitude nor the firing pattern changed during atropine-induced decrement of the food-induced depolarization (n = 6), demonstrating that the effect of atropine was limited to synaptic inputs. (*iii*) In three of four preparations the FMP was induced by extracellular stimulation of the stomatogastric nerve(s) (18) at the end of the period of atropine exposure. This indicates that atropine had no effect on the electrically induced FMP. Collectively these experiments indicate that atropine acts mainly and perhaps exclusively on synaptic inputs to the PCNs.

Having established that food-induced depolarization of the PCNs is mediated by cholinergic synapses, we tested the effects of bath application of AcCho. Bath application of AcCho (final concentration, 0.1 mM) to the isolated CNS of naive (untrained) specimens immediately induced the cyclic FMP (Fig. 2). The FMP was again identified by criteria established earlier (18), including (*i*) a continuous motor program, rather than the episodic program characteristic of egestion (n = 7), (*ii*) a protractor duty cycle that ranged from 26% to 45%, characteristic of feeding but different from egestion (n = 2/2), and (*iii*) salivary duct discharge in phase with protractor activity (n = 2/2), also uniquely diagnostic of feeding. Bath application of AcCho (0.1–1 mM) to the isolated buccal ganglion never activated the FMP (n = 4), indicating that AcCho induction of the FMP is mediated by neurons located in the brain—e.g., the PCNs. Bath application of AcCho also depolarized the PCNs of naive animals, and this depolarization preceded the onset of the FMP, as would be expected (see below).

Next we applied 10 mM AcCho directly to the somata of individual PCNs under visual control while recording their responses intracellularly. Pressure ejection of AcCho induced a large (10–20 mV), transient membrane depolarization with short latency (Fig. 3A). The somata of directly adjacent PCNs were unaffected, however, indicating that over the time course of the experiment, contact with AcCho was

FIG. 2. AcCho elicits the FMP from the isolated nervous system (brain and attached buccal ganglion) of *Pleurobranchaea*. AcCho (final concentration 0.1 mM) was applied at the bar, causing a typical feeding rhythm (18) recorded extracellularly from the salivary duct (SD), buccal protractor nerve (r1), and buccal retractor nerve (r3).

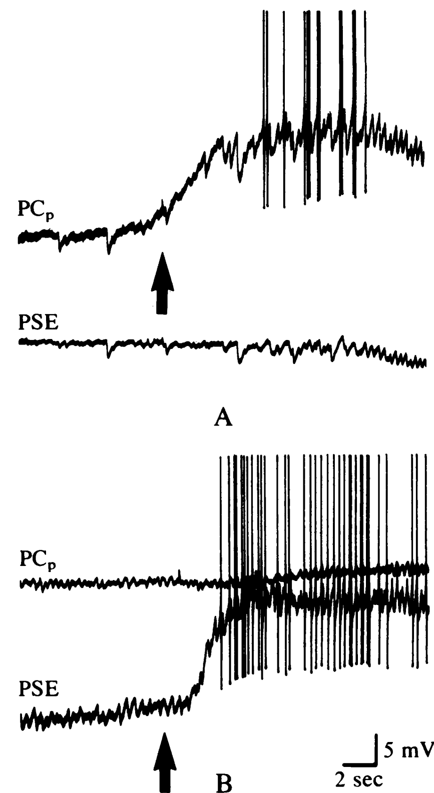

FIG. 3. Localization of AcCho affects single neurons, shown by pressure ejection of 10 mM acetylcholine (AcCho, upward arrows) onto the somata of individual, adjacent PCNs of the same naive preparation during intracellular recordings. (*A*) Ejection onto a phasic paracerebral neuron (PC_p; ref. 6). (*B*) Subsequent ejection onto the adjacent polysynaptic excitor (PSE), an identified subclass of the PCNs (6).

restricted to the soma directly beneath the pressure ejection pipette (compare Fig. 3A with Fig. 3B). Similarly, iontophoretic application of AcCho to single PCN somata caused immediate depolarization that was restricted to the soma directly beneath the pipette. Therefore, AcCho-induced depolarization of the PCN soma is mediated by AcCho receptors located in the soma membrane and not by activation of presynaptic neurons.

