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Serotonergic modulation of nonspiking local interneurones in the terminal abdominal ganglion of the crayfish

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Summary

The modulatory effect of serotonin on local circuit neurones forming the uropod motor control system of the crayfish *Procambarus clarkii* Girard was analysed electrophysiologically. Bath application of 10 μmol l^{-1} serotonin caused a decrease in the tonic spike activity of the exopodite reductor motor neurone. The inhibitory effect of serotonin on the motor neurone was dose-dependent and its spike discharge was completely suppressed for long periods by 1 mmol l^{-1} serotonin perfusion. Nonspiking local interneurones in the terminal abdominal ganglion showed either a membrane depolarization (*N*=6) or hyperpolarization (*N*=9) of 10–30 mV in amplitude when 100 μmol l^{-1} serotonin was perfused for 3–5 min. By contrast, spiking local interneurones and intersegmental ascending interneurones showed no observable excitatory responses to the perfusion of serotonin but instead some showed a small membrane hyperpolarization of 2–5 mV. These results indicate that the nonspiking interneurones could contribute substantially to the level of tonic excitation of the uropod motor neurones.

Sensory stimulation elicited depolarizing or hyperpolarizing potentials in the nonspiking interneurones and excitatory postsynaptic potentials (EPSPs) and spikes in the spiking interneurones. The sensory responses of spiking interneurones increased during bath application of serotonin and were reduced after 20–30 min of washing with normal saline. In the nonspiking interneurones, the amplitude of both depolarizing and hyperpolarizing potentials increased without any direct correlation with the serotonin-mediated potential change. This effect of serotonin was long-lasting and continued to enhance the responses of the nonspiking interneurones after washing. This post-serotonin enhancement persisted for over 1 h.

Key words: crayfish, *Procambarus clarkii*, serotonin, interneurone, nonspiking.

Introduction

Serotonin (5-hydroxytryptamine; 5-HT) is known to have a modulatory action on synaptic transmission in the central nervous system and to affect feeding, sexual and aggressive behaviours in both vertebrates and invertebrates (for reviews, see Weiger, 1997). Serotonin, for example, modulates the neural circuit for lateral giant (LG)-mediated escape in the crayfish (Glanzman and Krasne, 1983; Yeh et al., 1996, 1997; Teshiba et al., 2001). It enhances or depresses the synaptic responses of LG during sensory stimulation depending upon the social status of an animal. Serotonin also has modulatory effects on the more tonic motor responses of crayfish including the abdominal postural system (for a review, see Kravitz, 1988). Direct injection of serotonin into the systemic circulation of crayfish and lobster results in long-lasting tonic postural flexion, while the injection of another monoamine, octopamine, enhances the tonic postural extension (Livingstone et al., 1980). Bath application of serotonin causes an excitation of excitatory flexor motor neurones and inhibition of the antagonistic extensor motor neurones by acting on flexion evoking command fibres (Harris-Warrick and Kravitz, 1984; Harris-Warrick, 1985). Ma et al. (1992) have shown that serotonergic neurones in the lobster act as gain-setters by using direct stimulation of individual serotonin-containing neurones. Although direct activation of serotonin-containing neurones has little effect on the spike activity of the flexor motor neurones, the effect of the flexor command fibres in producing motor output is enhanced by the activation of serotonin-containing neurones. One of the most important modulatory roles of serotonin is, therefore, to enhance or reduce the responsiveness of neurones to normal ongoing physiological processes.

Nonspiking local interneurones are widely distributed in the central nervous system of the arthropods and are essential neural elements producing and modulating movements (e.g. Burrows, 1992; Nagayama et al., 1984, 1994). In the terminal abdominal ganglion of the crayfish, there are approximately 30 pairs of nonspiking interneurones that have unilateral and bilateral anatomy (Nagayama and Hisada, 1987, 1988; Nagayama et al., 1997). The majority of these nonspiking interneurones have a unilateral structure and are classified into

PL and AL groups by their gross morphology and somatic position. The PL interneurones are further classified into three identified sets of interneurones while the AL interneurones form three subgroups (Nagayama et al., 1997). The PL and AL interneurones play a major role in gaining control of the activity of motor neurones innervating the uropod muscles, by receiving both peripheral and central inputs and controlling the tonic background activity of the uropod motor neurones (Nagayama, 1997; Namba et al., 1994). To understand further the underlying organizational principles on which these circuits are based, it is important to understand how serotonin affects the activity of the nonspiking interneurones and modulates their synaptic responses. We have, however, little information about serotonergic modulation of nonspiking interneurones in arthropods. In this paper I show for the first time that nonspiking interneurones are depolarized or hyperpolarized by bath application of serotonin, although spiking interneurones of both intersegmental and local groups are not affected significantly. Furthermore, the synaptic interactions between sensory afferents and nonspiking interneurones are enhanced and prolonged by serotonin.

Materials and methods

Adult male and female crayfish, *Procambarus clarkii* (Girard) (7–10 cm body length from rostrum to telson) were used in all experiments. They were obtained commercially (Sankyo Labo Service, Japan) and kept in group in laboratory tanks supplied with flowing fresh water before use. There were no significant differences in results between the sexes. The abdomen was isolated and pinned ventral side up in a small chamber containing cooled physiological saline (van Harreveld, 1936). The swimmerets were removed and the terminal (sixth) abdominal ganglion exposed by removing the sixth sternite and peeling off the surrounding soft cuticle and the ventral aorta. The ganglion was stabilized on a silver platform and treated with protease (Sigma type XIV; Sigma, St Louis, MO, USA) for approximately 30 s without any change in firing rate of the motor neurones.

To monitor uropod motor activity and to stimulate sensory afferents innervating the exopodite, the soft cuticle overlying the uropod muscles was removed along the lateral edge of the protopodite and exopodite. The underlying hypodermis, ventral blood vessel, and connective tissue were removed to expose the muscles and nerves. Motor neurones innervating the uropod muscles all originate in the terminal abdominal ganglion. They were identified according to the criteria previously described (Nagayama et al., 1983; Nagayama, 1999). The exopodite reductor motor neurone exits from the second nerve root. The activity of this motor neurone was recorded at the bifurcation to the reductor and adductor exopodite muscles with the use of an extracellular suction electrode. To stimulate the sensory afferents innervating the exopodite electrically, another suction electrode was placed on the second root sensory bundle ipsilateral to the recording electrode for the motor neurones.

Intracellular recordings were made in the terminal ganglion neuropil with glass microelectrodes filled with either 2 mol l^{-1} potassium acetate (40–50 MΩ) or a 3% solution of Lucifer Yellow CH dissolved in 0.1 mol l^{-1} lithium chloride (100–200 MΩ). Penetrations of nonspiking local interneurones were confirmed by criteria previously described (Nagayama et al., 1997). Stable and long recordings (more than 1 h) are prerequisite for experiments of bath application, so the responses of nonspiking interneurones were mainly characterized by using microelectrodes filled with potassium acetate. Since the PL and AL nonspiking interneurones form opposing parallel connections in the local circuit (Nagayama and Hisada, 1987; Namba et al., 1994), they are physiologically identified by the combination of their response to sensory stimulation and output effect upon reductor motor neurone. Penetrations of intersegmental ascending interneurones and spiking local interneurones were confirmed by the intracellular injections of Lucifer Yellow. They were later identified by their gross morphology according to criteria based on Nagayama et al. (1993a,b).

