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Visualization of modulatory effects of serotonin in the silkmoth antennal lobe

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Summary

A unique serotonin-immunoreactive neuron innervates every glomerulus of the contralateral antennal lobe (AL), the primary olfactory center, of the male silkmoth *Bombyx mori*. In order to examine the possible modulatory effects of serotonin in the AL, we utilized high-speed optical imaging with a voltage-sensitive dye combined with bath application of serotonin. We found that serotonin at 10^{-4} mol l^{-1} caused significant and reversible increases in the optical responses in both the macroglomerular complex (MGC) and the ordinary glomeruli (Gs) evoked by electrical stimulation of the antennal nerve. Optical responses in both the MGC and Gs were also significantly longer lasting following serotonin application. Serotonin exerted a significantly greater enhancing effect in the toroid glomerulus of the MGC than in the cumulus, and the effects of serotonin were also non-homogenously distributed in the Gs. Our results are evidence that serotonin acts in both the MGC and Gs to modulate the responses of neuronal populations.

Key words: insect, *Bombyx mori*, moth, olfaction, optical recording, voltage-sensitive dye, brain.

Introduction

Insect antennal lobes (ALs) have been popular models for the study of olfactory processing. The accessibility of individual neurons, the relative simplicity of the neural network and the striking similarities to the vertebrate homolog, the olfactory bulb (Hildebrand and Sheperd, 1997), have made investigation of the insect AL fruitful. In the male silkmoth *Bombyx mori*, olfactory receptor neurons in the antennae send axons into the AL where they make synapses with postsynaptic neurons in specialized compartments known as glomeruli. The male silkmoth AL comprises approximately 57 ordinary glomeruli (Gs) and three sexually dimorphic glomeruli called the macroglomerular complex (MGC; So and Kanzaki, 2000) (see Fig. 1B). The silkmoth MGC glomeruli have been termed cumulus, toroid and horseshoe, after their counterparts in the hawkmoth *Manduca sexta*. *B. mori* and *M. sexta* projection neurons (PNs: AL output neurons) that innervate the cumulus respond to the minor pheromone component, while those innervating the toroid respond to the major component (R. Kanzaki, unpublished observations; Hansson et al., 1991). Intracellular recording has given insight into the olfactory coding mechanisms used by AL local interneurons (LNs) and PNs (Kanzaki and Shibuya, 1986; Christensen and Hildebrand, 1988, 1997; Christensen et al., 1989, 1996; Kanzaki et al., 1989).

However, olfactory information is embedded in the responses of neuronal populations. Gaining an understanding of how populations of neurons respond to olfactory stimuli is clearly vital to deciphering mechanisms of olfactory processing. Novel techniques such as multi-unit extracellular recording arrays and optical imaging allow for the simultaneous monitoring of neuronal responses in spatially distinct regions of the AL. These techniques will lead to a greater understanding of olfactory coding mechanisms in the insect brain. For example, neural ensemble recordings in *M. sexta* revealed that there is strong heterogeneity among the response profiles of closely spaced individual neurons in the AL (Christensen et al., 2000). Furthermore, optical imaging with a voltage-sensitive dye in the bumblebee (*Bombus terrestris*) AL has shown that odor-induced oscillations are localized to distinct glomeruli (Okada and Kanzaki, 2001). Imaging of population responses in the AL will also be extremely useful in understanding how neuromodulators can affect the dynamics of olfactory processing.

Serotonin, a biogenic amine, may play a vital role in olfactory coding processes in the insect AL. A pair of unique serotonin-immunoreactive (SI) neurons that innervate both ALs has been identified in many insects (Schürmann and Klemm, 1984; Kent et al., 1987; Rehder et al., 1987; Homberg and Hildebrand, 1989; Breidbach, 1990; Salecker and Distler, 1990; Sun et al., 1993; Hill et al., 2002). The *B. mori* SI neuron innervates every glomerulus in the contralateral AL, fires long-duration spontaneous action potentials and responds to mechanosensory stimuli to the antennae (Hill et al., 2002). Electron microscopic examination of the processes of the *M. sexta* SI neuron has shown a predominance of output synapses in the contralateral AL (Sun et al., 1993), suggesting that the SI neuron may serve a centrifugal role. Numerous studies have revealed that serotonin modulates the responses of AL