Pharmacology of the AcCho Response. To further characterize the AcCho response, we performed established pharmacological tests for distinguishing nicotinic from muscarinic receptors. Bath application of muscarinic agonists depolarized the PCNs. In comparison with AcCho, comparable concentrations of the agonists caused comparable depolarization of the PCNs (e.g., Fig. 4A). Muscarinic agonists that were bath-applied included 0.1 mM acetyl-β-methylcholine (AcMeCho) (n = 3; Fig. 4A) and 0.05 mM oxotremorine (n = 2). Similarly, pressure ejection of muscarinic agonists directly onto single PCNs induced strong depolarization (5–15 mV). Muscarinic agonists that were pressure ejected included 1 mM oxotremorine (n = 3) and 10 mM pilocarpine (n = 3). In contrast to results with muscarinic agonists, bath application of 0.1 mM nicotine (n = 3) and pressure ejection of 10 mM nicotine (n = 1) were without effect.

The application of cholinergic antagonists generally confirmed the above results—that is, bath application of 0.1 mM atropine (n = 3), a muscarinic antagonist, blocked the subsequent response to bath-applied AcCho (0.1–1 mM). Similarly, bath application of 0.5 mM trifluoperazine, a muscarinic antagonist (19) with other effects as well (20–22), blocked the response to bath-applied AcCho (n = 4). However, bath application of 0.1 mM hexamethonium, a nicotinic

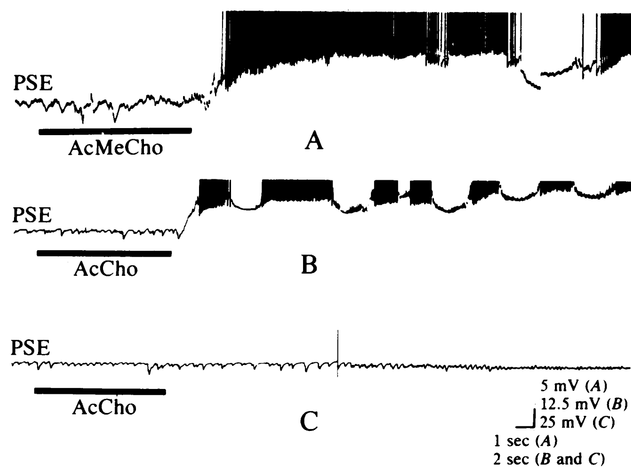

FIG. 4. Associative training suppresses the cholinergic response of PCNs. Shown are the intracellular responses of PCNs in an isolated brain removed from a naive (*A*), control (*B*), and experimental (*C*) preparation. Bars mark application to the bath of AcMeCho (*A*), a muscarinic agonist (final concentration, 0.1 mM), or 0.01 mM AcCho (*B* or *C*). PSE, polysynaptic excitor.

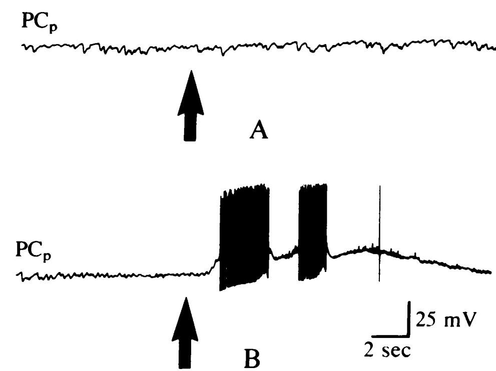

FIG. 5. Suppression of the cholinergic response of PCNs following training, demonstrated by pressure ejection of AcCho (arrows) onto a single PCN soma in a brain removed from a trained animal. Shown are intracellular responses of PCNs to 10 mM AcCho (*A*) and immediately subsequent application of 100 mM AcCho (*B*). An AcCho response is elicited by the higher concentration (*B*), although it is shorter and less intense than normal. PC_p, phasic paracellular neurons.

antagonist, blocked the AcCho response in four of six cases but did not interfere with the response in the other two cases. The results collectively indicate that the cholinergic response of the PCNs exhibits mainly muscarinic characteristics.

