

5-HYDROXYTRYPTAMINE MODULATES PHEROMONE-EVOKED LOCAL FIELD POTENTIALS IN THE MACROGLOMERULAR COMPLEX OF THE SPHINX MOTH *MANDUCA SEXTA*

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Summary

Extra- and intracellular recordings from an intact brain preparation were used to study the effects of 5-hydroxytryptamine (5-HT or serotonin) on projection neurons in the sexually dimorphic macroglomerular complex (MGC) in the antennal lobe of the male moth *Manduca sexta*. The MGC is a group of three identified glomeruli specialized for synaptic processing of primary afferent information about the multi-component sex pheromone of the female. We investigated the modulatory effects of 5-HT on pheromone-evoked local field potentials in the MGC. The magnitude and duration of these potentials, which are thought to be generated by a population of pheromone-sensitive projection neurons of the MGC, were increased by 5-HT. Using intracellular

recordings from the neurites of individual MGC projection neurons, we found that 5-HT increased the number of action potentials in response to pheromonal stimulation. These findings correlate well with earlier experiments that used other recording techniques. Our results are further evidence that 5-HT modulates a population of pheromone-sensitive MGC projection neurons that relay information about the pheromonal stimulus from the MGC to higher-order centers in the protocerebrum and are therefore pivotal for mate-finding and odor-guided behavior.

Key words: evoked potential, glomerulus, neuromodulation, olfaction, pheromone, serotonin, *Manduca sexta*.

Introduction

The macroglomerular complex (MGC) in each of the paired primary olfactory centers, the antennal lobes, of the brain of the male sphinx moth *Manduca sexta* consists of three sexually dimorphic glomeruli specialized for synaptic processing of primary afferent information about the multi-component sex pheromone of the female (Hansson et al., 1991; Heinbockel and Hildebrand, 1998). Because of its dedication to processing sensory inputs tuned to identified odorants (components of the pheromone blend) of known behavioral function, the MGC has served as an experimental model for extensive studies of the organization and physiology of olfactory glomeruli (Hildebrand, 1995, 1996; Hildebrand and Shepherd, 1997; for a more general review about olfactory information processing in insects, see Masson and Mustaparta, 1990).

The antennal lobes of male *M. sexta* contain approximately 60 'ordinary' spheroidal glomeruli and three sexually dimorphic glomeruli, which constitute the MGC (Rosparis and Hildebrand, 1992). Like other glomeruli in the antennal lobe, each MGC glomerulus houses terminals of antennal olfactory receptor cells, neurites of projection (output) neurons and local

interneurons (confined to the antennal lobe), and terminals of centrifugal neurons that project to the antennal lobes from elsewhere in the brain. In *M. sexta*, each antennal lobe possesses the soma of a single 5-HT-immunoreactive neuron that projects from the protocerebrum, where it has complex arborizations, to the contralateral antennal lobe, where it has widely ramifying, multiglomerular terminals (Kent et al., 1987; Homberg and Hildebrand, 1989; Sun et al., 1993). The processes of this 5-HT-containing neuron exhibit predominantly output synapses in the glomerular neuropil (Sun et al., 1993).

The three glomeruli of the MGC have been termed the cumulus, toroid 1 and toroid 2 on the basis of their shapes (Hansson et al., 1991; Heinbockel et al., 1994, 1995; Homberg et al., 1995). The cumulus and toroid 1 both receive direct input from olfactory receptor cells tuned to one of the two essential components of the sex pheromone (Kaissling et al., 1989; Christensen et al., 1995). MGC projection neurons (MGC-PNs) with arborizations in the toroid or cumulus or both and axons projecting to the protocerebrum respond to either one or

both of these two key pheromone components (Hansson et al., 1991). MGC-PNs with arborizations confined to toroid 1 respond preferentially to E10,Z12-hexadecadienal (bombykal or Bal), MGC-PNs with arborizations confined to the cumulus respond preferentially to E10,E12,Z14-hexadecatrienal (EEZ) and MGC-PNs with arborizations in both the cumulus and toroid 1 typically respond to both Bal and EEZ (Hansson et al., 1991). When the ipsilateral antenna is stimulated with the pheromone blend, an MGC-PN typically depolarizes and fires a burst of action potentials that is generally followed by a period of membrane hyperpolarization (Christensen and Hildebrand, 1987). In some MGC-PNs, excitation is preceded by a brief hyperpolarization, the amplitude of which varies from one MGC-PN to another (Christensen and Hildebrand, 1987; Christensen et al., 1996; Heinbockel et al., 1999).

Extracellular recordings from the MGC of *M. sexta* have revealed that pheromonal stimulation of the ipsilateral antenna evokes specific potentials in this highly specialized sensory neuropil (Heinbockel et al., 1998). These potentials typically consist of a sustained component superimposed by rapid oscillations. There is evidence from previous studies in *M. sexta* (Heinbockel et al., 1998) and other preparations (see below) suggesting that these potentials might result from the summed activity of pheromone-sensitive MGC neurons, especially MGC-PNs.