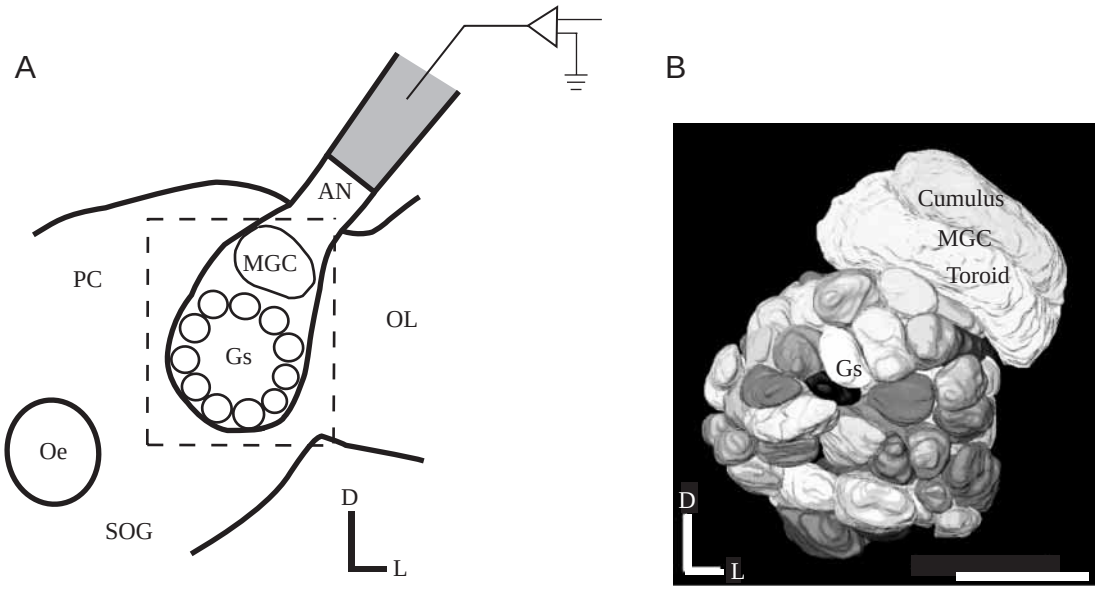

Fig. 1. (A) Schematic illustration of electrical stimulation of the antennal nerve (AN). (B) Three-dimensional reconstruction of the *Bombyx mori* antennal lobe (AL). The two main compartments of the macroglomerular complex (MGC) – the cumulus and toroid – can be seen, as well as many ordinary glomeruli (Gs). The reconstruction of the AL represents the region in (A) outlined by the broken lines. Scale bar, 100 μm. OL, optic lobe; Oe, oesophagus; PC, protocerebrum; SOG, suboesophageal ganglion; D, dorsal; L, lateral.

neurons. For example, in *M. sexta*, serotonin enhances the responses of individual AL neurons to both olfactory and electrical stimulation, (Kloppenburg and Hildebrand, 1995; Kloppenburg et al., 1999; Kloppenburg and Heinbockel, 2000), as well as enhancing the amplitude and duration of pheromone-evoked local field potentials in the MGC (Kloppenburg and Heinbockel, 2000). *In vitro*, serotonin inhibits two types of K^+ currents, as well as a voltage-activated Ca^{2+} current, in *M. sexta* AL neurons (Mercer et al., 1995). It has been proposed that the effects of serotonin on the K^+ currents may underlie the serotonin-induced increases of AL neuron excitability (Kloppenburg et al., 1999).

Are the modulatory effects of serotonin homogenously distributed throughout the AL or are there area-specific differences? Does serotonin affect neural processing in the Gs as well as in the MGC? In order to answer these questions we used a voltage-sensitive dye and a high-speed optical imaging system to visualize possible modulatory effects of serotonin in the silkmoth AL. Here, we report that optical responses in both the MGC and Gs were significantly greater, and significantly longer lasting, following serotonin application. Furthermore, we found that serotonin had a significantly greater effect in the toroid than in the cumulus, and that the enhancing effects of serotonin were also non-homogenously distributed in the Gs.

Materials and methods

Animals and dissection

Male silkmoths, *Bombyx mori* L., were used 3–6 days following eclosion. The legs were removed and the moths were placed in a dissection chamber. The head capsule was opened and muscles and tracheae were removed. The AL was carefully desheathed using finely sharpened forceps, and the brain was removed from the head and placed in a chamber for incubation with the voltage-sensitive dye (VSD) (RH414, Molecular Probes, Eugene, OR, USA; 1–3 mg ml^{-1}) for 5–10 min in the dark at room temperature (approx. 24°C). Following incubation with the VSD, the brain was rinsed with saline and placed in a separate recording chamber in which the brain was immersed in physiological saline solution. The composition of the physiological saline used was: 140 mmol l^{-1} NaCl, 5 mmol l^{-1} KCl, 7 mmol l^{-1} $CaCl_2$, 1 mmol l^{-1} $MgCl_2$, 5 mmol l^{-1} TES (*N*-tris(hydroxymethyl)-methyl-2-aminoethane sulfonic acid), 4 mmol l^{-1} $NaHCO_3$, 5 mmol l^{-1} trehalose and 100 mmol l^{-1} sucrose (pH 7.4). Serotonin (Sigma, St Louis, MA, USA) was dissolved in physiological saline.

Optical imaging

A suction electrode (Fig. 1A) was used to stimulate the antennal nerve (AN; 30–200 μA, 0.5 ms). The camera unit of the optical imaging system (Fuji HR Deltaron 1700, Fuji Film Microdevice, Tokyo, Japan) has a resolution of 128 photopixels×128 photopixels. Using the 10× objective, each pixel represents an area of 7 μm×7 μm. Excitation and emission filters used in this optical system passed light lengths of 535±25 nm and >615 nm, respectively. A dichroic mirror was placed in the light path of a metal halide lamp (KMH-250, BMH-250; Kiyohara Optical Laboratory, Tokyo, Japan). Eight responses to electrical stimulation of the AN were averaged in each recording session. Images were captured every 0.6 ms.

Serotonin application and wash

Following acquisition of the control optical responses, serotonin (10^{-4}–10^{-5} mol l^{-1}) was bath applied for 12 min.