Effect of Associative Training on the AcCho Response. Having established that the response of the PCNs to applied AcCho is mediated by muscarinic-like receptors located on the PCN soma, we determined the effect of associative training on this cholinergic response. This entailed quantitative comparison of the AcCho response of PCNs in brains removed from naive, control, and conditioned animals. The majority of the experiments utilized bath application of AcCho, because the concentration of AcCho can be controlled more precisely. Confirmatory experiments involving pressure ejection onto single somata were also performed.

Bath application of the muscarinic agonist AcMeCho (Fig. 4*A*) or of AcCho to the isolated CNS removed from naive (untrained) animals invariably depolarized the PCNs, usually causing bursts of PCN action potentials and initiating the FMP. In all cases, maximal depolarization was obtained within 45 sec after AcCho application. For naive specimens, the mean depolarization (± SEM) attained in a 5-sec period centered on 45 sec after AcCho application was 14.1 ± 2.0 mV (n = 7). Using the same measure, bath application of AcCho to the isolated CNS of control animals caused a mean PCN depolarization of 12.8 ± 4.4 mV (n = 8; Fig. 4*B*). In contrast, bath application of equivalent concentrations of AcCho to the isolated CNS of previously trained animals caused a mean depolarization of 1.6 ± 0.9 mV (n = 16; Fig. 4*C*). The mean AcCho-induced depolarization in PCNs of trained animals was significantly different from the means of naive and control specimens (Mann–Whitney U tests, $P \leq 0.001$ in both cases). The means of naive and control animals were not significantly different from each other (Mann–Whitney U test, $P > 0.1$).

In most cases bath application of 0.1 mM AcCho to the isolated CNS of trained animals caused no response from PCNs. In contrast, bath application of higher concentrations (1 mM and up) induced PCN depolarization and the FMP, although both were weaker than in naive or control animals. This finding indicates that the responsiveness of PCNs to AcCho is diminished rather than abolished by associative training in the food-avoidance paradigm.

Experiments comparable to the above were also performed using direct pressure ejection of AcCho onto exposed and impaled PCN somata rather than bath application. Pressure ejection of 10 mM AcCho invariably depolarized the PCNs of naive animals (n = 3; Fig. 3). In contrast, 10 mM AcCho was without effect on PCNs in the brain of a trained animal (Fig. 5*A*). As in the case of bath application, pressure ejection of higher concentrations of AcCho (100 mM) to the PCN of a trained animal caused a small depolarization and weak cyclic PCN rhythm (Fig. 5*B*). This result employing direct application of AcCho to PCN somata of trained animals confirms the more extensive findings employing bath application of AcCho.

DISCUSSION

The major finding of this study is that associative training in a food-avoidance paradigm reduces the responsiveness of feeding command interneurons in the brain (the PCNs) to their normal neurotransmitter, AcCho. The finding here that the cholinergic response of the PCNs is suppressed by associative training explains our previous observation in intact animals that training eliminates the excitatory response of the PCNs to food stimuli (16, 17). This in turn would reduce the feeding response to food, accounting for the learned suppression of feeding behavior following training. Therefore, the demonstrated reduced responsiveness of the PCNs to AcCho is presumably causal to the observed behavioral learning.