Coherent, rhythmic activity patterns evoked by odor stimulation have been observed in the olfactory systems of several vertebrate and invertebrate species (Gray, 1994; Gelperin et al., 1996; Laurent, 1996). In insects, these odor-evoked potential oscillations in the antennal lobe have been implicated in coding of odor information (Laurent and Davidowitz, 1994; Laurent, 1996; Laurent et al., 1996a,b) and are thought to be generated by the synchronous activity of antennal lobe projection neurons (Laurent and Davidowitz, 1994).

Somatic whole-cell patch-clamp recordings from MGC-PNs in the male antennal lobe of *M. sexta* showed that 5-HT increases neuronal excitability and the response to stimulation with pheromone (Kloppenborg et al., 1999). At the cellular level, the action of 5-HT is mediated, at least in part, by a decrease in the magnitude of two voltage-activated K^+ currents, a transient current, I_A , and a sustained current, $I_{K(v)}$ (Kloppenborg et al., 1999). On the basis of our earlier studies, we investigated the effect of 5-HT on (i) neuritic intracellular responses of MGC-PNs to pheromonal stimulation and (ii) the pheromone-evoked local field potentials that are thought to be generated mainly by a population of MGC-PNs.

Materials and methods

Animals and physiological preparation

Manduca sexta (Lepidoptera: Sphingidae) were reared on an artificial diet modified from that of Bell and Joachim (1976) at 25–26 °C and 40–50% relative humidity under a long-day photoperiod regimen (17 h:7 h light:dark). All chemicals, unless stated otherwise, were obtained from Sigma (St Louis, MO, USA).

Extracellular and intracellular recordings were obtained from intact brain preparations. In an attempt to perform all experiments on animals in a reproducible physiological state, all moths in this study were handled identically. Adult male moths (1–3 days post-eclosion) were prepared in the evening prior to the day of the experiment. 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. For dissection, the moths were anesthetized by cooling on ice or in a refrigerator (at approximately 4 °C) for 20–30 min. 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 and the cibarial-pump musculature. With the antennae and their innervation intact, the head was then separated from the rest of the animal and pinned in a Sylgard-coated recording chamber (volume approximately 1 ml). To facilitate the insertion of a recording electrode and the penetration of pharmacological agents into the tissue, parts of the antennal lobe were desheathed manually using very fine forceps.

During the experiment, the preparation was superfused constantly (at approximately 2 ml min⁻¹) with saline solution modified from that of Pichon et al. (1972), containing (in mmol l⁻¹): 149.9 NaCl, 3.0 KCl, 6.0 CaCl₂, 10.0 Tes, pH 6.9, and 25.0 sucrose to balance the osmolarity with that of the extracellular fluid. 5-HT (serotonin creatinine sulfate from Sigma or Research Biochemicals, Natick, MA, USA) was added to the superfusion saline solution at a final concentration of 10⁻⁴ mol l⁻¹. The threshold for detectable excitation of projection neurons by 5-HT was 10⁻⁵ mol l⁻¹, and a maximal effect was observed at 10⁻⁴ mol l⁻¹ (Kloppenborg and Hildebrand, 1995). 5-HT was applied only once in each preparation.

Intracellular recordings and staining

Intracellular recording electrodes were fabricated from borosilicate glass capillaries (outer diameter 1.0 mm, inner diameter 0.5 mm; World Precision Instruments, Sarasota, FL, USA) with a Flaming-Brown puller (model P-2000, Sutter Instrument Co., San Rafael, CA, USA). The tip of each electrode was filled with a solution of an intracellular marker (see below), and the shaft of the electrode was filled with filtered (0.2 µm pore size) 2.5 mol l⁻¹ KCl or potassium acetate solution. The electrodes typically had resistances of 50–100 MΩ. Recordings were made from neurites in the neuropil of the MGC. Signals were amplified by an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA, USA), and intracellular recording and current injection were carried out in bridge mode. Data were digitized at 5–10 kHz using a TL1 board (Axon Instruments) and pClamp 6 software (Axon Instruments) running on a PC and stored on disk.