During this 12-min incubation period, the saline solution containing serotonin was changed 3–4 times. Following bath application of serotonin, optical responses in the AL to electrical stimulation of the AN were acquired (the same amount of current was used as in the control). Serotonin was washed off with normal saline solution for 15 min, changing the solution in the recording chamber every 2 or 3 min. After washing, the optical response in the AL was acquired, again using the same amount of current as the control.

Data analysis

Following the collection of the raw optical data, we used programs to calculate the change in fluorescence divided by the background fluorescence level ($\Delta F/F$) and to compensate for photobleaching. We also processed the raw data with a finite impulse response (FIR) filter, which cut off signals above 90 Hz.

In order to directly visualize and to understand the time course of the effects of serotonin, we used programs to rotate and shift optical data files to match the control position (in most cases, the brain was in a slightly different position following serotonin application and wash). The rotation and shifting of the images resulted in a good correlation between the images (in all cases $r>0.9$). The average optical response in 100 pixels of the AN (70 μm×70 μm) was used to normalize the optical responses in the AL. The serotonin-effect optical files were calculated by subtracting the control responses from the serotonin responses and then dividing by the maximum control values for each pixel. This was necessary because the control optical responses were not of uniform strength throughout the AL, therefore the strength of the serotonin effect depended upon the strength of the control optical response in each pixel.

The duration of the optical response was calculated by measuring the time from the point where the rising phase of the control response reached 50% of its peak amplitude to the point where the falling phase fell to this value. As the rising phases were virtually identical in the control and serotonin optical responses, and as previous imaging studies have shown that the rising phase of the optical response is purely due to the activity of sensory axons (Ai et al., 1998), we measured the duration of the control and serotonin optical responses using 50% of the control peak amplitude as the reference points. This was applicable to the control and serotonin optical data but not to the wash response. The wash response consistently had a slightly different rising phase than either the control or serotonin responses; therefore, this method for measuring the duration of the response could not be applied to the wash responses.

To determine statistical significance we used a paired *t*-test. An asterisk indicates a significant difference ($P<0.05$), and two asterisks indicate a highly significant difference ($P<0.005$).

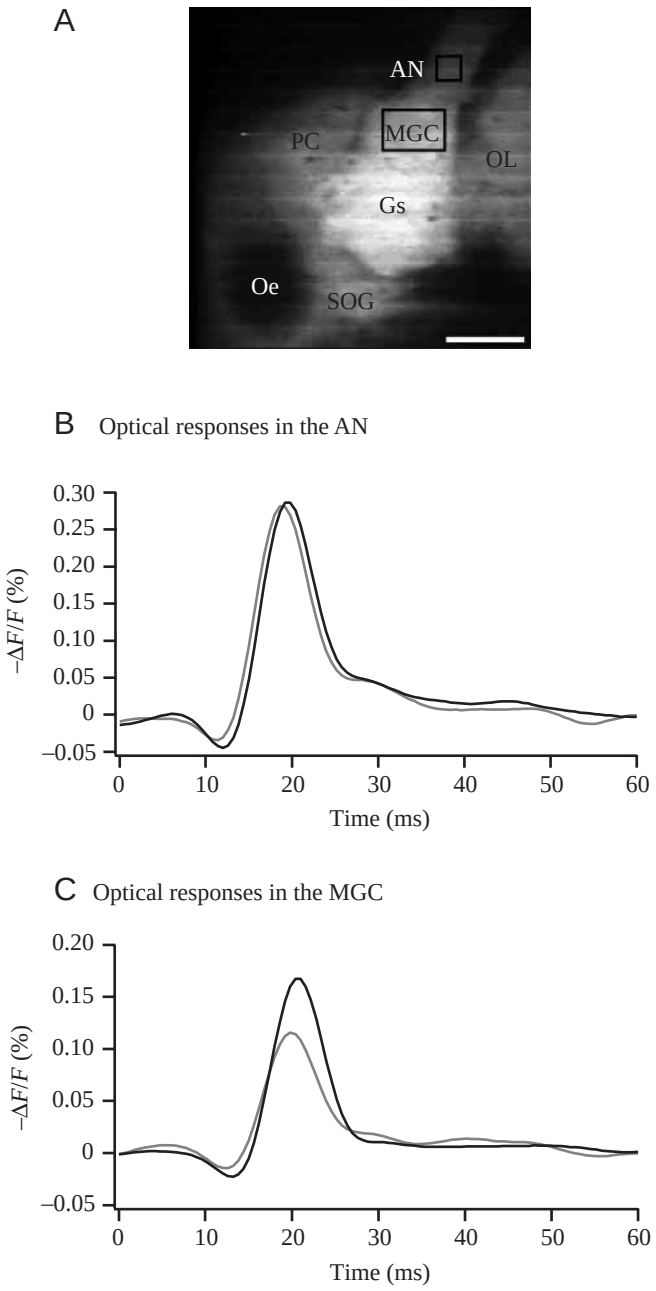

Fig. 2. Serotonin enhances the optical response in the antennal lobe (AL) but not the antennal nerve (AN). (A) Real image of the brain demonstrating the regions from which measurements of the optical responses were made in the AN (100 pixels, representing an area of 70 μm×70 μm) and macroglomerular complex (MGC; 384 pixels, representing an area of 168 μm×112 μm). Scale bar, 200 μm. OL, optic lobe; Oe, oesophagus; PC, protocerebrum; SOG, suboesophageal ganglion; Gs, ordinary glomeruli. (B) Serotonin had virtually no effect on the optical response in the AN. Gray line, control optical response; black line, optical response following serotonin application. (C) The optical response in the MGC was greatly enhanced by serotonin. ΔF, change in fluorescence; F, background fluorescence.