That AcCho normally serves as the neurotransmitter mediating food-induced depolarization of the PCNs is supported by the following observations. (*i*) The cholinergic antagonist atropine reversibly abolishes food-induced depolarization of the PCNs (Fig. 1). The finding that in the presence of atropine the FMP can still be driven by electrical stimulation of the stomatogastric nerve(s) suggests that the role of AcCho is limited to the detection of food and initiation of feeding but does not include central generation of the FMP. (*ii*) Application of AcCho to the PCN somata depolarizes the PCNs (Fig. 3) and selectively induces the FMP (Fig. 2). This further supports the role of AcCho in initiating feeding and shows that depolarization of the PCNs by applied AcCho is mediated by cholinergic receptors in the soma membrane rather than by activation of central neurons presynaptic to the PCNs.

Two lines of evidence indicate that the cholinergic response of the PCNs is mediated by muscarinic-like receptors. (*i*) Muscarinic agonists (AcMeCho, oxotremorine, pilocarpine) induce strong depolarization of the PCNs. In contrast,

nicotine is without effect. (*ii*) Muscarinic antagonists (atropine, trifluoperazine) block the response of the PCNs to applied AcCho and also block the normal depolarizing response of the PCNs to food stimulation of the oral veil and tentacles. The finding that a nicotinic antagonist (hexamethonium) blocked the response to applied AcCho in two-thirds of the cases may mean that hexamethonium at the concentrations used blocks the muscarinic-like responses of the PCNs. Alternatively, the muscarinic-like receptors of the PCNs may exhibit slightly different pharmacological properties from classical muscarinic receptors in vertebrates, as suggested for nicotinic-like receptors in *Aplysia* (23, 24). The demonstration here that atropine blocks not only synaptic inputs to the PCNs but also blocks the response of the PCN soma to applied AcCho indicates that synaptic and somatic cholinergic receptors are muscarinic-like.

This work shows that associative learning entails modifications in neurons that are postsynaptic to both the CS (food stimuli) and the US (aversive electric shock). Our data indicate that the CS and the US interact at a common postsynaptic target (the PCNs) to reduce responsiveness to AcCho and thus reduce the subsequent responsiveness of the PCNs to the CS alone. This is a modified form of Hebb's postulate of learning (25), by which coactivation of two neurons modifies their functional relationship. In this case coactivation results from convergence of the CS and US on a common target.

The learning mechanism we have demonstrated differs in two fundamental respects from those proposed to date in molluscan model systems. First, the presynaptic mechanisms proposed in *Aplysia* for habituation (26, 27), sensitization (28), and an analog of classical conditioning (29) entail training-induced modification of neurotransmitter release by modulation of presynaptic voltage-gated ion channels. Learning in *Hermissenda* also involves changes in voltage-gated ion channels (30). In contrast, our data involve a training-induced modification of postsynaptic neurotransmitter responsiveness, ultimately by modulation of the action of neurotransmitter-gated ion channels. Such postsynaptic modifications could arise through changes in the muscarinic-like receptors, alteration of possible second messenger cascades that they induce, or modification of the ion channels they activate.

A second way in which the present results are unique is that learning models suggested for other molluscs entail changes in sensory neurons that mediate the CS. Such a learning mechanism would appear to lack specificity, however, inasmuch as all subsequent behavior utilizing the same sensory modality would be modified. In contrast, the learning-induced changes described here occur in central interneurons, mediating select behaviors that are modified by training. Such an arrangement would permit greater learning specificity, flexibility, and increased convergence and interaction with other pathways and neurotransmitters. Such a postsynaptic mechanism of learning may be more generally applicable to higher organisms, including mammals, where central rather than peripheral correlates to learning have long been known (31, 32) and where AcCho has been indirectly implicated in learning (2, 4, 33–35).