Whenever feasible after the relatively long physiological experiments, the neuron was injected ionophoretically with either Neurobiotin (Vector Laboratories, Burlingame, CA, USA; 3–5% in KCl with 0.05 mol l⁻¹ Tris buffer, pH 7.4) or

biocytin (Sigma, 3–5 % in 2 mol l^{-1} KCl with 0.05 mol l^{-1} Tris buffer, pH 7.4). To inject biocytin, alternating hyperpolarizing and depolarizing current pulses (1 nA, 1 min) were used for a total of 10 min. For injection of Neurobiotin, depolarizing current (1 nA) was employed for 10 min. Neurons stained with Neurobiotin or biocytin were visualized using Cy3-conjugated streptavidin. The brains were fixed overnight in 2.5 % formaldehyde (in 0.1 mol l^{-1} phosphate buffer with 3 % sucrose) and subsequently rinsed in 0.2 mol l^{-1} phosphate buffer. The tissue was incubated on a shaker at approximately 4°C for 3 days with Cy3-conjugated streptavidin (Jackson Immunologicals, West Grove, PA, USA) diluted 1:100 in 0.2 mol l^{-1} phosphate buffer containing 0.3 % Triton X-100. The brains were dehydrated and cleared in methyl salicylate. All brains were inspected as whole mounts in a fluorescence microscope (Nikon, Melville, NY, USA). Clearly stained neurons were investigated further as whole mounts using a laser scanning confocal microscope (BioRad MRC 600, Hercules, CA, USA).

Evoked potential recordings

Methods for recording evoked potentials in the insect brain have been described elsewhere (e.g. Mercer and Erber, 1983; Kaulen et al., 1984; Kloppenburg and Erber, 1995; Laurent and Naraghi, 1994; Heinbockel et al., 1998). Recording electrodes (patch pipettes) were produced from borosilicate glass ($100 \mu\text{l}$ micropipettes, outer diameter 1.71 mm, inner diameter 1.32 mm; VWR Scientific, West Chester, PA, USA) using a Flaming-Brown puller (P-87, Sutter Instrument Co.). The electrodes were filled with filtered ($0.2 \mu\text{m}$ pore size) extracellular saline solution and had resistances of 1–5 M Ω . The signals were recorded by an Axoclamp-2A amplifier in bridge mode. Data were digitized (5–10 kHz) and stored on disk with the aid of the software program pClamp 6 (Axon Instruments) and a Digidata board 1200B (Axon Instruments) running on a PC. For analysis of electrophysiological data, the software programs pClamp 6 (Axon Instruments), Axograph 3 (Axon Instruments) and Origin 4 and 5 (Microcal Software, Inc., Northampton, MA, USA) were used.

Odor stimulation

The antenna ipsilateral to the recording electrode was stimulated with clean air or with air carrying odorants (see Matsumoto and Hildebrand, 1981). Odor stimuli were delivered to the antenna by pulsing air from a compressed air cylinder through cartridges (0.5 ml acid-cleaned glass syringe barrels) containing filter paper impregnated with odorant. Odor cartridges could be changed during the course of the experiment. The air-puffs were controlled by a solenoid-driven valve (General Valve, Fairfield, NJ, USA) triggered by pClamp 6 software and the corresponding computer interface.

As odor stimuli, we used a mixture of 10 ng of E10,Z12-hexadecadienal (Bal; one of the two key components of the sex pheromone of female *M. sexta*) and 10 ng of E11,Z13-pentadecadienal ('C15'), a chemically more stable mimic of the second key component (E10,E12,Z14-hexadecatrienal, or

'EEZ') of the pheromone (Christensen et al., 1989; Kaissling et al., 1989; Tumlinson et al., 1989). These synthetic compounds were gifts from Dr J. H. Tumlinson (USDA, Gainesville, FL, USA). Odorants were dissolved in hexane and applied to the filter paper (1 cm \times 2 cm) as $100 \mu\text{l}$ of solution. Throughout this study, concentrations of odorants refer to the source concentration on the filter paper in the stimulus cartridge. The concentration that actually reached the antennal sensilla was unknown.

Cartridges containing clean filter paper alone (blank control) were used to confirm that the elicited responses were not due simply to mechanical stimulation. Because odorants were dissolved in hexane, we also tested all preparations with cartridges loaded with filter paper originally bearing pure hexane (solvent control). Since the solvent evaporates over 1–2 min, responses to this solvent control did not differ from responses to a blank cartridge (containing clean filter paper). Because all odorants were applied in hexane to filter paper at least 1 h before use, it is very unlikely that any of the responses obtained were due to hexane. To minimize sensory adaptation, each odor stimulus was separated from the previous and following ones by interstimulus intervals of at least 1 min.

Statistical analyses

Student's *t*-test was used to assess the significance of differences between mean values of variables measured under control conditions, during 5-HT application and after washing in 5-HT-free saline. A Bonferroni correction was used to adjust for repeated *t*-tests, and significance was accepted at $P=0.025$. Throughout this paper, all calculated ranges are reported as the standard error of the mean (S.E.M.).

Results

The effects of exogenously applied 5-HT on sex-pheromone-evoked local field potentials in the MGC were analyzed by means of extracellular recordings. Evidence from previous studies suggested that the extracellularly recorded, pheromone-evoked potentials might reflect mainly the population response of MGC-PNs (see Laurent and Davidowitz, 1994; Heinbockel et al., 1998), so we recorded MGC-PN activity intracellularly in current-clamp mode from neurites of these neurons in a parallel series of experiments.