Results

Electrical stimulation of the AN evoked optical responses in both the MGC and the Gs of the AL (see Fig. 1A for experimental set-up). Enhancing effects of serotonin were observed in the AL but not in the AN. We measured the optical responses in the control and after serotonin application in both

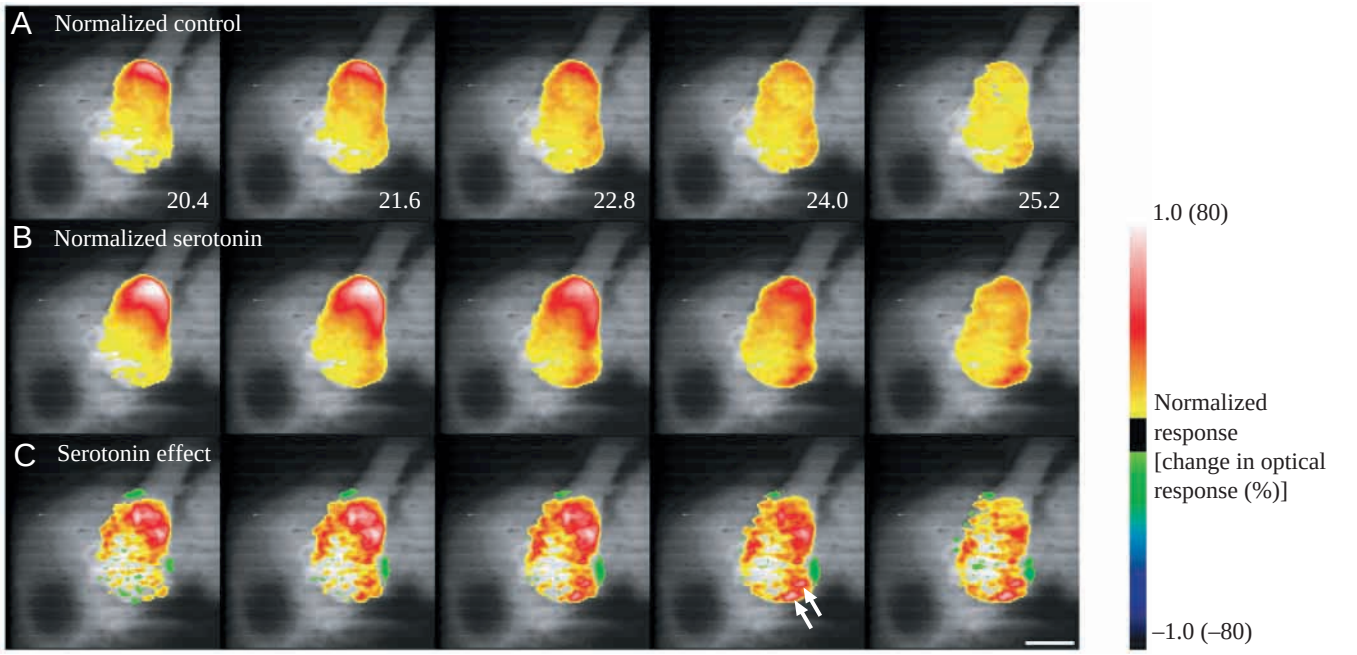

Fig. 3. The optical response in the antennal lobe (AL) in response to electrical stimulation of the antennal nerve (AN) was greater and longer lasting following serotonin application. (A) The normalized control optical response is first in the macroglomerular complex (MGC) and, in later frames, throughout the AL. The time given in each frame represents the time (ms) following the stimulus; the same values apply to the corresponding frames in (B) and (C). (B) After 12 min bath application with serotonin (10^{-4} mol l^{-1}), the normalized optical response is stronger throughout the AL and longer lasting. (C) The serotonin-effect images show that the enhancing effects are non-homogenously distributed throughout the AL. Arrows indicate two glomerulus-sized regions of the ordinary glomeruli (Gs) in which the enhancing effects of serotonin were particularly strong. Scale bar, 200 μm.

the AN (100 pixels, representing an area of 70 μm×70 μm) and in the MGC (384 pixels, representing an area of 168 μm×112 μm) (Fig. 2A). The optical response in the AN (Fig. 2B) was virtually unchanged by serotonin application, whereas the response in the MGC was greatly increased (Fig. 2C).

Following bath application of serotonin, in seven out of seven preparations, the normalized optical responses in the AL were greater, longer lasting and distributed in different areas of the AL compared with controls. The optical response following the wash with saline resembled that of the control (data not shown). Typical normalized control and serotonin optical responses are shown in Fig. 3 (images are 1.2 ms apart). In the control (Fig. 3A), electrical stimulation of the AN results in a wave of depolarization that traveled throughout the AL. Strong depolarizing responses were first observed in the MGC and, in later frames, throughout the entire AL. After bath application of serotonin for 12 min (Fig. 3B), the optical responses in the AL are stronger, localized to larger regions of the AL and the optical response is longer lasting. The serotonin effect (Fig. 3C) is localized mainly in the MGC and in various regions of the Gs. In the later frames, the serotonin effect can be observed to be strongest in regions of the Gs that may represent individual glomeruli (arrows in Fig. 3C).