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1. Folkers, E. & Spatz, H.-Ch. (1984) *J. Insect Physiol.* **30,** 957–965.
2. Davis, K. L. & Yesavage, J. A. (1979) in *Brain Acetylcholine and Neuropsychiatric Disease*, eds. Davis, K. L. & Berger, P. A. (Plenum, New York), pp. 205–213.
3. Friedman, E., Larer, B. & Kuster, J. (1983) *Pharmacol. Biochem. Behav.* **19,** 309–312.
4. Drachman, D. A. (1977) *Neurology* **27,** 783–790.
5. Kovac, M. P., Davis, W. J., Matera, E. M., Morielli, A. & Croll, R. P. (1985) *Brain Res.* **331,** 275–284.
6. Kovac, M. P., Davis, W. J., Matera, E. M. & Croll, R. P. (1983) *J. Neurophysiol.* **49,** 1517–1538.
7. Croll, R. P., Kovac, M. P., Davis, W. J. & Matera, E. M. (1985) *J. Neurosci.* **5,** 56–63.
8. Gillette, R., Kovac, M. P. & Davis, W. J. (1978) *Science* **199,** 798–801.
9. Gillette, R., Kovac, M. P. & Davis, W. J. (1982) *J. Neurophysiol.* **47,** 885–908.
10. Dreyer, F. & Peper, K. (1974) *Pflügers Arch.* **348,** 263–272.
11. Davis, W. J., Mpitsos, G. J. & Pinneo, J. M. (1974) *J. Comp. Physiol.* **117,** 99–125.
12. Mpitsos, G. J. & Davis, W. J. (1973) *Science* **180,** 317–320.
13. Mpitsos, G. J. & Collins, S. D. (1975) *Science* **188,** 954–957.
14. Mpitsos, G. J., Collins, S. D. & McClellan, A. D. (1978) *Science* **199,** 497–506.
15. Davis, W. J., Villet, J., Lee, D., Rigler, M., Gillette, R. & Prince, E. (1980) *J. Comp. Physiol. A* **138,** 157–165.
16. Davis, W. J. & Gillette, R. (1978) *Science* **199,** 801–804.
17. Davis, W. J., Gillette, R., Kovac, M. P., Croll, R. P. & Matera, E. M. (1983) *J. Neurophysiol.* **49,** 1557–1572.
18. Croll, R. P., Davis, W. J. & Kovac, M. P. (1985) *J. Neurosci.*, 48–55.
19. Richelson, E. (1977) *Nature (London)* **266,** 371–373.
20. Seeman, P. & Lee, T. (1975) *Science* **188,** 1217–1219.
21. Mazzei, G. J., Schatzman, R. C., Turner, R. S., Vogler, W. R. & Kuo, J. F. (1984) *Biochem. Pharmacol.* **33,** 125–130.
22. Slater, N. T., Hall, A. F. & Carpenter, D. O. (1985) *Brain Res.* **329,** 275–279.
23. Kehoe, J. S. (1972) *J. Physiol. (London)* **225,** 115–146.
24. Kehoe, J. S. (1972) *J. Physiol. (London)* **225,** 147–152.
25. Hebb, D. O. (1949) *Organization of Behavior* (Wiley, New York).
26. Castellucci, V. F. & Kandel, E. R. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 5004–5008.
27. Castellucci, V. F., Carew, T. J. & Kandel, E. R. (1978) *Science* **202,** 1306–1308.
28. Kandel, E. R. & Schwartz, J. H. (1982) *Science* **218,** 433–443.
29. Hawkins, R. D., Abrams, T. W., Carew, T. J. & Kandel, E. R. (1983) *Science* **219,** 400–405.
30. Alkon, D. L. (1984) *Science* **226,** 1037–1045.
31. Kubanis, P., Zornetzer, S. F. & Freund, G. (1982) *Pharmacol. Biochem. Behav.* **17,** 313–322.
32. Mason, S. T. (1983) *Neurosci. Biobehav. Rev.* **7,** 325–347.
33. LoConte, G., Bartolini, L., Casamenti, F., Marconcini-Pepu, I. & Pepeu, G. (1982) *Pharmacol. Biochem. Behav.* **17,** 933–937.
34. Bammer, G. (1982) *Neurosci. Biobehav. Rev.* **6,** 247–296.
35. Fisher, S. K., Klinger, P. D. & Agranoff, W. (1983) *J. Biol. Chem.* **258,** 7358–7363.