Effects of 5-HT on responses of single MGC-PNs to antennal stimulation with pheromone

MGC-PNs were recognized on the basis of both their well-described electrophysiological responses to pheromonal stimulation of the ipsilateral antenna and their morphological characteristics, revealed by intracellular staining. Fig. 1 shows a typical response (Fig. 1A) and glomerular arborizations (Fig. 1B) of an MGC-PN. Intracellular staining of this MGC-PN revealed arborizations spanning the cumulus and toroid 1. Stimulation of the ipsilateral antenna with the two-component pheromone blend evoked a membrane depolarization with a burst of action potentials followed by a delayed

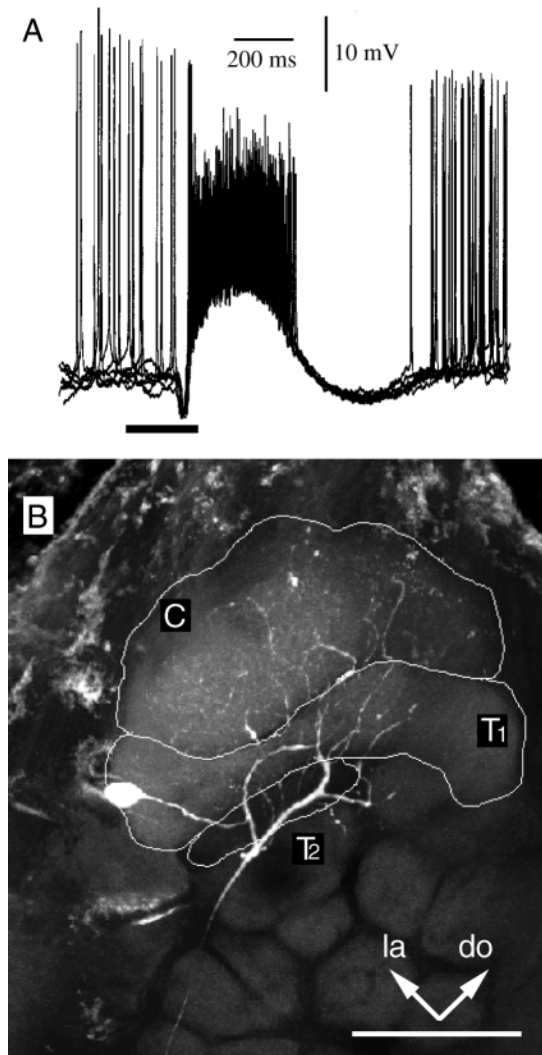


Fig. 1. Intracellular recordings showing the responses of a macroglomerular complex projection neuron to antennal stimulation with the sex pheromone blend (10 ng of Bal and 10 ng of C15; see Materials and methods). (A) Seven overlaid traces recorded at 5 min intervals over a period of 30 min to demonstrate the reproducibility of the response during the experiment. An initial rapid hyperpolarization was followed by strong depolarization of the membrane potential with a burst of action potentials and an after-hyperpolarization. The bar beneath the recordings marks the duration of the pheromone stimulation. (B) Arborization within the MGC of the same neuron as in A, revealed by intracellular staining. Laser scanning confocal image of a projection neuron with arborizations in both the cumulus and the toroid 1. Scale bar, 100 μm ; C, cumulus; T1, toroid 1; T2, toroid 2; la, lateral; do, dorsal.

hyperpolarization ($N=34$). Once a stable recording had been established, responses to the pheromone blend were highly reproducible upon repeated stimulation ($\leq 1 \text{ min}^{-1}$) and remained unaltered over at least 30 min.

Fig. 2A shows the response of an MGC-PN to antennal stimulation with pheromone before, during and after application of 5-HT. Its glomerular arborizations are shown in

Fig. 2B. The principal effect of 5-HT was to increase the excitatory components of the pheromone-induced response. As summarized in Fig. 3, in the presence of 5-HT, the number of action potentials was increased by $16 \pm 2\%$ ($P < 0.001$; $N=6$) and the duration of the burst was increased by $19 \pm 4\%$ ($P < 0.001$; $N=6$). This effect had developed fully approximately 3–5 min after application of 5-HT and was readily reversible when 5-HT-containing saline solution was replaced by normal saline solution. In some experiments, however, full recovery was not observed within the recording time (up to 40–50 min after starting the wash).

