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NEUROMODULATION BY 5-HYDROXYTRYPTAMINE IN THE ANTENNAL LOBE OF THE SPHINX MOTH *MANDUCA SEXTA*

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Summary

Using intracellular recording techniques, we have begun to examine the effects of 5-hydroxytryptamine (5-HT) on antennal-lobe (AL) neurones in the brain of the adult moth *Manduca sexta*. 5-HT modulated the responses of local interneurones and projection neurones, which were recognized on the basis of well-established electrophysiological criteria, to primary synaptic input elicited by electrical stimulation of the ipsilateral antennal nerve. 5-HT applied at low concentration (10^{-8} mol l^{-1}) reduced the excitatory responses evoked by electrical stimulation of the antennal nerve, whereas at high concentration (10^{-4} mol l^{-1}), 5-HT enhanced the responses. At 10^{-4} mol l^{-1}, 5-HT increased cell input resistance, led to broadening of action potentials and caused increased cell excitability in many AL neurones.

Key words: 5-hydroxytryptamine, serotonin, *Manduca sexta*, neuromodulation, antennal lobe, olfaction.

Introduction

In the nervous systems of invertebrates, 5-hydroxytryptamine (5-HT or serotonin) serves as a neurotransmitter (Osborne, 1982; Walker, 1984), a neuromodulator (Kupfermann, 1979; Kravitz *et al.* 1985; Pentreath *et al.* 1982) and a neurohormone (Kravitz *et al.* 1985; Trimmer, 1985; Davis, 1987). 5-HT plays an important role in learning in some animals (reviewed by Byrne, 1987) and has been implicated in the regulation of neuronal development (Lauder, 1988, 1990).

Specific antisera against 5-HT have been used to stain neurones possessing 5-HT-like immunoreactivity (5-HT-LIR) in the central nervous system (CNS) of various species of insects (Nässel, 1987; Homberg and Hildebrand, 1989*a*,*b*; Homberg *et al.* 1989). In the brain, a relatively small subset of neurones exhibits 5-HT-LIR. These neurones typically have wide-field arborizations extending to many or all major neuropile regions of the brain.

A single 5-HT-immunoreactive neurone resides in each antennal lobe (AL) of *M. sexta* (Kent *et al.* 1987). Each of these 5-HT-immunoreactive neurones has extensive ramifications in the ipsi- and contralateral protocerebrum and arborizations throughout the glomerular neuropile of the contralateral AL. The neurones are born during embryonic development and, throughout larval, pupal and adult stages of life, are associated with the primary olfactory centres of the brain, which undergo extensive reorganization and development during metamorphic adult development. Recent studies, using immunocytochemistry combined with electron-microscopic techniques, have shown that, in the adult moth, each of these 5-HT-immunoreactive neurones makes direct synaptic contact with AL interneurones, and a majority of these contacts are output synapses from the 5-HT-immunoreactive neurone (Sun *et al.* 1993).

In contrast to the well described morphology of 5-HT-immunoreactive neurones in the insect brain, the physiological roles of 5-HT in the insect CNS are much less well understood. From behavioural and electrophysiological studies in various species, however, there is strong evidence that 5-HT acts as a central neuromodulator or neurotransmitter in insects (e.g. Usherwood *et al.* 1980; Mercer and Menzel, 1982; Linn and Roelofs, 1986; Nässel, 1987; Neumann *et al.* 1987; Bermudez *et al.* 1992; Linn *et al.* 1992).

In the work presented here, we have begun to study the role of 5-HT in the ALs, the primary olfactory centres in the brain *M. sexta*. This system has been investigated extensively as a model for studies of olfactory information processing, and it offers the experimental advantage that each AL is innervated by only one identified 5-HT-immunoreactive neurone.

Materials and methods

Manduca sexta (Lepidoptera: Sphingidae) were reared on an artificial diet (modified from that of Bell and Joachim, 1976) at 25 °C and 50–60 % relative humidity under a long-day photoperiod regimen (17 h:7 h light:dark) as described previously (Sanes and Hildebrand, 1976; Tolbert *et al.* 1983).

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Adult moths of both sexes, 1–5 days post-eclosion, were used for these studies.

Physiological preparation

In an attempt to perform all experiments on insects in a reproducible physiological state, we prepared all moths in the same way in the evening prior to the day in which they would be used for experiments. Each moth was immobilized in a plastic tube (Christensen and Hildebrand, 1987), the scales were removed from its head, and the insect was kept at room temperature (approximately 20 °C) overnight. Immediately before the experiment, the head capsule was opened by cutting a window between the two compound eyes and the bases of the antennae. The brain was exposed by removing the palps, cibarial pump and antennal muscles. The head, with the antennae and their innervation of the ALs intact, was then removed and pinned in a Sylgard-coated recording chamber (volume <0.5 ml). For electrical stimulation of the antennal nerve, a pair of stainless-steel hook electrodes was positioned under the nerve near the AL. To facilitate insertion of the recording electrode and penetration of pharmacological agents into the tissue, parts of the ALs were desheathed manually.