Bath application of serotonin caused significant increases in the optical responses in both the MGC (48.7±8.7%; $P<0.001$, $N=7$) and in the Gs (40.0±8.0%; $P<0.005$, $N=5$) compared with the controls (Fig. 4). These effects reversed significantly with washing in both the MGC and Gs ($P<0.05$ in both cases).

In order to determine if serotonin significantly affected the duration of the optical responses in the MGC and Gs, we measured the time from the point where the optical response reached 50% of the peak value of the control response (see Materials and methods for details) to where it dropped to 50% of this value. Serotonin significantly enhanced the duration of the optical response in both the MGC, from 6.6±0.2 ms to 8.3±0.4 ms ($P<0.005$, $N=7$), and in the Gs, from 8.3±0.2 ms to 10.1±0.2 ms ($P<0.005$, $N=5$) (Fig. 5).

The effects of serotonin varied depending on the region of the AL (Fig. 6). Optical responses were measured in six compartments of the MGC (each compartment representing an area of 81 μm×81 μm) and in five compartments of the Gs (each compartment representing an area of 144 μm×144 μm) (see inset in Fig. 6). Among preparations, there was variation in the regions that had the strongest enhancement due to serotonin. In all preparations, the enhancing effects of serotonin were non-homogenously distributed throughout the AL. Fig. 6 shows the effects of serotonin in six regions of the MGC and five regions of the Gs in one preparation. In this preparation, the modulatory effects of serotonin were stronger in the toroid than in the cumulus compartments of the MGC, and the enhancing effects were greater in some Gs regions (i.e. Gs3) than in others (i.e. Gs2 and Gs4).

Serotonin had a stronger enhancing effect in the toroid than

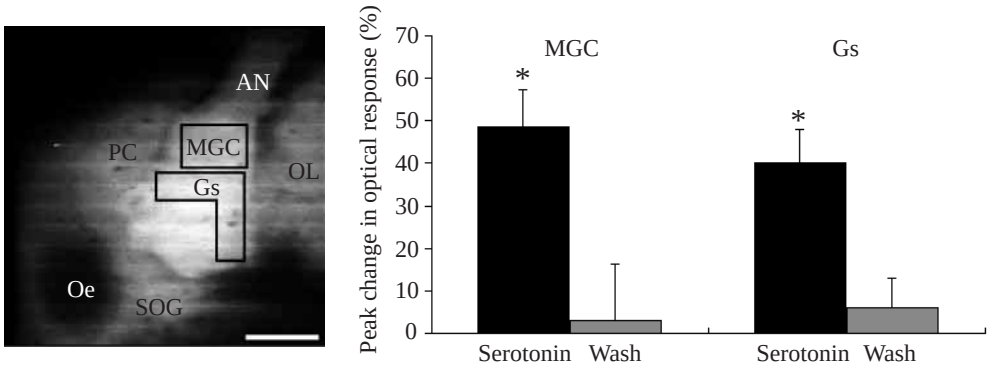

Fig. 4. Serotonin application resulted in reversible enhancement of the optical responses in both the macroglomerular complex (MGC) and the ordinary glomeruli (Gs). The average peak percentage increase in the optical response in the MGC was 48.7±8.7% (N=7) and in the Gs was 40.0±8.0% (N=5). These both represent significant increases from the control values (P<0.001 and P<0.005, respectively). The enhancing effects reversed significantly with washing in both the MGC and Gs (P<0.05). Scale bar, 200 μm. OL, optic lobe; Oe, oesophagus; PC, protocerebrum; SOG, suboesophageal ganglion. Error bars represent S.E.M.

in the cumulus (Fig. 7A). The average peak increase in the optical response in the toroid (54.9±8.0%; N=7) was significantly greater than in the cumulus (42.6±10.2%; P<0.05, N=7). Additionally, the effects of serotonin in the central cumulus were significantly greater than in the lateral cumulus (49.3±11.4% *versus* 39.0±11.4%; P<0.05, N=7; Fig. 7B). There were no significant differences among the enhancing effects of serotonin in the medial, central and lateral toroid (Fig. 7B).

In the Gs, the enhancing effects of serotonin were also non-homogenously distributed (Fig. 7C). The enhancement due to serotonin was significantly greater in Gs3 than in Gs1, Gs4 and Gs5 (60.8±8.8% *versus* 32.0±11.8%, 37.7±5.8% and 21.2±13.8%, respectively; P<0.05, N=5).

Application of a lower concentration of serotonin (10^{-5} mol l^{-1}) did not result in a significant enhancement of the optical responses in the AL. Following application of serotonin at 10^{-5} mol l^{-1}, the peak optical responses in the MGC and Gs were 3.5±14.4% weaker (N=3) and 9.2±10.9% stronger (N=3), respectively; these did not represent significant changes.

Discussion

Optical imaging with a voltage-sensitive dye revealed that serotonin enhanced the optical responses in both the MGC and the Gs, that the effects of serotonin were stronger in the toroid than in the cumulus, and that the enhancing effects were also non-homogenously distributed in the Gs. Additionally, the optical responses in both the MGC and Gs were longer lasting following serotonin application. The enhancing effects of serotonin could be due to effects of serotonin at several different loci. Serotonin could be acting at the sensory terminals to increase the release of neurotransmitter, it could be acting postsynaptically on LNs or PNs or it could be acting on the LN terminals. Our optical-imaging technique monitors changes in membrane potential in the AL caused by the activities of populations of sensory fibers, LNs and PNs. Although it is possible with this method to separate the activities of sensory fibers from postsynaptic (AL) neurons (Ai et al., 1998), it is not possible to pinpoint where serotonin is acting to cause the enhancement of the optical responses in the AL.