Effects of 5-HT on pheromone-evoked potentials

Stimulation of the ipsilateral antenna with the pheromone blend evoked potential changes in the MGC that could be recorded extracellularly. A detailed description of these pheromone-evoked potentials, including their oscillatory component, has already appeared (Heinbockel et al., 1998). In brief, these potentials consisted of an initial slope that often (but not always) reached a plateau during the remainder of the stimulus (typically 300 ms). After the end of stimulation, the potential returned slowly to the pre-stimulus level. These signals typically had an amplitude of 1–2 mV. In the following, this component of the evoked potential is referred to as the 'sustained component'. In many recordings, this sustained component was overlaid by rapid membrane potential oscillations (30–50 Hz). Fig. 4 shows an example of these pheromone-evoked potentials. Blank control and solvent control stimuli presented during each experiment (see Materials and methods) elicited no response or significantly smaller responses (not shown; see Heinbockel et al., 1998). These results suggest that the potentials evoked within the MGC by pheromonal stimulation are generated by pheromone-sensitive neurons. The responses were highly reproducible and remained unaltered over at least 30 min if the stimulus was applied at a low frequency ($\leq 1 \text{ min}^{-1}$; Fig. 4).

The amplitude and duration of potentials evoked by pheromonal stimulation were increased by 5-HT (Figs 5, 6). The sustained and oscillatory components of the signal could be separated by digital filtering and subtraction protocols (see Figs 5, 6; for methodological details, see Heinbockel et al., 1998). To quantify the effects of 5-HT on the sustained component (Fig. 5), the following variables were measured: (i) peak amplitude, (ii) the duration from the onset of depolarization to the point at which the potential returned to baseline, and (iii) the integral from the onset of depolarization to the point at which the potential returned to baseline (Fig. 5B). The amplitude of the evoked potentials was increased by $63 \pm 10\%$ ($P < 0.01$; $N=5$), and the duration of the response was increased by $37 \pm 4\%$ ($P < 0.01$; $N=5$). Consequently, the integral of the signal was increased by $77 \pm 17\%$ ($P < 0.01$; $N=5$). Analysis of the fast oscillatory component (Fig. 6A,B) revealed that the mean amplitude of the oscillations was increased by 5-HT by $25 \pm 4\%$ ($P < 0.01$; $N=5$) and that the duration of the oscillatory component was increased by $26 \pm 4\%$ ($P < 0.01$; $N=5$). All effects were