The brain was superfused constantly with physiological saline solution (approximately 2 ml min^{-1}), modified from that of Pichon *et al.* (1972) and containing (in mmol l^{-1}) 149.9 NaCl, 3.0 KCl, 3.0 $CaCl_2$, 10.0 Tes, pH 6.9, and 25.0 mmol l^{-1} sucrose to balance osmolarity with that of the extracellular fluid. In certain experiments, tetrodotoxin (TTX; 10^{-8} to 10^{-4} mol l^{-1}) and $CdCl_2$ (0.5×10^{-3} to 3×10^{-3} mol l^{-1}) were added in order to block Na^+ and Ca^{2+} channels, respectively (see, for example, Hayashi and Levine, 1992; Laurent *et al.* 1993; Trimmer and Weeks, 1993). 5-HT (5-HT creatinine sulphate or 5-HT hydrochloride from Sigma or Research Biochemicals International) was added to the superfusion saline at concentrations in the range 10^{-8} to 10^{-4} mol l^{-1}. The superfusion system was designed so that we could switch from normal saline to 5-HT-containing saline solution without disturbing its constant flow.

Stimulation

Brief (0.1 ms, <5 mA) pulses of electric current, applied to the antennal nerve *via* the stainless-steel hook electrodes, were used to stimulate primary afferent inputs to the AL (Harrow and Hildebrand, 1982; Christensen and Hildebrand, 1987; Waldrop *et al.* 1987). Single pulses were used to analyze the characteristics of the evoked response, and trains of five pulses with a frequency of 20 s^{-1} were applied to test for monosynaptic connectivity between impaled neurones and sensory afferents (Waldrop *et al.* 1987).

Current pulses were injected *via* the recording electrode to elicit single action potentials or trains of spikes and to measure the cell input resistance. Single spikes elicited in this way were used to analyze changes in action potential waveform resulting from the exogenous application of 5-HT. To avoid the potential problem that high-frequency spontaneous bursts of spikes can induce activity-dependent inactivation of delayed-rectifier-like K^+ currents (Aldrich *et al.* 1979*a*,*b*), neurones were hyperpolarized to suppress spontaneous activity. Single spikes were elicited by injection of depolarizing rectangular current pulses (1 ms, ⩽10 nA) or ramp-like depolarizing currents (115 ms, slope 0.01 nA ms^{-1}). The cell input resistance was measured from the *I–V* relationship, derived by injection of hyperpolarizing current pulses (125 ms, −2.0 to −0.4 nA or −1.9 to −0.3 nA in 0.4 nA steps) into the cell. All stimuli were controlled by computer (i80386 microcomputer running pCLAMP software, Axon Instruments).

Physiological recording

Borosilicate glass capillary electrodes (o.d. 1.0 mm, i.d. 0.78 mm, Sutter Instrument Co.) were produced with a Flaming-Brown puller (P-87, Sutter Instrument Co.). The electrodes were filled with filtered (0.2 μm pore size) 2 mol l^{-1} KCl solution and had resistances of 20–100 MΩ, measured in the tissue. For experiments in which current was injected (e.g. measurement of cell input resistance), it was necessary to use electrodes with low resistance (20–30 MΩ) in order to keep the recordings stable over the course of the experiment. Recordings were made from neurites in the neuropile of the AL, and impalements (and hence recordings) typically could be maintained for approximately 30 min. Signals were amplified with an Axoclamp-2A amplifier (Axon Instruments). Intracellular recordings and current injections were carried out in bridge mode. Data were stored on magnetic tape (Vetter Instruments FM tape recorder) or on computer diskettes with the aid of the software programs pCLAMP (Axon Instruments) or Experimenter's Workbench (BrainWave Systems). The same programs were used for data analysis.

Results

The effects of exogenously applied 5-HT were examined in 91 AL neurones, each in a different moth. Of those neurones, 45 (or 50 %) gave clear responses to applied 5-HT. The findings reported here were obtained from the 5-HT-responsive neurones.

Characterization of the impaled neurones

At the beginning of each recording, the response characteristics of the neurone were examined to ascertain whether it was a local interneurone or a projection neurone (Homberg *et al.* 1989; Christensen *et al.* 1993). Recognition of neuronal type was based on the following criteria (Matsumoto and Hildebrand, 1981; Waldrop *et al.* 1987; Christensen *et al.* 1993; T. A. Christensen, personal communication): (a) *spontaneous activity*, projection neurones typically exhibit constant background firing, whereas local interneurones often show bursting spontaneous activity; (b) *action potential amplitude*, intracellular recordings from projection neurones show spikes of a single amplitude, whereas recordings from local interneurones typically include spikes of multiple amplitudes; (c) *response to electrical stimulation of the antennal nerve*, the responses of projection neurones typically

are complex and often comprise a sequence of inhibitory and excitatory components that includes early inhibition, whereas most local interneurones give simpler responses in which short- or longer-latency excitation is the first observable component; and (d) *response to high-frequency electrical stimulation of the antennal nerve*, the responses of many local interneurones can follow high-frequency electrical stimulation (e.g. 20 stimuli s^{-1}) of the antennal nerve, apparently because the local interneurones receive monosynaptic inputs from the sensory afferents, whereas projection neurones typically cannot follow high-frequency electrical stimulation of the antennal nerve.