For bath application of serotonin, the chamber (8 ml volume) was constantly perfused with fresh saline at a rate of 4 ml min^{-1} using a microtube pump (MP-3; Eyela, Tokyo, Japan). After physiological characterisation, interneurones were rested for more than 2 min with a continuous perfusion of normal saline. Serotonin of the required concentration was dissolved in normal saline and then perfused for 3–5 min. The preparations were then washed out with normal saline. In some preparations, small quantities of serotonin at concentrations of 0.1 μmol l^{-1} were applied *via* pressure microinjection from micropipettes into the lateral neuropil of the terminal ganglion near the intracellular recording site. The tips of micropipettes were broken manually under a microscope to be approximately 5 μm in outer diameter and serotonin was ejected from the penetrated micropipette by N_2 gas pressure controlled by pneumatic picopump (PV830, WPI) at 69–138 kPa for 100 ms.

All recordings were stored on a PCM data recorder and displayed on a Gould electrostatic chart recorder. Interneurones in which the response did not recover after washing were excluded from the results. The results are based on 15 stable recordings from nonspiking interneurones and 10 spiking interneurones of both ascending (8) and local (2) groups from 75 crayfish.

Results

Motor response to bath application of serotonin

The tonic spike activity of the exopodite reductor motor neurone (Nagayama et al., 1983) decreased in frequency after bath application of serotonin in a dose-dependent manner (Fig. 1). The reductor motor neurone spiked spontaneously at a rate of 5–25 impulses s^{-1} in different crayfish. The spike discharge decreased gradually after 2 min of bath application of serotonin (for 3 min) and was gradually inhibited after 5 or 6 min, remaining at that level for several minutes before recovering gradually. The spike discharge of the reductor

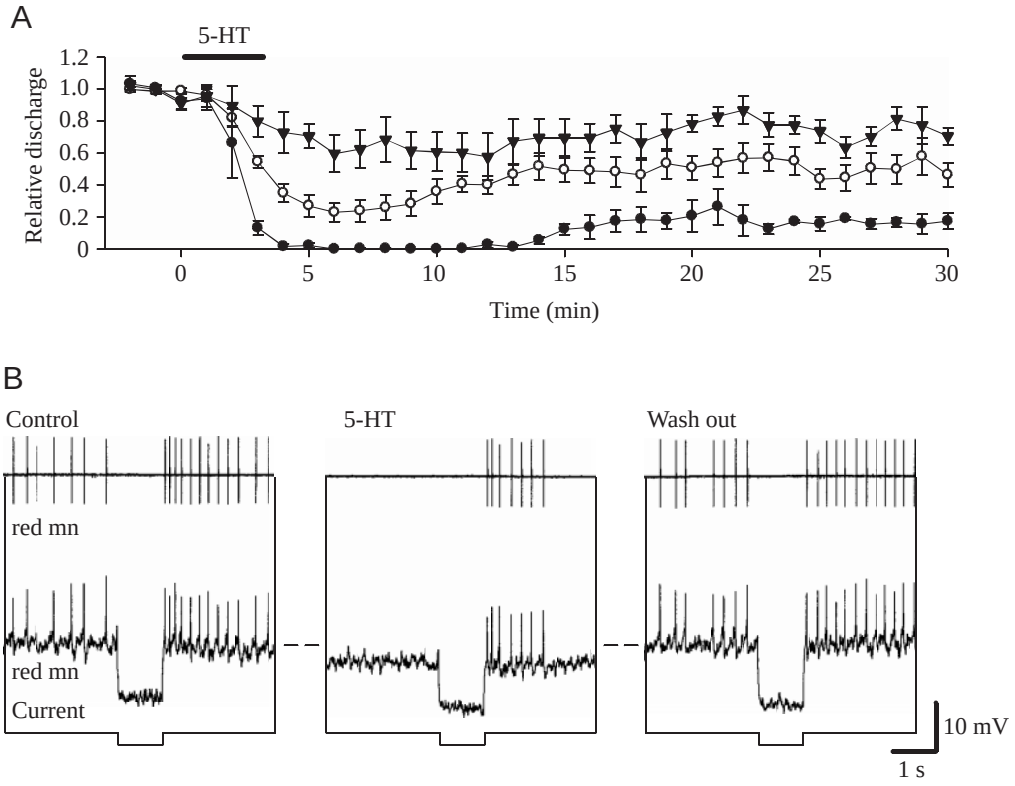

Fig. 1. Serotonin-mediated inhibition of the exopodite reductor motor neurone. (A) The relative change in spike frequency of the reductor motor neurone during bath application of serotonin plotted every 1 min (mean ± S.E.M.). The number of tonically occurring spikes of the motor neurone is expressed as an average rate relative to the initial 3 min period prior to serotonin (5-HT) perfusion. The thick bar indicates the period of serotonin application. ▼, 10 μmol l^{-1} (*N*=4); ○, 100 μmol l^{-1} (*N*=6); ●, 1 mmol l^{-1} (*N*=4) serotonin perfusion. (B) Input resistance of the reductor motor neurone (red mn) measured by a brief injection of 1 nA hyperpolarizing current was reduced during serotonin (5-HT; 1 mmol l^{-1})-mediated membrane hyperpolarization. The dashed line indicates the resting membrane potential level.

motor neurone was almost completely abolished after bath application of 1 mmol l^{-1} serotonin (*N*=4; Fig. 1A, filled circles), while the number of spikes in the motor neurone were reduced to approximately 60% and 20% of the initial level after bath application of 10 μmol l^{-1} (*N*=4; Fig. 1A, filled triangles) and 100 μmol l^{-1} (*N*=6; Fig. 1A, open circles) serotonin, respectively.

Fig. 1B shows the response of the reductor motor neurone during serotonin perfusion. The motor neurone showed a continuous hyperpolarization with no spikes after bath application of 1 mmol l^{-1} serotonin that recovered by washing with normal saline. The input resistance of the motor neurone, measured by brief pulses of 1 nA hyperpolarizing current, was reduced by 20–30% (23±5.8%, mean ± S.D., *N*=3) during membrane hyperpolarization mediated by serotonin.