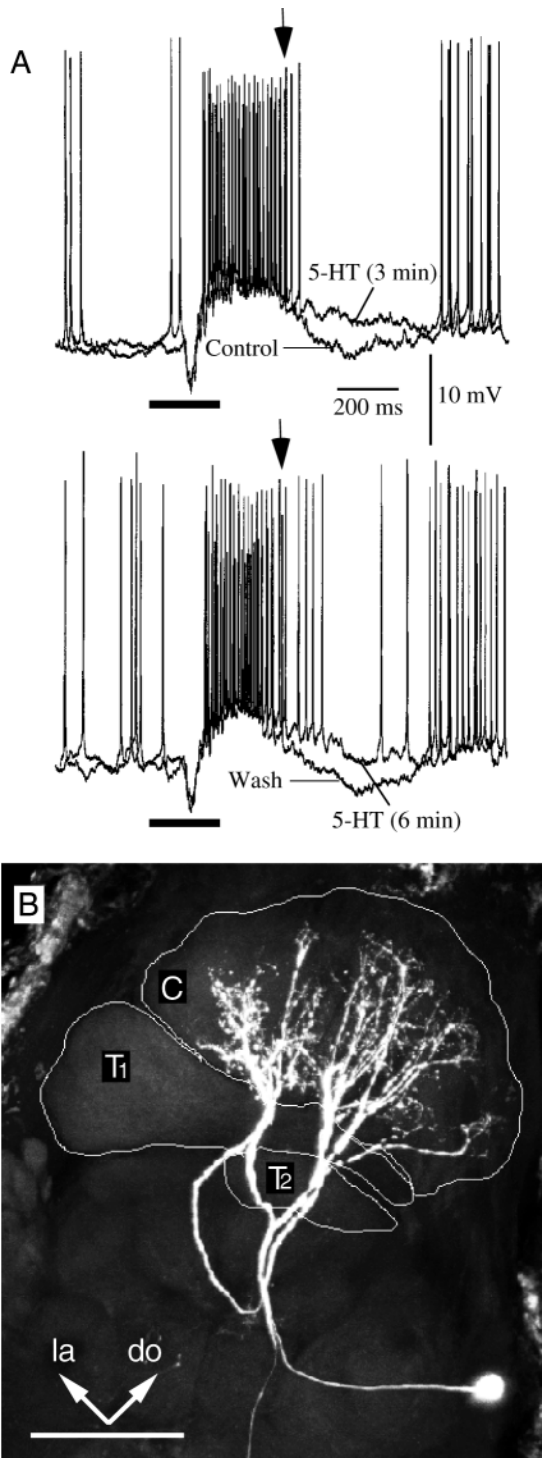


Fig. 2. (A) Intracellular recordings of responses of a macroglomerular complex projection neuron to antennal stimulation with the pheromone blend before, during and after application of 5-HT (10^{-4} mol l $^{-1}$). The top two traces show the responses before (Control) and during 5-HT application, while the bottom two traces depict the responses during and after 5-HT application (Wash). Arrows indicate the position of the last spike of the response to the pheromone blend recorded prior to application 5-HT (top recording, Control) and after washing in 5-HT-free saline (bottom recording, Wash). Application of 5-HT increased the number of spikes, the length of the spike train and the duration of the depolarization elicited by stimulation with the pheromone blend (10 ng of Bal; 10 ng of C15). All effects were reversed by washing with 5-HT-free saline. The bar beneath the two recordings marks the duration of pheromone stimulation. (B) Arborizations of this projection neuron within the MGC revealed by intracellular staining. Scale bar, 100 μ m; C, cumulus; T1, toroid 1; T2, toroid 2; la, lateral; do, dorsal.

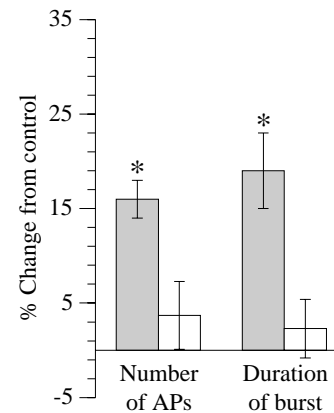


Fig. 3. Effects of 5-HT on mean percentage change in the number of spikes and the length of the spike train. Serotonin (shaded columns) significantly increased the number of action potentials (APs) (by $16 \pm 2\%$; $P < 0.001$; $N = 6$) and the length of the spike train (by $19 \pm 4\%$; $P < 0.001$; $N = 6$). Both effects reversed significantly after washing with saline (open columns; $P < 0.001$ for both variables). Values are means \pm S.E.M. An asterisk indicates a value significantly different from the control ($P \leq 0.025$).

reversible, and 5-HT had no significant effect on the frequency of the fast oscillatory component.

Discussion

Using extracellular recordings of evoked potentials in the MGC and intracellular recording from MGC-PNs, we have been able to monitor the modulatory effects of 5-HT on the

responses of a population of MGC neurons to stimulation of the antennae with biologically relevant odors, namely sex pheromones. We assume that exogenously applied 5-HT mimics the effects of 5-HT released from the processes of the centrifugal 5-HT-immunoreactive neurons that innervate each antennal lobe (Kent et al., 1987; Homberg and Hildebrand, 1989; Sun et al., 1993). The majority of contacts between serotonergic processes and antennal lobe neurons are output synapses from the 5-HT-containing cell and are well placed to modulate the activity of projection neurons (Sun et al., 1993).

An earlier study has shown that local field potentials in the MGC of *M. sexta* can be evoked by stimulation of the ipsilateral antenna with sex pheromone (Heinbockel et al., 1998). These potentials are thought to be generated by pheromone-sensitive neurons located in this male-specific region of the antennal lobe, and changes in these evoked

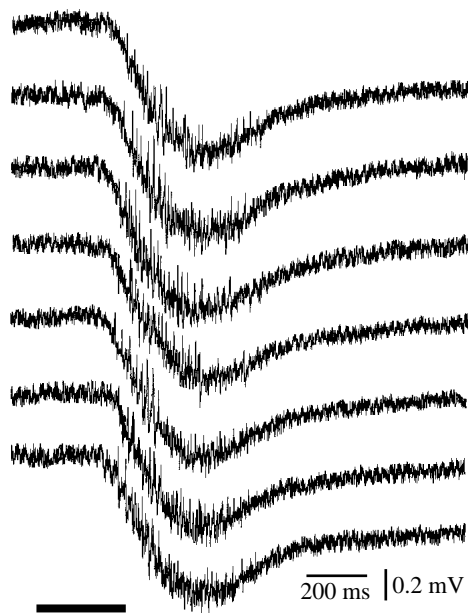


Fig. 4. Potentials evoked by pheromone stimulation and recorded extracellularly in the macroglomerular complex. Seven traces recorded at 5 min intervals over a period of 30 min are shown to demonstrate the reproducibility of the response over time. The bar beneath the recording marks the duration of stimulation with the pheromone blend (10 ng of Bal; 10 ng of C15).

potentials are likely to reflect modifications of the responses of many pheromone-sensitive neurons (for a discussion of the generation of evoked potentials in insect brains, see also Mercer and Erber, 1983; Kaulen et al., 1984; Kloppenburg and Erber, 1995). Thus, the 5-HT-induced increase in magnitude and duration of the potentials evoked upon antennal stimulation with pheromone suggest an increase in activity of a larger population of pheromone-sensitive neurons. This finding correlates well with our earlier studies, in which 5-HT was found to affect, in similar ways, the response properties of many antennal lobe neurons both *in situ* and *in vitro*. Serotonin increases both the number and duration of action potentials elicited from antennal lobe neurons by electrical stimulation (Kloppenburg and Hildebrand, 1995; Mercer et al., 1996). By means of whole-cell patch-clamp recording, we recently found that 5-HT increased both the duration of the membrane depolarization and the number of action potentials elicited by pheromonal stimulation (Kloppenburg et al., 1999).