All neurones described in this paper could be classified as local interneurones or projection neurones according to these criteria, permitting us to use low-resistance, KCl-filled electrodes, which have stable resistances and therefore yield recordings of high quality for longer periods of time than do dye-filled electrodes. Precise classification of AL neurones, however, would have required intracellular staining.

Concentration dependence

The dose-dependence of the effect of 5-HT was studied at concentrations in the range 10^{-8} to 10^{-4} mol l^{-1}. Although there were significant differences in the responses of local interneurones and projection neurones to antennal nerve shocks, the effects of 5-HT on both types of AL neurones were similar and dependent on the dose of 5-HT used. At low concentration (10^{-8} mol l^{-1}), 5-HT generally had subtle effects. The depolarization and/or number of action potentials elicited by standardized electrical stimulation of the antennal nerve was reduced. In some cases, these responses were abolished completely. At 10^{-4} mol l^{-1}, 5-HT caused a strong enhancement of the depolarization and/or number of spikes elicited by electrical stimulation of the antennal nerve. Concentrations of 5-HT between 10^{-8} and 10^{-4} mol l^{-1} elicited graded, intermediate effects.

The effects of different concentrations of 5-HT on two local interneurones and two projection neurones are shown in Fig. 1. In the local interneurone (Fig. 1A), the electrical stimulation of the antennal nerve elicited depolarization and action potentials. This response was reduced slightly by 10^{-8} mol l^{-1} 5-HT, whereas 10^{-4} mol l^{-1} 5-HT caused a clear increase in the depolarization and the number of elicited spikes. The effects of 5-HT reversed after approximately 4 min of washing with physiological saline solution.

The effects of various concentrations of 5-HT on a projection neurone are shown in Fig. 1B. This neurone responded to antennal-nerve shock with an initial spike followed by hyperpolarization of the cell membrane. A low concentration of 5-HT (10^{-8} mol l^{-1}) suppressed the early spike and increased the amplitude of the hyperpolarization that followed. Increasing the concentration of 5-HT to 10^{-6} mol l^{-1} had little apparent effect on the cell, whereas a further increase to 10^{-4} mol l^{-1} caused a distinct depolarization and firing of action potentials and the abolition of the hyperpolarization previously evoked by antennal nerve shock. The effect of 5-HT (10^{-4} mol l^{-1}) on local interneurones (Fig. 1C) and projection neurones (Fig. 1D) was completely reversible after washing (3–10 min) with physiological saline and was readily reproducible upon repeated application of 5-HT.

In general, the effects of 5-HT at higher concentration (10^{-4} mol l^{-1}) were much more pronounced than those at low concentration (10^{-8} mol l^{-1}). The remainder of this paper presents additional description and analysis of the effects of this higher concentration, but this focus does not reflect a judgment about its physiological significance.

Neuronal input resistance and excitability

For both local interneurones and projection neurones, cell input resistance increased under the influence of 5-HT (10^{-4} mol l^{-1}) in more than 80 % of the neurones studied (Fig. 2A–C). In the neurones that showed significant effects in response to the application of 5-HT, an increase of 37±22 % (mean ± S.D., N=6, P<0.01 using the paired t-test) was observed (Fig. 2C). This increase in input resistance was sometimes accompanied by an increase in spike amplitude. The increase in excitability described above was also apparent in experiments using this protocol. 5-HT increased the number of spikes elicited by a small-amplitude depolarizing current pulse (Fig. 2A). This effect of 5-HT was observed in several neurones but was not always as pronounced as shown in Fig. 2A.

5-HT had a similar effect on the input resistance after TTX and Cd^{2+} had been added to the superfusion saline solution in order to block Na^+ and Ca^{2+} channels (Fig. 3). Under these conditions, the input resistance increased by 32±13 % (mean ± S.D.; N=4; P<0.02 using the paired t-test) (Fig. 3C). In these experiments, because TTX and Cd^{2+} were applied before the neurone was impaled, the criteria for differentiation between local and projection neurones could not be applied.