Hyperpolarizing and depolarising response of nonspiking interneurones during bath application of serotonin

During bath application of serotonin at 100 μmol l^{-1} for 3–5 min, 9 out of 15 nonspiking local interneurones showed a membrane hyperpolarization while the remaining six interneurones showed a depolarization accompanied by a decrease in the spike frequency of the reductor motor neurone. Fig. 2A shows an example of serotonin-mediated hyperpolarization of a nonspiking interneurone. The membrane potential began to shift negatively during serotonin application and reached 30 mV in amplitude after 7 min from the beginning of serotonin perfusion. This sustained hyperpolarization of the interneurone was maintained for approximately 10 min, recovered gradually and returned to the initial level after approximately 60 min of washing. The effective period of serotonin-mediated depolarization of the nonspiking interneurones was rather shorter than that of serotonin-mediated hyperpolarization, and the membrane potential of the interneurones frequently recovered to initial levels within 20 min of washing (Fig. 2B). The peak amplitude of hyperpolarization of the nonspiking interneurones was 18.6±5.7 mV (*N*=9) and the time course of recovery was 47±19 min, which were statistically different ($P<0.05$, student *t*-test) from those of serotonin-mediated depolarization of the nonspiking interneurones. The peak amplitude of depolarization was 12.8±3.3 mV (*N*=6) and the time couse of recovery was 26±11 min. To compare the effect of serotonin more quantitatively, small quantities of serotonin of 0.1 μmol l^{-1} in concentration were ejected directly into the neuropil near the recording site of the nonspiking interneurones (Fig. 2C,D). Serotonin-mediated hyperpolarization of the nonspiking interenurones (Fig. 2C) ranged between 20 and 94 ms (46±29.7 s, mean ± S.E.M., *N*=5), which was significantly longer ($P<0.05$, student *t*-test) than the serotonin-mediated depolarization of the nonspiking interenurones (Fig. 2D) that ranged between 5 and 23 s (13.8±7.0 s, *N*=5).

Five out of six nonspiking interneurones that showed serotonin-mediated depolarization made inverting connections with the reductor motor neurone. Thus, they could decrease tonically occurring spikes of the reductor motor neurone by their depolarization of the membrane potential. For example, depolarizing current (2nA) injected into one of these interneurones reduced the number of spikes of the reductor motor neurone (Fig. 3A). After bath application of

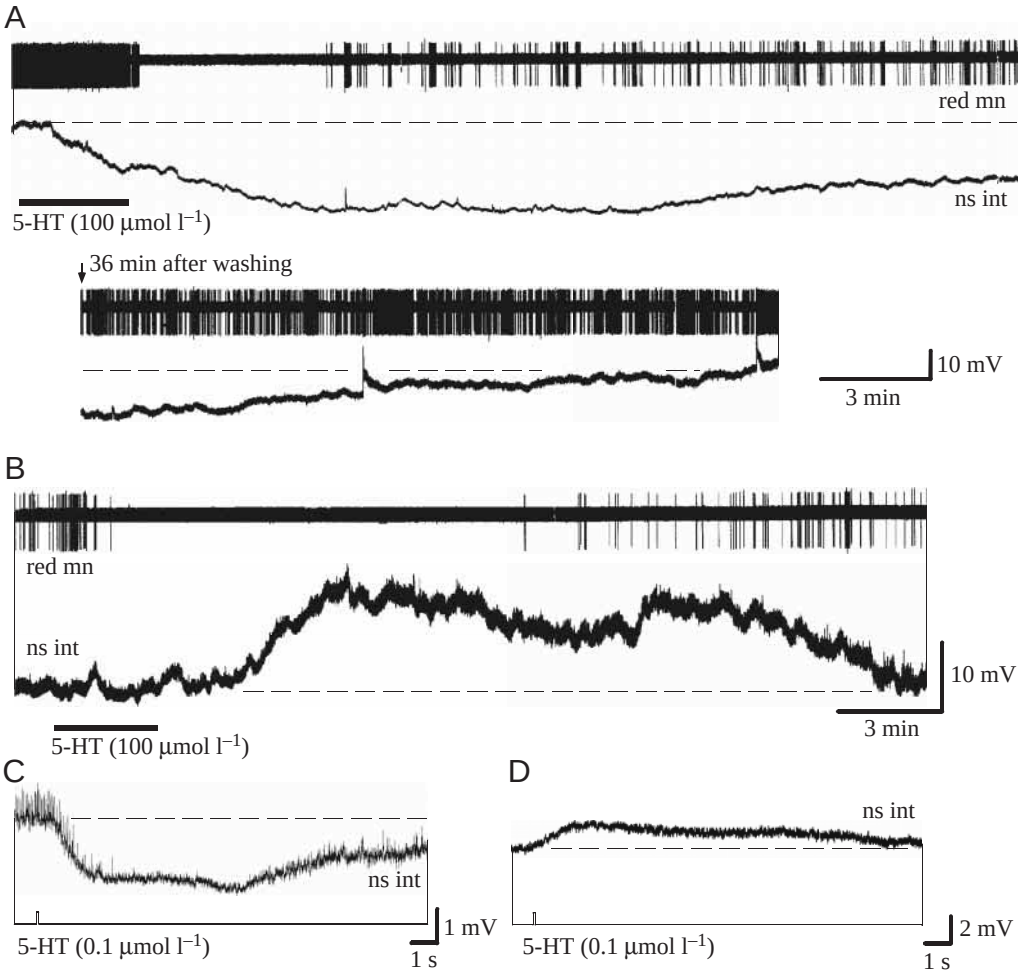

Fig. 2. Serotonin-mediated membrane potential changes of nonspiking interneurones (ns int). (A) Serotonin-mediated hyperpolarization of a nonspiking interneurone. Bath application of serotonin (5-HT; 100 μmol l^{-1}) for 3 min (indicated by thick bar) inhibited the motor neurone and elicited a hyperpolarization of the nonspiking interneurone. (B) Serotonin-mediated depolarization of a nonspiking interneurone. Bath application of 100 μmol l^{-1} serotonin for 3 min (indicated by thick bar) mediated a membrane depolarization of the nonspiking interneurone and suppressed the tonic discharge of the reductor motor neurone (red mn). (C) Brief application (100 ms) of 0.1 μmol l^{-1} serotonin caused a long-lasting membrane hyperpolarization of the nonspiking interneurone. (D) Brief application (100 ms) of 0.1 μmol l^{-1} serotonin caused a membrane depolarization. The dashed line indicates the resting membrane potential level.

100 μmol l^{-1} serotonin (for 5 min), the membrane potential of this interneurone was depolarized by approximately 12 mV, staying at that depolarized level for several minutes, then gradually declining to the initial level within 15 min after washing (Fig. 3C, filled circles). When the interneurone was depolarized by serotonin, the spike frequency of the reductor motor neurone decreased simultaneously (Fig. 3C, open circles). The spike activity of the motor neurone decreased during sustained membrane depolarization of the interneurone and gradually increased following the recovery of the membrane potential of the interneurone. Before serotonin perfusion, the passage of a 1 nA hyperpolarizing current into this interneurone had no effect upon the activity of the reductor motor neurone (Fig. 3B). At the peak of serotonin-mediated depolarization (3 min after washing, 8 min total), the same current injected into the interneurone caused an increase in the spike frequency of the motor neurone (from 7.25 impulses s^{-1} to 10 impulses s^{-1}; Fig. 3Di). An increase in the spike frequency of the motor neurone continued to be observed during the falling phase of depolarization of the interneurone (Fig. 3Dii). After the resting membrane potential recovered to the initial level after 10 min of washing, the same hyperpolarizing current injected into the interneurone had no significant effect upon the motor neurone (Fig. 3Diii). Thus, the membrane depolarization of the nonspiking interneurones mediated by serotonin contributed to reduce the spike discharge of the reductor motor neurone during bath application of serotonin.