The concentrations of serotonin used in this study (10^{-4}–10^{-5} mol l^{-1}) are similar to the concentrations used by other studies examining the effects of serotonin on moth AL neurons (Kloppenburg and Hildebrand, 1995; Kloppenburg et al., 1999; Kloppenburg and Heinbockel, 2000). While it is impossible to determine the exact concentration of serotonin that reaches the synaptic regions, it is safe to assume that, due to the glial sheath encasing each glomerulus and the presence of serotonin uptake mechanisms, it is a few orders of magnitude lower than the bath applied concentration.

An ultrastructural study of the SI putative AL feedback neuron in *M. sexta* demonstrated the presence of dense-cored vesicles in the contralateral AL and also found a low number of synaptic sites (Sun et al., 1993). Taken together, these data suggest the possibility of non-synaptic release of serotonin. Serotonin could potentially diffuse throughout a glomerulus and bind to receptors in outer regions of the glomerulus where the SI neuron does not branch (Kent et al., 1987; Sun et al., 1993; Hill et al., 2002). Thus, serotonin released from the SI neuron could potentially bind to receptors on the sensory terminals (located in the outer regions of glomeruli). The enhancement of the optical responses in the MGC and the Gs reported here could be due, in part, to serotonin acting on the

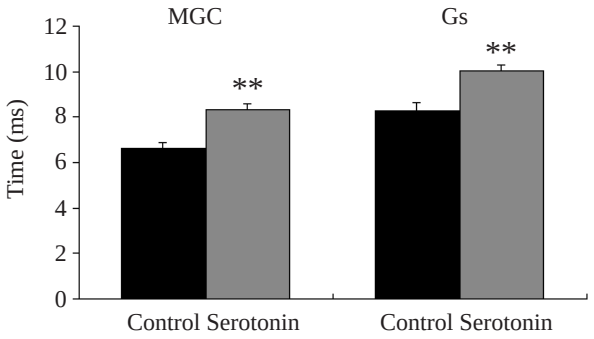

Fig. 5. Serotonin increased the duration of the optical response in both the macroglomerular complex (MGC) and the ordinary glomeruli (Gs). Serotonin application significantly increased the duration of the optical response in the MGC from 6.6±0.2 ms to 8.3±0.2 ms (P<0.005, N=7) and in the Gs from 8.3±0.2 ms to 10.1±0.2 ms (P<0.005, N=5). Error bars represent S.E.M.