Resting potential and spontaneous activity

The resting membrane potential of the AL neurones, under the conditions of the experiments in this study, was between −35 and −60 mV. 5-HT did not have a reproducible effect on the resting potential and spontaneous activity of tested neurones. In a few recordings we observed a depolarization of the cell membrane (<5 mV) after application of 10^{-4} mol l^{-1} 5-HT for more than 3 min. This effect was not reversible in the limited recording time (approximately 30 min, including the approximately 15 min wash), and we could not determine whether this was an effect of 5-HT or a decrease in the quality of the recording. Nevertheless, we never observed hyperpolarization in response to any of the applied concentrations of 5-HT.

Broadening of action potentials

To investigate changes in spike width, we injected hyperpolarizing current into the impaled neurone to suppress spontaneous activity. The membrane potential was kept constant under manual control. Brief rectangular, depolarizing current pulses (≤1 ms, ≤10 nA) or ramps of depolarizing

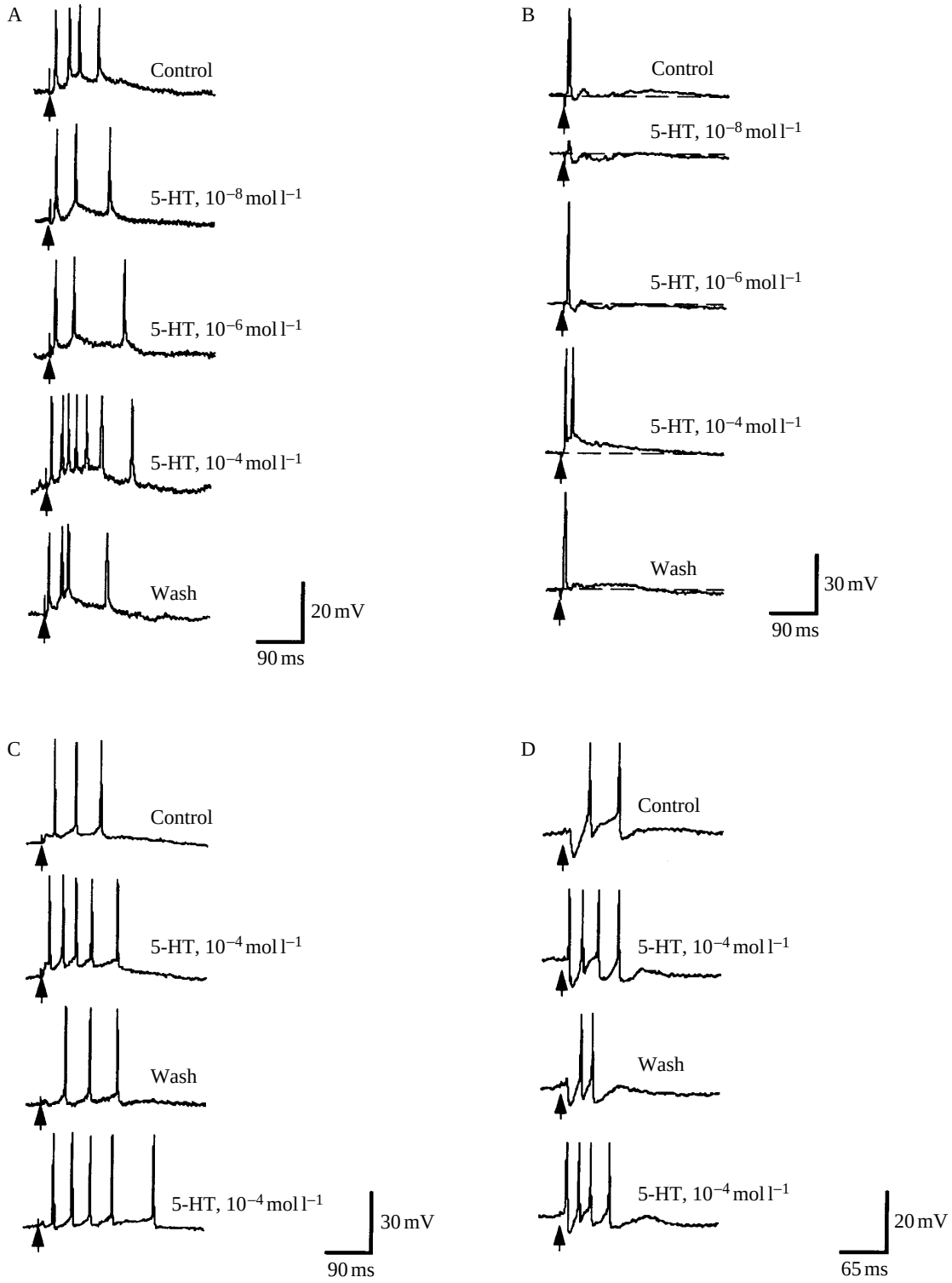

Fig. 1. Responses from two local interneurones (A,C) and two projection neurones (B,D) before, during and after superfusion with physiological saline solution containing 5-HT. Responses were evoked by electrical stimulation (arrows) of the ipsilateral antennal nerve. (A,B) At low concentration (10^{-8} mol l^{-1}), 5-HT decreased responses to antennal nerve shock, whereas at high concentration (10^{-4} mol l^{-1}), it increased responses to the primary afferent input. The effects of 5-HT could be reversed by washing with physiological saline solution. (C,D) Effect of repeated application of 5-HT (10^{-4} mol l^{-1}). 