Five out of nine interneurones that showed serotonin-mediated hyperpolarization made noninverting connections with the reductor motor neurone. Their excitatory effect upon the motor neurone was cancelled by the serotonin-mediated hyperpolarization. The remaining four nonspiking interneurones that showed a serotonin-mediated hyperpolarization made inverting connections with the reductor motor neurone. Two of them had bidirectional effects upon the motor neurones, which suggested that they released inhibitory transmitter continuously at resting potential. The serotonin-mediated hyperpolarization of these interneurones thus increased the spike activity of the reductor neurone in part by decreasing the amount of inhibitory transmitter.

Modulation of sensory responses of the nonspiking interneurones during bath application of serotonin

The interneurone shown in Fig. 2A received depolarizing postsynaptic potentials of approximately 6 mV in amplitude in response to the electrical stimulation of the second nerve root sensory bundle, which contains mechanosensory afferents that

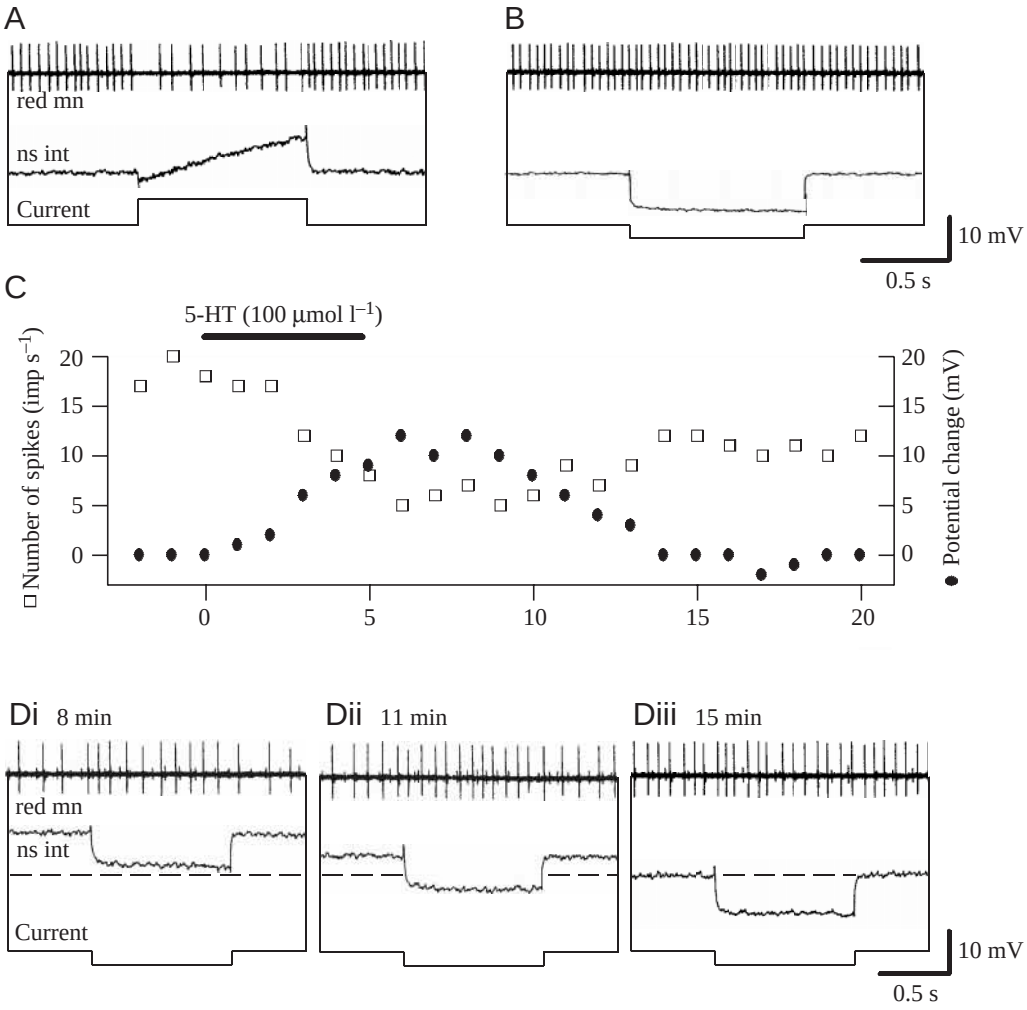

Fig. 3. Serotonin-mediated depolarization of a nonspiking interneurone (ns int). (A,B) Effect of current injected into a nonspiking interneurone upon the reductor motor neurone (red mn). The passage of a 2 nA depolarizing current into the interneurone decreased the tonic spike frequency of the motor neurone (A) while the injection of a 1 nA hyperpolarizing current had little effect upon the motor neurone (B). (C) Bath application of serotonin (5-HT; 100 μmol l^{-1}) for 5 min (indicated by thick bar) decreased the number of tonically occurring spikes of the motor neurone (impulses s^{-1}; □) and elicited a depolarization of the nonspiking interneurone (●). (D) Temporal relationship between the serotonin-mediated depolarization of the interneurone and the effect of hyperpolarizing current injected into the interneurone upon the motor neurone. The passage of a 1 nA hyperpolarizing current into the interneurone increased the tonic spike level of the motor neurones only during serotonin-mediated depolarization. The time indicated on each trace (Di–iii) shows the elapsed time after serotonin application. The dashed line indicates the resting membrane potential level.