The functional role of the modulatory effects of 5-HT on pheromone-evoked potentials in the MGC of *M. sexta* has yet to be elucidated. Responses of individual neurons have been interpreted to be neural substrates for odor-guided behavior, and the synchronized activity in olfactory neuropils has been implicated in distinguishing and coding antennal information about different odorants (Laurent and Davidowitz, 1994; Laurent et al., 1996a,b). Rapid oscillations and evoked potentials have been observed in the olfactory systems of several insect species (e.g., bees, locusts and moths) that have

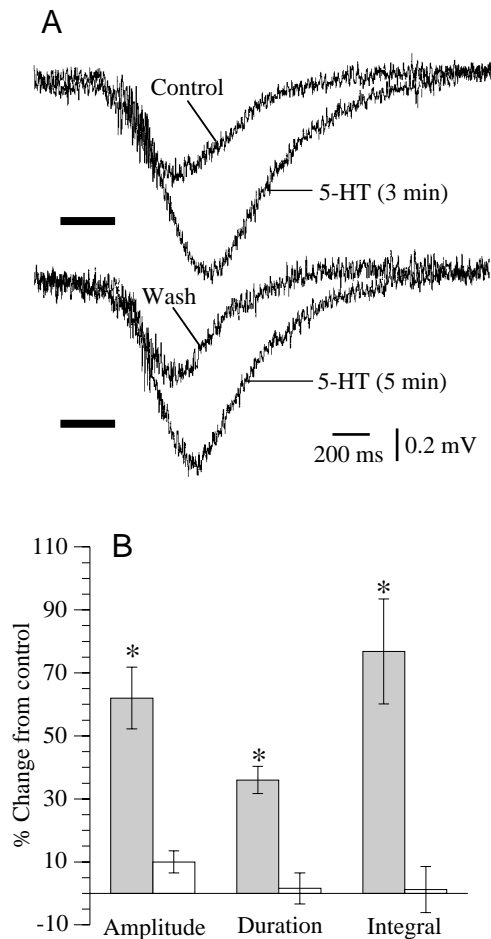


Fig. 5. (A) Extracellular recordings of pheromone-evoked potentials in the macroglomerular complex before, during and after application of 5-HT. The bar beneath the recording marks the duration of the pheromone stimulation (10 ng of Bal; 10 ng of C15). (B) Mean percentage change induced by 5-HT of several parameters of the pheromone-evoked potentials in the MGC (shaded columns). Addition of 5-HT to the bathing medium resulted in significant increases in amplitude ($63 \pm 10\%$; $P < 0.01$; $N = 5$), duration ($37 \pm 4\%$; $P < 0.01$; $N = 5$) and integral ($77 \pm 17\%$; $P < 0.01$; $N = 5$) of the evoked potentials (shaded columns). These effects were reversed by washing in saline (for all parameters, $P < 0.025$; open columns). Values are means \pm S.E.M. An asterisk indicates a value significantly different from the control ($P \leq 0.025$).

either broadly tuned or highly specialized olfactory systems (Wehr and Laurent 1996; Laurent et al., 1996b; Stopfer et al., 1997; Heinbockel et al., 1998). Synchronized oscillatory activity has been shown to occur during both long (1 s) and short (0.3 s) odor stimuli and for combinatorial representation of monomolecular and complex odors (Laurent and Davidowitz, 1994; Laurent and Naraghi, 1994; Laurent et al., 1996a,b; Heinbockel et al., 1998). These results suggest a general role for coherent oscillatory signals in the processing of olfactory information in different behavioral contexts or states of the animal. Synchronized oscillatory activity of neuronal assemblies might show better tuning to shorter odor

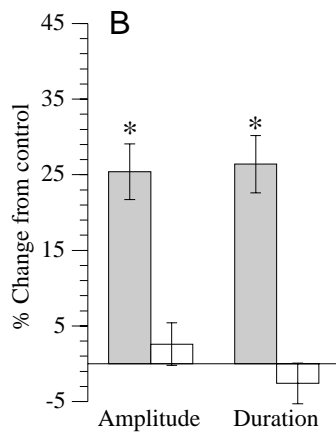
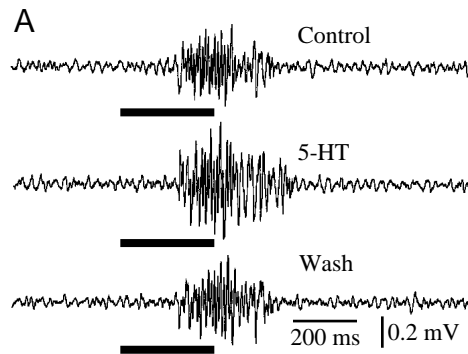