5-HT increased the responses of both types of antennal lobe neurones. In the local interneurone (C), 5-HT enhanced depolarization and increased the number of spikes elicited by antennal nerve shock. In the projection neurone (D), 5-HT increased the number of evoked spikes. In both neurones, the effects of 5-HT could be reversed by washing with physiological saline solution and were reproducible during a second application of 5-HT. Although the responses of local interneurones and projection neurones to electrical stimulation of the antennal nerve differ, the effects of 5-HT were similar in both types of AL neurones.

current (115 ms, slope 0.01 nA ms^{-1}) were used to elicit single spikes in the neurones. Spike widths, measured at 50 % amplitude relative to the resting potential, increased under the influence of 10^{-4} mol l^{-1} 5-HT in 9 out of the 21 neurones studied, including both local interneurones and projection neurones (Fig. 4). In the neurones that gave clear responses to 5-HT, the increase in spike width was 11.3±2.2 % (mean ± S.D., *N*=9, *P*<0.001 by paired *t*-test; Fig. 4C). In contrast to the other described effects, this spike broadening was not fully reversible during the limited recording time (approximately 30 min, including a wash of approximately 15 min).

In Fig. 4B the spike widths of 26 neurones are plotted as a function of time. The spike broadening of the nine 5-HT-sensitive neurones coincided with application of 5-HT. During periods of washing, these neurones recovered partially from the effects of 5-HT. Twelve of the neurones did not show such spike-broadening during application of 5-HT, and the variation in spike width during the recording time was similar to that of an untreated control group of five neurones.