innervate the exopodite (Fig. 4A). The amplitude of the depolarizing postsynaptic potentials increased to approximately 10 mV after 8 min of serotonin perfusion (5 min after washing) superimposed on a membrane hyperpolarization of approximately 30 mV in amplitude (Fig. 4B). This change in the size of the postsynaptic potentials is characteristic of typical chemical synaptic transmission. After 20 min of washing, the serotonin-mediated hyperpolarization of the interneurone began to recover gradually (Fig. 2A), but the amplitude of the depolarizing postsynaptic potentials in the interneurone during sensory stimulation increased further. The amplitude of depolarizing postsynaptic potentials was approximately 20 mV, with a membrane hyperpolarization of 15 mV after approximately 45 min of washing (Fig. 4C). Despite the membrane potential being restored to its initial level after approximately 60 min of washing, the depolarizing postsynaptic potentials during sensory stimulation remained large (Fig. 4D). At the same time, the spike frequency of the reductor motor neurone also increased during sensory stimulation in comparison with the response of the motor neurone before serotonin perfusion (cf. top traces in Fig 4A and D). This enhancement in amplitude of the depolarizing postsynaptic potentials was observed in all nonspiking interneurones (N=4) that showed serotonin-mediated hyperpolarization. Only one out of six nonspiking interneurones that showed serotonin-mediated depolarization received depolarizing postsynaptic potentials from the sensory afferents (Fig. 5). Before bath application of 100 μmol l^{-1} serotonin (3 min), the depolarizing postsynaptic potentials were approximately 7 mV in amplitude (Fig. 5A). After serotonin perfusion, the postsynaptic potentials firstly slightly decreased in amplitude superimposed on a serotonin-mediated depolarization (Fig. 5B). The interneurone was depolarized by 10 mV in amplitude after 6 min of washing (9 min in total) and the depolarizing postsynaptic potentials of the interneurone began to increase in amplitude (Fig. 5C). After approximately 20 min washing, the interneurone was still depolarized but sensory stimulation evoked depolarizing postsynaptic potentials of over 10 mV in amplitude (Fig. 5D).

The remaining 10 nonspiking interneurones received hyperpolarizing postsynaptic potentials during sensory stimulation (Fig. 6). The amplitude of the hyperpolarizing

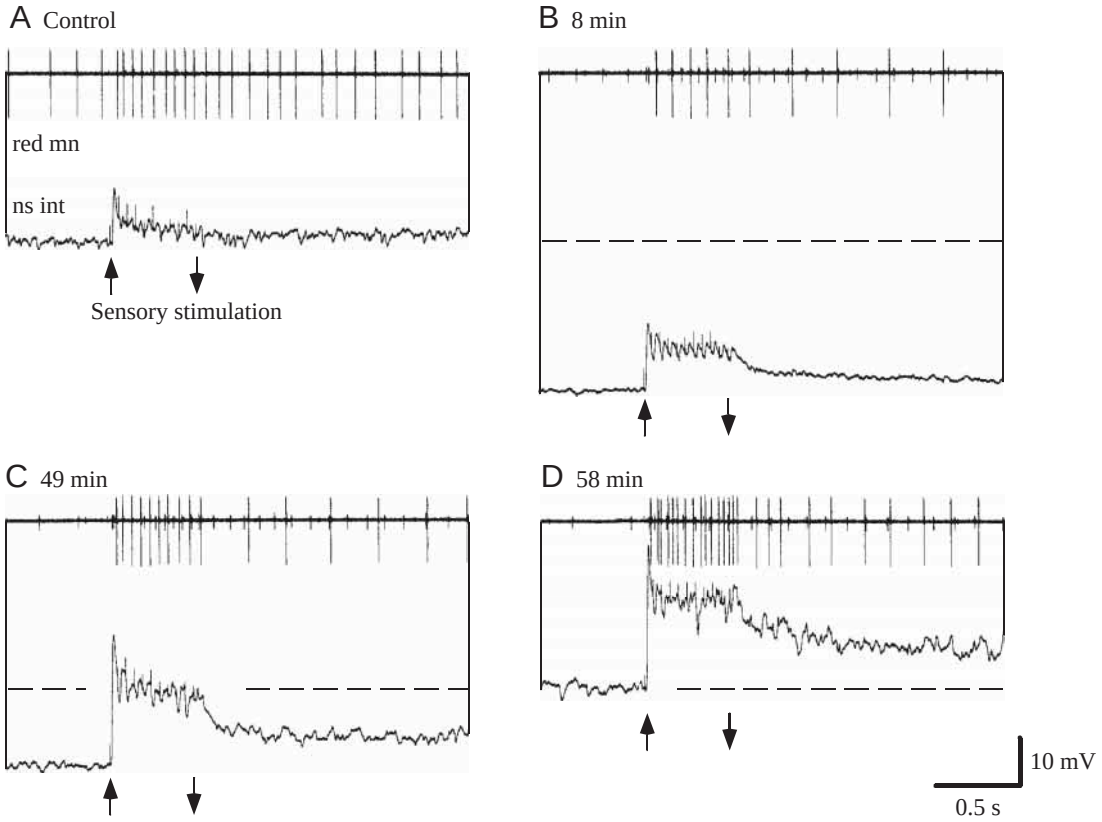

Fig. 4. Post-serotonin enhancement of the depolarizing response of a nonspiking interneurone (ns int) that showed a serotonin-mediated hyperpolarization during sensory stimulation. Bath application of 100 μmol l^{-1} serotonin for 3 min mediated a hyperpolarization of the membrane potential of the interneurone shown in Fig. 2A. The amplitude of the depolarizing postsynaptic potentials in the interneurone elicited by sensory stimulation at 20 Hz of 11 stimuli (indicated by arrows) increased after serotonin perfusion. The time indicated on each trace (A–D) shows the elapsed time after serotonin application. The dashed line indicates the resting membrane potential level; red mn, reductor motor neurone.

postsynaptic potentials in the nonspiking interneurones that showed either serotonin-mediated hyperpolarization (N=5) or depolarization (N=5) was enhanced and prolonged after serotonin perfusion. Before serotonin application, sensory stimulation elicited hyperpolarizing postsynaptic potentials of approximately 6 mV in amplitude in one of these interneurones (Fig. 6Ai). After serotonin application (3 min), the membrane potential of the interneurone began to shift negatively (Fig. 6Aii). After 3 min of washing (in total, 6 min after serotonin application), the interneurone was hyperpolarized by 15 mV and the hyperpolarizing postsynaptic potentials of the nonspiking interneurone mediated by sensory stimulation were reduced in size to approximately 50% of their initial amplitude (Fig. 6Aiii). This change in amplitude of the hyperpolarizing postsynaptic potentials is also characteristic of typical chemical synaptic transmission. The hyperpolarizing response of the interneurone during sensory stimulation was, however, gradually enhanced after approximately 10 min of serotonin perfusion. The amplitude of the hyperpolarizing postsynaptic potentials in the interneurone increased to approximately 75% of the initial postsynaptic potentials, although the level of hyperpolarization of the interneurone was similar to that at 6 min (cf. Fig. 6Aiii and iv). The membrane potential of the interneurone was still hyperpolarized after approximately 30 min of serotonin perfusion while the amplitude of the hyperpolarizing postsynaptic potentials of the interneurone during sensory stimulation was considerably larger than that of the initial postsynaptic potentials (Fig. 6Av). The hyperpolarizing postsynaptic potentials of another interneurone that showed serotonin-mediated depolarization were also enhanced by bath application of serotonin. Before serotonin application, hyperpolarizing postsynaptic potentials of approximately 5 mV in amplitude were observed in the interneurone during electrical stimulation of sensory afferents (Fig. 6Bi). Bath application of 100 μmol l^{-1} serotonin for 3 min caused a membrane depolarization of the interneurone that was restored to the initial level after approximately 20 min of washing. Subsequent sensory stimulation evoked hyperpolarizing postsynaptic potentials of approximately 10 mV in amplitude (Fig. 6Bii).