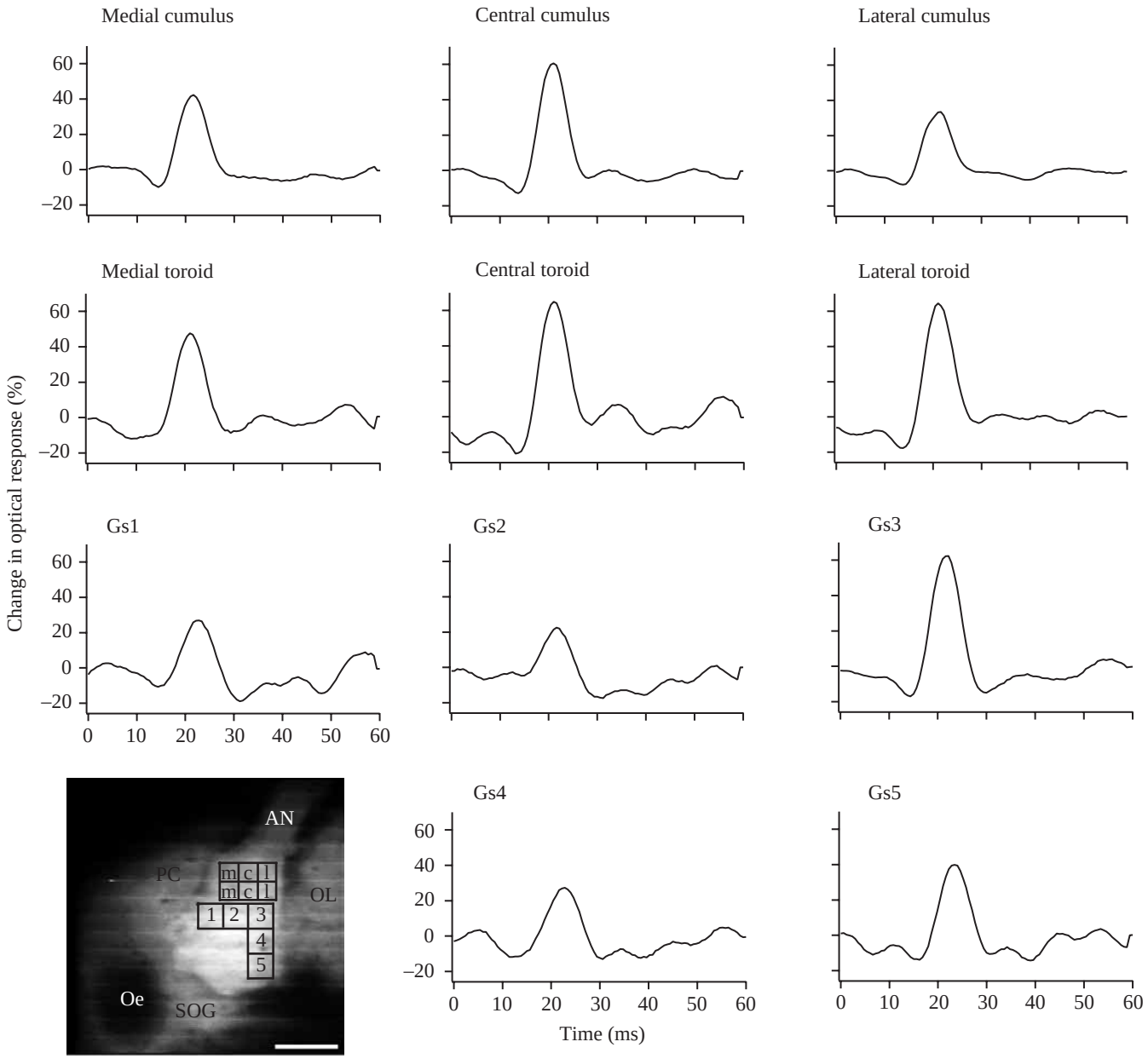

Fig. 6. The enhancing effects of serotonin were non-homogenously distributed throughout the antennal lobe (AL). The inset shows a real image of the brain, demonstrating the regions from which measurements of optical responses were made in individual compartments of the macroglomerular complex (MGC; each compartment represents an area of 81 μm×81 μm) and the ordinary glomeruli (Gs; each compartment represents an area of 144 μm×144 μm). In this preparation, greater enhancing effects were observed in the toroid regions than in the cumulus regions of the MGC. Additionally, in some areas of the Gs, the enhancing effects of serotonin were stronger (i.e. Gs3) than in others (i.e. Gs2 and Gs4). The area-specific differences in the enhancing effects of serotonin varied among preparations. Scale bar, 200 μm. OL, optic lobe; Oe, oesophagus; PC, protocerebrum; SOG, suboesophageal ganglion; c, central; l, lateral; m, medial.

sensory terminals. For example, by altering Ca^{2+} influx into the presynaptic terminal, serotonin could lead to an increase in neurotransmitter release. Such presynaptic effects of serotonin have been described in the crayfish (*Procambarus clarkii*) neuromuscular junction, where serotonin increases the number of vesicles released (Southard et al., 2000) and increases the amount of reliable vesicles (Wang and Zucker, 1998). A similar effect in *B. mori* could lead to an increased release of transmitter from sensory terminals, which could lead to a larger number of action potentials in postsynaptic neurons. Such increases in postsynaptic action potentials could underlie the increases in the amplitude and duration of the optical responses reported here.

Serotonin could also be acting on the postsynaptic neurons, the LNs and PNs. Studies of *M. sexta* AL neurons *in vitro* have demonstrated that serotonin leads to an increase in input resistance, spike broadening, a decreased response latency and an increase in the number of spikes elicited by electrical