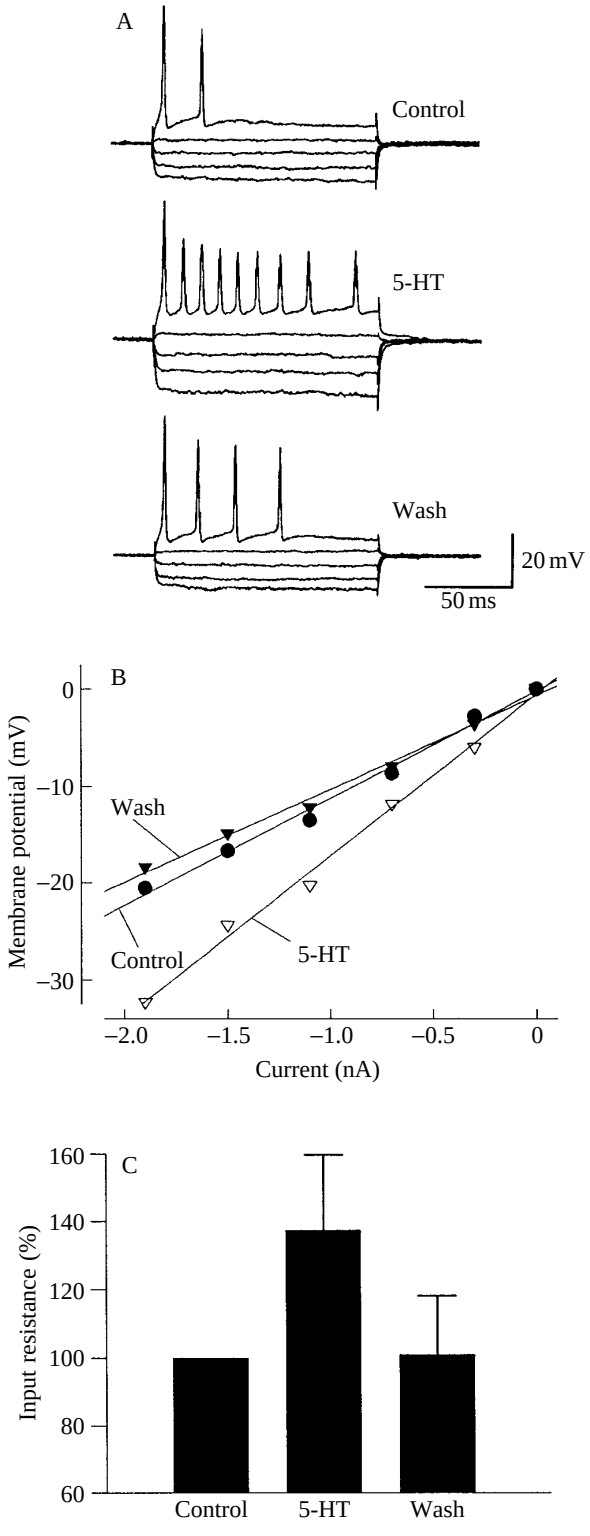

Fig. 2. Effect of 5-HT on current–voltage (*I–V*) relationships. (A) Membrane excitability: 5-HT (10^{-4} mol l^{-1}) increased the cell excitability in this experiment. A 125 ms, 0.5 nA depolarizing current pulse elicited two spikes in the neurone prior to application of 5-HT. During 5-HT application, spikes occurred throughout the pulse. Cell input resistance: the change in membrane potential resulting from a constant-amplitude current pulse was increased during application of 5-HT (hyperpolarizing current injection 125 ms, −1.1 to −0.3 nA in 0.4 nA steps). (B) The *I–V* relationships of the neurone indicate an increase in cell input resistance during application of 5-HT (hyperpolarizing current injection 125 ms, −1.9 to −0.3 nA in 0.4 nA steps). (C) Relative change of cell input resistance in six neurones before, during and after application of 5-HT (10^{-4} mol l^{-1}). The input resistance of the investigated cells was 9.9±2.8 MΩ (mean ± S.D., control value shown as 100 %). The cell input resistances were calculated from the slopes of *I–V* plots similar to the one shown in Fig. 2B (hyperpolarizing current injection: 125 ms, −2.0 to −0.4 nA or −1.9 to −0.3 nA in 0.4 nA steps). The change in input resistance caused by 5-HT is expressed as a change of percentage (37±22 %, mean ± S.D.) relative to the control. Significance: control to 5-HT, *P*<0.01 (paired *t*-test); 5-HT to wash, *P*<0.01; *N*=6.

Time course of the 5-HT effects

Effects of 5-HT appeared within a few seconds and reached their maximum within 3 min after the onset of application of 5-HT. No change in the effects of 5-HT at this concentration was observed if the amine was applied continuously over a period of 10 min. Responses started to return to control levels within 1 min after cessation of application of 5-HT, when superfusion was switched to normal saline solution. After 3–10 min, the effects of 5-HT had vanished. The time course of recovery from the 5-HT-induced spike broadening apparently differs from time courses of recovery from the other described effects. Under the present experimental conditions, the spike-broadening effect was not fully reversible within the limited recording time (approximately 30 min, including a wash of approximately 15 min), whereas the increase in cell input resistance and in the response elicited by antennal stimulation were fully reversible within 10 min (Figs 1–3). In neurones for which the effects on spike-broadening, input resistance and response to primary synaptic input were tested, the spikes usually remained broadened when the other effects had already fully reversed.

Discussion

The modulatory effects of 5-HT on olfactory interneurones in the AL of *M. sexta* were investigated by means of intracellular recordings from cells that could be identified as either local interneurones or projection neurones by electrophysiological criteria. Neurones that could not be classified by these criteria are not described in this report. Additional, more precise characterization of the AL neurones would require intracellular staining with injected dye, a procedure not used in the present investigation because we required stable electrode resistances throughout the duration of the intracellular recording.