Effect of serotonin on spiking interneurones

The responses of eight ascending interneurones, including three VE-1, two NE-1, RO-1, RO-4 and RO-5, as well as two spiking local interneurones of a medial group were examined during bath application of serotonin. Most showed no significant change in membrane potential after bath application

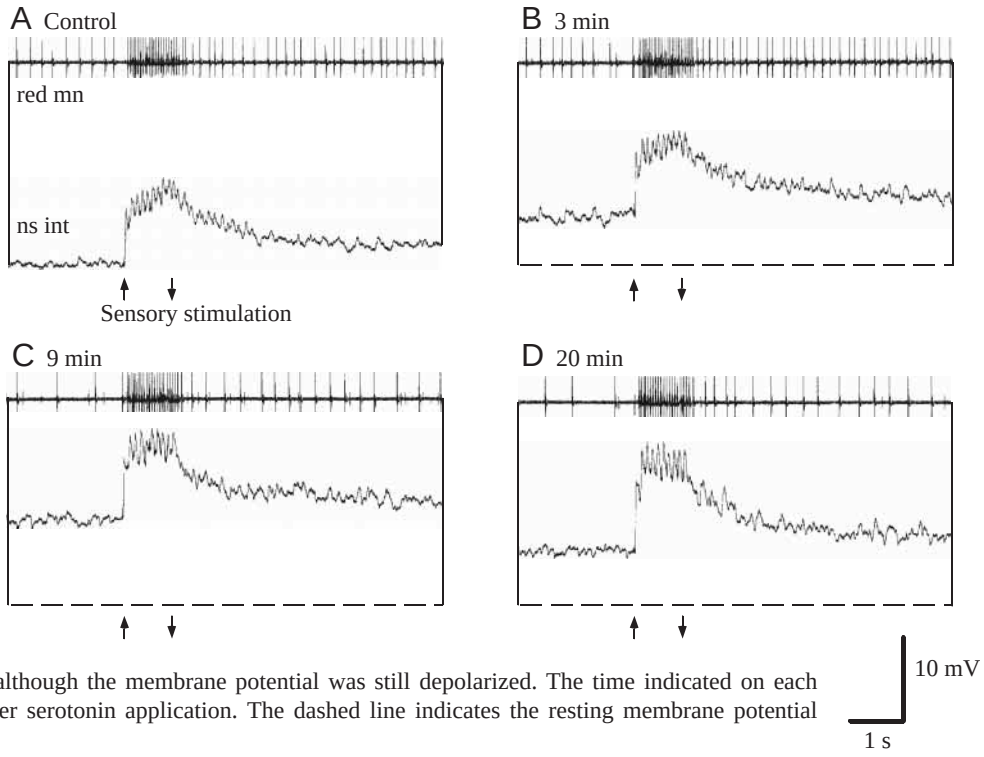

Fig. 5. Post-serotonin enhancement of the depolarizing postsynaptic potentials of a nonspiking interneurone (ns int) that showed a serotonin-mediated depolarization during sensory stimulation. Bath application of 100 μmol l^{-1} serotonin for 3 min mediated a depolarization of the membrane potential of the nonspiking interneurone and decreased the tonic spike discharge of the reductor motor neurone (red mn). After serotonin perfusion, the amplitude of depolarizing postsynaptic potentials of the interneurone in response to the sensory stimulation at 20 Hz of 11 stimuli (indicated by arrows) initially decreased slightly in association with serotonin-mediated depolarization. The depolarizing postsynaptic potentials then increased in amplitude, although the membrane potential was still depolarized. The time indicated on each trace (A–D) shows the elapsed time after serotonin application. The dashed line indicates the resting membrane potential level.

of either 100 μmol l^{-1} or 1 mmol l^{-1} serotonin but some interneurones showed a small membrane hyperpolarization of 2–5 mV in amplitude. For example, identified ascending interneurone, VE-1 (Nagayama et al., 1993a) was only slightly hyperpolarized by bath application of 1 mmol l^{-1} serotonin, although the tonically occurring spikes of the reductor motor neurone were completely suppressed for more than 10 min (Fig. 7A). No spiking interneurones were depolarized or produced spikes following bath application of serotonin.

Electrical stimulation of the second nerve root sensory bundle at 20 Hz elicited excitatory responses in the ascending interneurone VE-1 (Fig. 7B). The stimulus intensity was set so that about half of the electric pulses elicited spikes in the interneurone (Fig. 7Bi). When 1 mmol l^{-1} serotonin was applied for 3 min, the excitability of VE-1 to sensory stimulation gradually increased (Fig. 7Bii). With the same intensity of stimulation, VE-1 responded with spikes to every electrical pulse with no significant depolarization of the resting potential (Fig. 7Biii). The excitability of the interneurone then gradually decreased and returned to the initial level after approximately 20 min of washing with normal saline (Fig. 7Biv–vi).

Discussion

The effect of nonspiking local interneurones during bath application of serotonin

The tonic spike activity of the exopodite reductor motor neurone was reduced during bath application of serotonin. This study strongly suggests that the decrease in the activity of the reductor motor neurone was caused by an activity change of the nonspiking local interneurones mediated by serotonin. Firstly, spiking neurones of both ascending and local groups (Nagayama et al., 1993a,b) showed no excitatory response during bath application of serotonin (e.g. Fig. 7A), which indicates no active control from spiking interneurones during serotonin-mediated inhibition of the motor activity. Secondly, some nonspiking interneurones showed a depolarization of 10–30 mV in amplitude during bath application of serotonin (e.g. Fig. 2). Since small changes in the membrane potential of the nonspiking interneurones are sufficient for generating large changes in the activity of the motor neurones (Nagayama et al., 1994), a serotonin-mediated depolarization of the nonspiking interneurones could affect the tonic spike activity of the reductor motor neurone. The observations that five out of six nonspiking interneurones showing a depolarizing response to serotonin made inverting connections with the reductor motor neurone suggested that the spike activity of the reductor motor neurone could be reduced by these nonspiking interneurones. Four out of nine nonspiking interneurones that showed a serotonin-mediated hyperpolarization made inverting connections with the reductor motor neurone. These results suggest that some of the interneurones could partially increase the activity of the reductor motor neurone if they released inhibitory transmitter continuously at their resting potential. In fact, many nonspiking interneurones show GABA-like immunoreactivity (Nagayama et al., 1997). However, the remaining five nonspiking interneurones that showed a serotonin-mediated hyperpolarization made noninverting connections with the motor neurone. Thus, the membrane responses of the majority of nonspiking interneurones caused a depression of the tonic activity of the motor neurone.