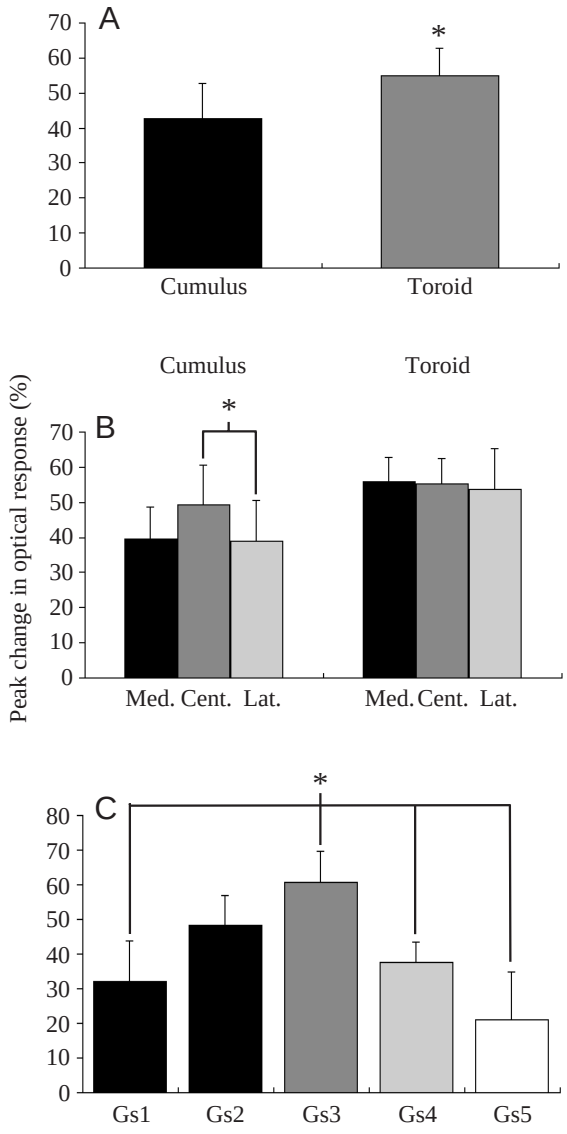

Fig. 7. The enhancing effects of serotonin were greater in some antennal lobe (AL) regions than in others. (A) The average peak increase in the optical response was significantly greater in the toroid (54.9±8.0%, N=7) than in the cumulus (42.6%±10.2, P<0.05, N=7). (B) The enhancing effects of serotonin were significantly greater in the central cumulus than in the lateral cumulus (49.3±11.4% *vs* 39.0±11.4%; P<0.05, N=7). There were no significant differences in the enhancing effects of serotonin in the medial, central and lateral toroid. (C) The enhancing effects of serotonin were non-homogenously distributed in the Gs. The enhancing effects of serotonin in Gs3 were significantly greater than in Gs1, Gs4 and Gs5 (60.8±8.8% *vs* 32.0±11.8%, 37.7±5.8% and 21.2±13.8%, respectively; P<0.05, N=5). Error bars represent S.E.M.

stimulation (Mercer et al., 1995). Mercer et al. proposed that these effects are due, at least in part, to effects on three types of K^+ channels. The observed enhancement of the optical responses in the MGC and Gs in the present study could result from these kinds of effects on populations of LNs and PNs. If a population of AL neurons had increased input resistances, increased excitability and fired broader spikes, one would expect that stronger and longer-lasting membrane potential depolarizations would be observed throughout the AL.

We observed that the enhancing effects of serotonin were non-homogenously distributed in the AL. This phenomenon could, in theory, be due simply to differences in the penetration of serotonin into different parts of the AL. However, we feel that this is unlikely due to the fact that the entire AL was desheathed in each preparation. Also, as serotonin was bath applied, it should have penetrated equally throughout the AL. It seems unlikely that the glial sheaths encasing some glomeruli pose more of a diffusional barrier than others. While it is feasible that the glomeruli situated in the interior of the AL may not have been exposed to as high a concentration of serotonin as more peripherally located glomeruli, our imaging technique mainly records membrane potential changes from the surface of the brain, so these considerations are essentially moot.

The non-homogeneity of the effects of serotonin in the AL could be due to differences in the distribution and density of serotonin receptors in different regions of the AL. The observed differences in the enhancing effects of serotonin in the MGC are particularly interesting because the SI neuron in several insect species has branches in both the cumulus and the toroid (Kent et al., 1987; Sun et al., 1993; Hill et al., 2002). In *B. mori* and *M. sexta*, the toroid is the region of the MGC in which PNs that respond to the major pheromone component branch, while the cumulus is the compartment in which PNs that respond to the minor component branch (R. Kanzaki, unpublished observations; Hansson et al., 1991). The behavioral implications of the present data are that serotonin release into both the cumulus and the toroid could lead to a greater increase in the moth's sensitivity to the major pheromone component compared with the minor component.

This is the first report of serotonin enhancing neural responses in the Gs. Intracellular recording from Gs PNs in *B. mori* and *M. sexta* have revealed that they respond to general odors (Kanzaki and Shibuya, 1987; King et al., 2000). Therefore, serotonin released into the AL could potentially enhance the responses of PNs to general odors as well as to pheromones. General odors play very important roles in the lives of insects, in behaviors such as locating food sources and host plants for oviposition (Willis and Arbas, 1991). The finding that serotonin enhances neuronal responses in the Gs as well as the MGC could have been predicted from the branching pattern of the *B. mori* SI neuron, which branches in every glomerulus of the contralateral AL (Hill et al., 2002). The non-homogenous distribution of the enhancing effects of serotonin in the Gs could also stem from differences in the distribution and density of serotonin receptors in the Gs. Alternatively, it is possible that serotonin enhances the activity of populations of both inhibitory and excitatory LNs with branches in many Gs. The net serotonin effect would therefore depend on the relative activation of inhibitory and excitatory LNs in each region of the AL. At any rate, due to the non-homogeneity of the serotonin effect in the Gs, release of serotonin throughout the Gs (i.e. by the SI neuron) could result in greater increases in sensitivity to

some general odors than to others. It would be worthwhile recording intracellularly from Gs PNs and/or LNs and examining whether or not serotonin differentially modulates the responses to different general odors.

The serotonin-induced enhancement of neural responses in the AL described here and previously in *M. sexta* (Kloppenburg and Hildebrand, 1995; Mercer et al., 1995; Kloppenburg et al., 1999; Kloppenburg and Heinbockel, 2000) may in part underlie behavioral modification in moths caused by exogenous application of serotonin. In the moth *Trichoplusia ni*, it has been reported that serotonin applied exogenously extends the time window during which pheromone-induced behavior can be elicited (Linn and Roelofs, 1986). In *B. mori*, exogenous application of serotonin increases the sensitivity of the moth to pheromone, while application of serotonin receptor antagonists decreases the sensitivity (L. Gatellier, unpublished observations). The serotonin-induced behavioral changes cited above could stem, in part, from the effects of serotonin in the AL. Enhanced neuronal responses in the AL would be relayed to higher olfactory centers in the protocerebrum that are involved in the generation of descending signals that produce pheromone-oriented behaviors (Kanzaki et al., 1991, 1994).

In the future, we plan to use optical-imaging techniques to examine the effects of serotonin application on pheromone and general odor-evoked optical responses in the AL. Optical imaging is a technique well-suited to the study of olfactory systems and will continue to increase our understanding of olfactory processing mechanisms and the role(s) that neuromodulation plays in olfaction.

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