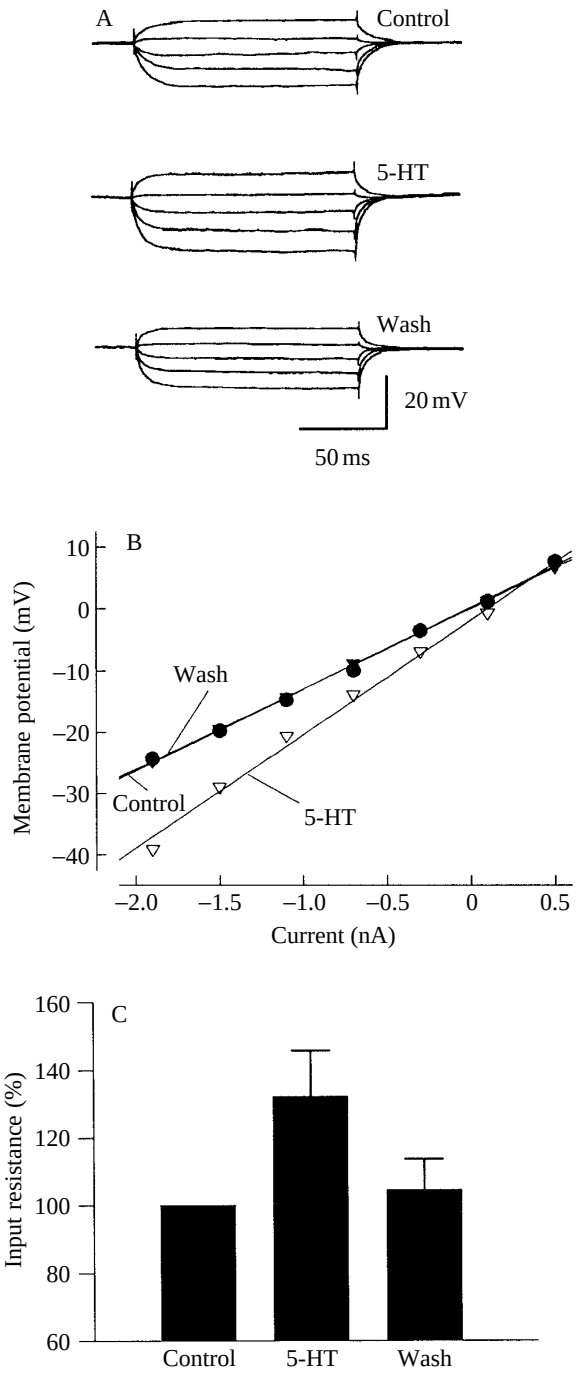

Fig. 3. Effects of 5-HT on current–voltage relationships during application of TTX and Cd^{2+}. (A) The change in membrane potential resulting from a constant-amplitude current pulse was increased during application of 5-HT (current injection 125 ms, −1.1 to +0.5 nA in 0.4 nA steps). (B) The *I–V* relationships of the neurone indicate an increase in cell input resistance during application of 5-HT (current injection: 125 ms, −1.9 to +0.5 nA in 0.4 nA steps). (C) Relative change of cell input resistance in four different neurones before, during and after application of 5-HT (10^{-4} mol l^{-1}). The input resistance of the investigated cells was 10.2±3.7 MΩ (mean + S.D., control value shown as 100 %). The input resistances were calculated from the slopes of *I–V* plots similar to the one shown in B (current injection: 125 ms, −1.9 to +0.5 nA in 0.4 nA steps). The change in input resistance caused by 5-HT is expressed as change of percentage (32±13 %, mean ± S.D.) relative to the control. Significance: control to 5-HT, $P<0.02$ (paired *t*-test); 5-HT to wash, $P<0.02$; $N=4$.

Dose–response relationships

The effects of 5-HT on AL neurones were dose-dependent. It should be kept in mind that the applied concentrations of 5-HT, which were in the range 10^{-8} to 10^{-4} mol l^{-1}, did not necessarily reflect the actual concentrations experienced by the neurones. Nevertheless, high and low concentrations of 5-HT had different effects. It is possible that the different effects of 5-HT were mediated by different receptors, and pharmacological experiments could provide useful insights into the characteristics of the receptors involved. Receptor-binding studies have demonstrated that methysergide is an effective ligand for 5-HT receptors in the honeybee (Scheidler *et al.* 1989; Scheidler, 1991). Bermudez *et al.* (1992) showed that 5-HT antagonists, such as ketanserin, ritanserin and MDL72222, have antagonistic effects on certain 5-HT-activated currents in isolated somata from the thoracic ganglion of *Locusta migratoria*. The specificity of 5-HT agonists and antagonists in the neuropile of the insect CNS has yet to be determined. It is likely that several different types of amine receptors are expressed by AL neurones of *M. sexta*. Octopamine- and dopamine-like immunoreactivities as well as 5-HT immunoreactivity have been observed in this region of brain neuropile (U. Homberg, personal communication).

Possible cellular mechanisms

The electrophysiological experiments reported here demonstrated that 10^{-4} mol l^{-1} 5-HT increased the responses of a population of AL neurones to primary afferent input. This effect was accompanied by increases in cell excitability, cell input resistance and spike width. A similar 5-HT-induced increase in cell input resistance was apparent in preparations that had been treated with TTX and Cd^{2+} in order to block Na^+ and Ca^{2+} currents. Together with the results reported in Mercer *et al.* (1995), we interpret this as strong evidence that 5-HT has direct effects on the neurones we studied.

Similar effects of 5-HT have been found in molluscs and crustaceans. For example, in sensory neurones of *Aplysia californica*, spike broadening and changes in cell excitability result from modulation of K^+ channels by 5-HT (Klein *et al.* 1982). Whole-cell patch-clamp studies of cultured AL

neurones from *M. sexta* (Mercer *et al.* 1995) support the idea that modulation of K^+ channels may underlie the neuromodulatory effects of 5-HT observed in the present study. *In vitro* studies of a morphologically identifiable subset of AL neurones have shown that 5-HT decreases a fast A-type K^+ current as well as a slower-activating K^+ current that resembles the delayed rectifier (Mercer *et al.* 1992, 1995). The finding, described in the present report, that the different effects of 5-HT had different time courses has been confirmed in patch-clamp experiments *in vitro* (A. R. Mercer and P. Kloppenburg, unpublished observations), suggesting that more than one cellular mechanism is influenced by 5-HT.