Changes in the activity of the reductor motor neurone and the shift in membrane potential of some nonspiking interneurones were temporally correlated when serotonin was applied but, in other cases, the shift in membrane potential of the interneurones was faster or later than the activity change of the motor neurone. Approximately 30 nonspiking local interneurones are estimated to be in the terminal ganglion (Nagayama and Hisada, 1987), and all nonspiking interneurones found in this study showed substantial changes in their membrane potential caused by serotonin application that generally inhibited the motor neurone. The degree and course of inhibition of the motor neurone during serotonin application is therefore reflected in the net activity of the nonspiking interneurones.

More than 30 neurones in the crayfish and approximately 100 neurones in the lobster ventral nerve cord display serotonin-like immunoreactivity (Beltz and Kravitz, 1983; Real and Czternasty, 1990). In the terminal ganglion of the crayfish, at least one neurone with a cell body in a central medial region shows serotonin-like immunoreactivity (Real and Czternasty, 1990). Furthermore, several serotonin-like immunoreactive neurones in more anterior ganglia send descending axons into the terminal ganglion that give rise to extensive branching. Since nonspiking interneurones have numerous fine branches extending within both the ventral and dorsal neuropil (Nagayama et al., 1994), it is possible that serotonin-like immunoreactive neurones make synapses directly with the nonspiking interneurones. The identification of the serotonin-containing neurones and simultaneous intracellular recordings between them and the nonspiking interneurones are needed to further clarify this point.

Modulatory effects of serotonin on the sensory responses of nonspiking interneurones

The excitability of ascending interneurones during sensory stimulation was reversibly increased after bath application of serotonin without any significant change in membrane potential. A similar serotonergic modulation has been described in both invertebrates and vertebrates (e.g. Peck et al., 2001). The number of spikes in afferents of leech mechanoreceptors (Gascoigne and McVean, 1991) and a crayfish leg chordotonal organ (El Manira et al., 1991) are increased by serotonin, while the sensory responses of a lobster oval organ proprioceptor are

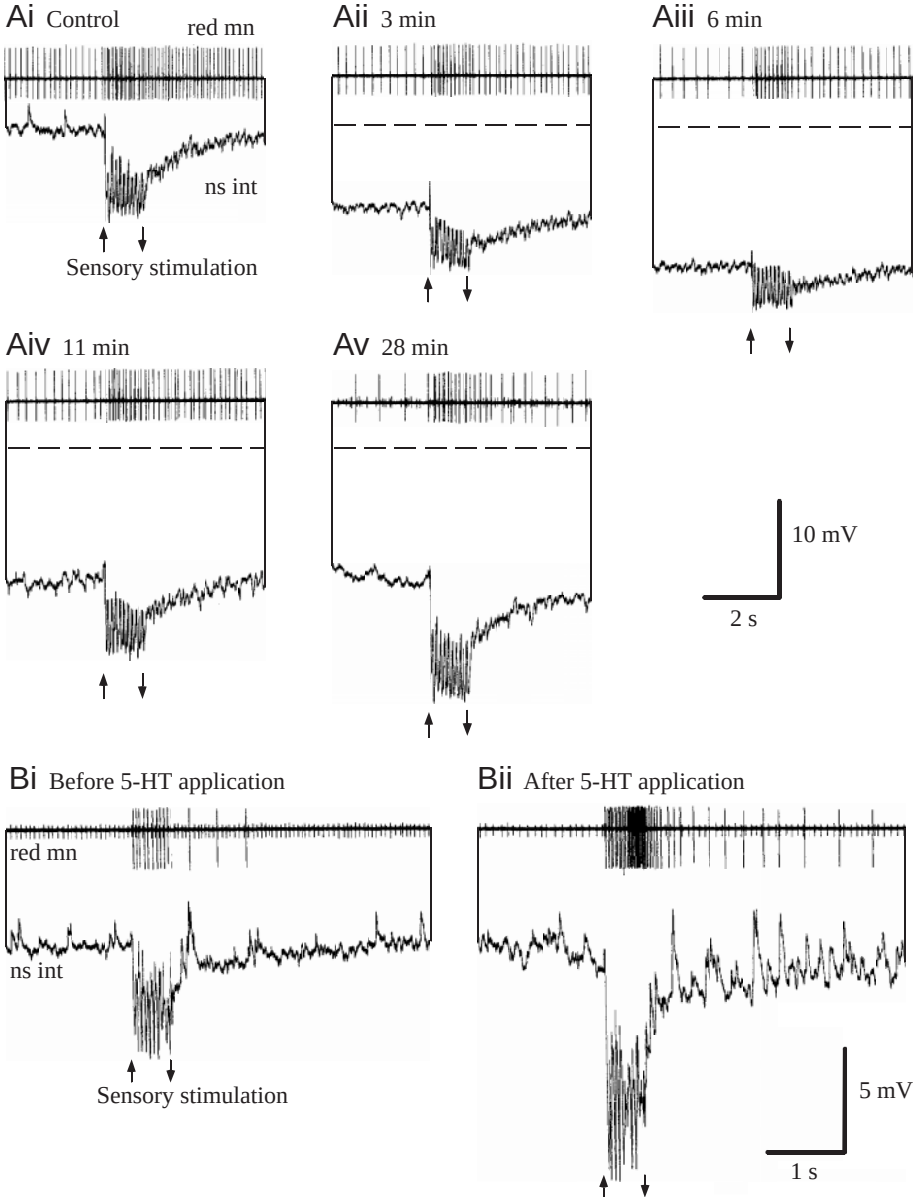

Fig. 6. Post-serotonin enhancement of the hyperpolarizing postsynaptic potentials of a nonspiking interneurone (ns int) during sensory stimulation. (A) Bath application of 100 μmol l^{-1} serotonin (5-HT) for 3 min mediated a hyperpolarization of the interneurone. After serotonin perfusion, the amplitude of hyperpolarizing postsynaptic potentials of the interneurone during sensory stimulation at 20 Hz of 11 stimuli (indicated by arrows) initially decreased in association with a serotonin-mediated hyperpolarization of the membrane. After approximately 10 min of washing, the amplitude of the evoked hyperpolarizing postsynaptic potentials increased, even though the membrane was still hyperpolarized. The time indicated on each trace (Ai–v) shows the elapsed time after serotonin application. The dashed line indicates the resting membrane potential level. (B) The amplitude of hyperpolarizing postsynaptic potentials of the interneurone during sensory stimulation at 20 Hz of 11 stimuli (indicated by arrows) increased after serotonin-mediated depolarization. red mn, reductor motor neurone.