Fig. 6. (A) Extracellular recordings of pheromone-induced potential oscillations in the macroglomerular complex before, during and after application of 5-HT. The bar beneath the recordings marks the duration of the pheromone stimulation (10 ng of Bal; 10 ng of C15). The traces were obtained from recordings similar to those shown in Figs 4 and 5. The slow, sustained component of the signals was digitally subtracted (as described by Heinbockel et al., 1998) to improve visualization of the high-frequency oscillation. (B) Both the mean amplitude of the oscillations ($25 \pm 4\%$, $P < 0.01$; $N = 5$) and the length of the period during which stimulus-induced oscillations occurred ($26 \pm 4\%$; $P < 0.01$; $N = 5$) were increased significantly by 5-HT (shaded columns). These effects were reversed significantly after washing with saline (for all parameters $P < 0.025$; open columns). Values are means \pm S.E.M. An asterisk indicates a value significantly different from the control ($P \leq 0.025$).

stimuli than individual neurons since, in moth projection neurons, oscillations of the membrane potential emerge only as a consequence of prolonged odor stimulation with pulse durations above 0.5 s (Christensen et al., 1998).

Recently, a population of downstream neurons was identified that depends on input from synchronized neuronal assemblies for its odor selectivity, while synchronization was not important for odor selectivity of individual neurons in the antennal lobe of locusts (MacLeod and Laurent, 1996; MacLeod et al., 1998). The 5-HT-induced changes in odor-evoked potentials, especially the effect on the oscillatory component, might reflect changes in the extent of

synchronization among neurons or in the number of synchronized neurons. Modulatory compounds such as 5-HT might influence the extent of synchronization among neurons in the MGC and potentially affect oscillatory synchronized activity used for odor coding.

Considering the evidence for a role of neural assemblies in odor coding, modulation of these olfactory networks might be critical for adapting and optimizing the olfactory system during changes in environmental conditions or for the changing needs of the organism. Stopfer and Laurent (1999) recently reported that repeated odor stimulation changes the population dynamics of projection neurons in the antennal lobe of the locust and suggested that these changes led to a more precise and readily classifiable odor representation, using relational information contained across neuronal assemblies.

In this context, it is important to note that in *Aplysia californica*, 5-HT has been strongly implicated in associative learning and memory, both at the cellular level and at the behavioural level (e.g. Kandel and Schwartz, 1982; Carew and Sahley, 1986; Byrne et al., 1993; Hawkins et al., 1993; Byrne and Kandel, 1996; Carew, 1996). Examination of the associative learning capabilities of moths has revealed that, like honeybees (Menzel, 1990, 1993; Mauelshagen and Greggers, 1993; Hammer and Menzel, 1995; Menzel and Müller, 1996), moths exhibit strong learning of behaviorally relevant olfactory cues (Hartlieb, 1996; Fan et al., 1997; K. Daly and B. Smith, personal communication). An increasing body of evidence suggests that the antennal lobes play a role in the formation of such memories (Menzel and Müller, 1996; Sigg et al., 1997), so it is tempting to speculate that 5-HT modulation of antennal lobe neurons may be involved not only in the short-term facilitation of olfactory responses, but also in long-term changes that affect the functional and structural plasticity of the antennal lobes of the brain.

It has been shown previously (Linn and Roelofs, 1986) that, in the moth *Trichoplusia ni*, exogenously applied 5-HT can extend the time window during which sex-pheromone-induced behavior can be elicited and random motor activity can be expressed. Recent findings show that, over a 24 h period, 5-HT levels in the antennal lobe of *M. sexta* fluctuate significantly: they are low during the middle of the subjective day, when the moth is inactive, and higher at night, particularly around subjective dawn and dusk (Kloppenborg et al., 1999), when activity levels, including the levels of odor-driven behavioral activities, are at a peak (Lingren et al., 1977). At night, especially around dawn and dusk, *M. sexta* adults are involved in mating and searching for host plants (Gilmore, 1938; Lingren et al., 1977), both of which involve goal-directed flight guided by olfactory cues (Arbas et al., 1993). The effects of 5-HT on *M. sexta* antennal lobe neurons described here and in previous studies (Kloppenborg and Hildebrand, 1995; Mercer et al., 1996; Kloppenborg et al., 1999) suggest that 5-HT released in the antennal lobes at these times would increase the responsiveness of the moth to olfactory stimuli. In the male antennal lobe, one of the cellular targets of 5-HT is a population of MGC-PNs in which 5-HT increases the

excitability by reducing the activity of two voltage-activated K^+ channels.

Taken together, these results suggest that, in the olfactory system of *M. sexta*, 5-HT is involved in modulating the physiological properties of antennal lobe neurons and that the modulatory effects of 5-HT are likely to regulate the performance odor-dependent patterns of behavior.

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