Possible significance of the neuromodulatory effects of 5-HT

The findings presented here demonstrate that 5-HT has neuromodulatory effects on olfactory interneurones in the AL of *M. sexta*. The functional significance of these effects for olfactory information processing in the AL and for olfaction-dependent behaviour, however, remains to be explored further. Linn and Roelofs (1986) have reported that, in the moth *Trichoplusia ni*, exogenously applied 5-HT can extend the time window during which pheromone-induced behaviour can be elicited and random motor activity can be expressed. In the moth *Lymantria dispar*, 5-HT enhances circadian-rhythm-dependent general motor activity, and in *M. sexta*, injection of 5-HT into a thoracic ganglion suppresses dopamine-induced flight motor activity (Claassen and Kammer, 1985). 5-HT reduces conditioned olfactory responses in the honeybee *Apis mellifera* (Mercer and Menzel, 1982; Menzel *et al.* 1988) and modulates the activity of motor neurones in the lobster *Homarus americanus* by acting on premotor interneurones (Harris-Warrick and Kravitz, 1984; Kravitz *et al.* 1985). Local application of 5-HT in the medulla externa and medulla terminalis of the visual system of the crab *Leptograpsus variegatus* enhances an optokinetic response (Erber and Sandeman, 1989). Peripheral modulatory effects of 5-HT have been found in mechanoreceptors of *H. americanus* (Pasztor and Bush, 1987, 1989), where 5-HT causes a reduction of receptor potential amplitude. These related findings provide ample motivation for further studies aimed at establishing the behavioural significance of neuromodulation by 5-HT in the antennal lobe of the moth.

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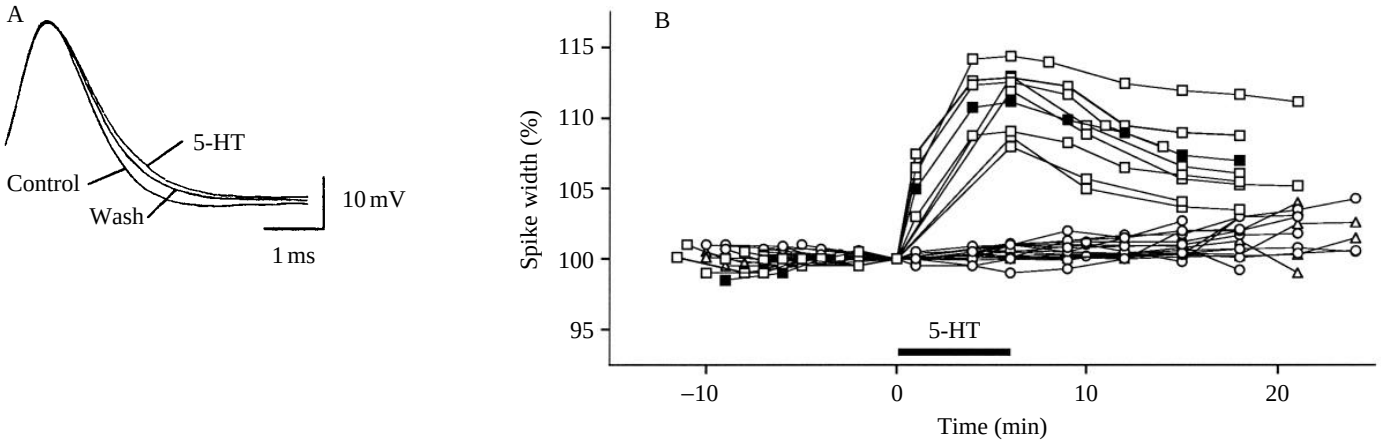

Fig. 4. Spike-broadening effect of 5-HT. (A) These recordings are from the neurone presented in Fig. 2. The neurone was hyperpolarized to suppress spontaneous activity. Spikes were elicited by depolarizing current injection. The spike width measured at 50 % spike amplitude (relative to the resting potential) was increased from 1.31±0.01 to 1.46±0.01 ms (N=3, P<0.001 using the paired t-test). (B) The spike widths of 26 neurones plotted as a function of time relative to 5-HT application. The spike width for each neurone at each time point is expressed as the percentage of the spike width for that cell measured at 0 min, the onset of application of 5-HT (which proceeded over the ensuing 6 min, indicated by the bar). Every point is a mean of three measurements (S.D. always <1 %). The spike width increased in nine neurones (open squares), whereas 5-HT had no effect on twelve neurones (circles). Five neurones served as controls (triangles). Instead of applying 5-HT to these neurones, we switched the perfusion system to a reservoir containing normal saline solution. Filled squares represent the neurone of Fig. 4A. (C) Relative change of cell input resistance in the nine neurones shown in B before, during and after application of 5-HT (10^{-4} mol l^{-1}). The spike width is expressed as change of percentage (mean + S.D.) relative to the control. Control and 5-HT represent the measurements taken at 0 min and 6 min, respectively. Wash represents the last measurements taken from each recording. 5-HT increases the spike width by 11.3±2.2 %. Significance: control compared with 5-HT, P<0.01 (paired t-test); 5-HT compared with wash, P<0.01; N=9.

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