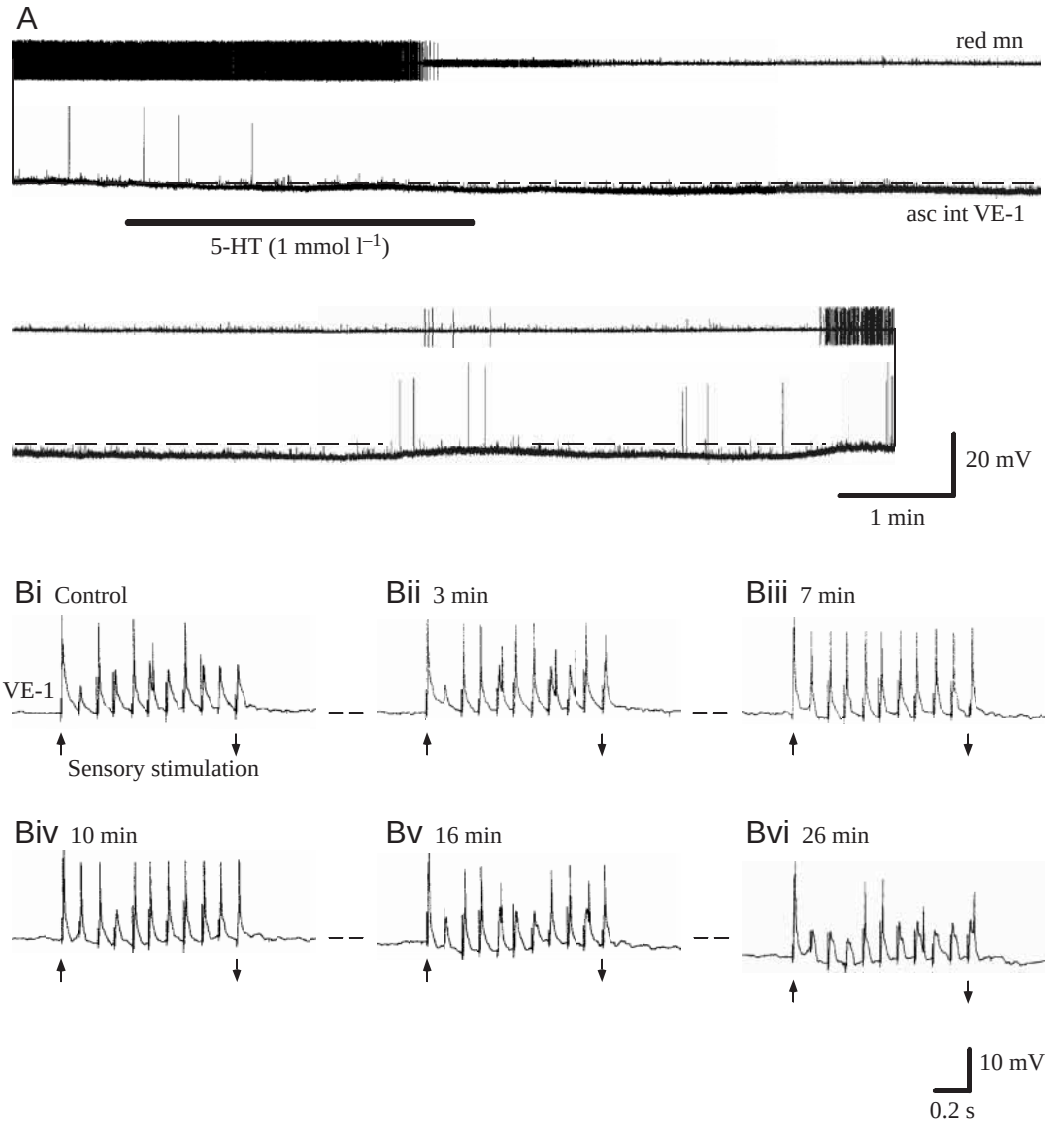

Fig. 7. Response of ascending interneurone (asc int) VE-1 to bath application of serotonin (5-HT). (A) Bath application of 1 mmol l^{-1} serotonin for 3 min (indicated by thick bar) elicited no significant changes in the membrane potential of VE-1, although the tonically occurring spikes of the reductor motor neurone (red mn) were completely suppressed for long periods. (B) The excitatory response of VE-1 to sensory stimulation at 20 Hz of 11 stimuli (indicated by arrows) was increased after serotonin perfusion, although the excitatory level of VE-1 returned to its initial level after approximately 20 min of washing. The time indicated on each trace (Bi–vi) shows the elapsed time after serotonin application. The dashed line indicates the resting membrane potential level.

depressed (Pasztor and Bush, 1989). In *Aplysia californica*, serotonin facilitates the connection between siphon sensory neurones and gill and siphon motor neurones by increasing transmitter released from presynaptic sensory neurones (Kandel and Schwartz, 1982; Glanzman et al., 1989). Although the amplitude of depolarizing or hyperpolarizing potentials in nonspiking interneurones elicited by sensory stimulation was also increased without any direct correlation with the serotonin-mediated potential change, the responses in nonspiking interneurones to sensory stimulation were enhanced and prolonged after washing. A similar post-serotonin enhancement has been reported in *Tritonia* swim interneurones (Katz and Frost, 1995). The slow excitatory postsynaptic potentials (EPSPs) of the dorsal flexion neurone mediated by the dorsal swim interneurone increased in amplitude for more than 10 h. Since spiking interneurones showed no post-serotonin enhancement, and hyperpolarizing responses of the nonspiking interneurones also continued to increase despite sensory afferents not making inhibitory synapses directly with the nonspiking interneurones (Nagayama and Sato, 1993; Nagayama, 1997; Ushizawa et al., 1996), the post-serotonin enhancement could not be due to the presynaptic effect of the release of transmitter from the sensory afferents. Certain postsynaptic mechanisms on the membrane of the nonspiking interneurones, such as an upregulation of the receptors, could perhaps occur during serotonergic modulation.

Behavioural significance of serotonergic modulation

The effect of serotonin on the synaptic responses of the lateral giant (LG) interneurones in the crayfish is known to be dependent on the social status of the animal (Yeh et al., 1996). In socially isolated or dominant crayfish, serotonin increases the response of LG to the sensory stimulation of tailfan afferents. By contrast, in socially subordinate crayfish, serotonin inhibits the response of LG (Yeh et al., 1997). Furthermore, the behavioural performance of the crayfish to mechanical stimulation of the abdomen was also different depending on the social status of the crayfish (Drummond

et al., 2002). In this study, six nonspiking interneurones showed depolarization and nine interneurones showed hyperpolarization, although their sensory responses were commonly enhanced by bath application of serotonin. There is, however, no close relationship in this study between serotonin-mediated membrane potential changes and sensory inputs or motor outputs of the nonspiking interneurones. For example, five out of nine nonspiking interneurones that made inverting connections with the reductor motor neurone showed serotonin-mediated membrane depolarization, while the remaining four interneurones showed hyperpolarization. At the moment, the relationship between the mode of serotonergic effect on the nonspiking interneurones and the social status of the crayfish is unclear. Since the nonspiking interneurones receive both peripheral and central inputs and continuously control the excitability of the uropod motor neurones (Nagayama and Hisada, 1987; Namba et al., 1994, 1997), the post-serotonin enhancement of the nonspiking interneurones in response to sensory stimulation and probably inter- and intra-segmental interactions affects the background excitability of the motor neurones. Thus, the effects of nonspiking interneurones can be gated or biased depending on the behaviour at a given state of the crayfish.

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