Spatial segregation of excitatory and inhibitory effects of 5-HT on crayfish motoneurons

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Bacqué-Cazenave J, Issa FA, Edwards DH, Cattaert D. Spatial segregation of excitatory and inhibitory effects of 5-HT on crayfish motoneurons. J Neurophysiol 109: 2793–2802, 2013. First published March 13, 2013; doi:10.1152/jn.01063.2012.—Altering neuronal membrane properties, including input resistance, is a key modulatory mechanism for changing neural activity patterns. The effect of membrane currents generated by either synaptic or voltage-dependent channels directly depends on neuron input resistance. We found that local application of serotonin to different regions of identified motoneurons (MNs) of the postural/walking network of isolated crayfish produced different changes in input resistance. Puff-applied 5-HT in the periphery of the initial segment produced exclusively inhibitory responses. In contrast, when 5-HT was puff-applied on the central arbor of the same depressor (Dep) MN, exclusively depolarizing responses were obtained. Both inhibitory and excitatory responses were direct because they persisted in low-calcium saline. We found numerous close appositions between 5-HT-immunoreactive processes and the initial segment of dextran-rhodamine-filled Dep MNs. In contrast, almost no close apposition sites were found in Dep MN arbor. It seems that the 5-HT controls the level of excitability of postural network MNs by two mechanisms acting at two different sites: inhibitory responses (consistent with an action involving opening of K⁺ channels) occur in the initial segment region and may involve classic synaptic transmission, whereas depolarizing responses (consistent with an action involving closing of K⁺ channels) occur on MN branches via apparent paracrine effects.

Crustacea; stretch reflex; neuromodulation; posture; serotonin; crayfish; 5-HT cell; paracrine

SEROTONIN (5-HT) CONTROLS a variety of behaviors in vertebrates as well as invertebrates. In vertebrates, this diversity is supported by a multiplicity of receptor subtypes, the localization of which seems functionally important (Riad et al. 2000; Rumajogee et al. 2006). In crayfish, serotonin (5-HT) influences aggressiveness (Huber and Delago 1998; Huber et al. 1997a,b), social status (Huber et al. 1997b, 2001), and escape behavior (Teshiba et al. 2001). At the cellular level, the effects of 5-HT depend on the animal’s social history (Le Bon-Jego et al. 2001; Yeh et al. 1996, 1997). In addition, the ionic mechanisms involved in the effects of 5-HT on LG in social isolates are complex. A low-5-HT concentration appears to decrease a potassium conductance, whereas a high-5-HT concentration appears to increase a chloride conductance (Krasne and Edwards 2002).

In the present study, we aimed at localizing and deciphering the ionic mechanisms involved in positive and negative effects of 5-HT on leg motoneurons (MNs) of isolated crayfish. Using an in vitro preparation of the crayfish locomotor network (El Manira and Clarac 1991), we show the existence of close appositions between 5-HT-immunoreactive processes and depressor (Dep) MNs close to their initial segment area and spike-initiating zone (SIZ) at the periphery of the neuropil. Using pressure ejection, we demonstrate that local application of 5-HT in the area where close apposition sites were found induces exclusively inhibitory effects on Dep MNs. By contrast, slow excitatory effects were observed when 5-HT was applied on central Dep branches on which no close apposition from 5-HT processes was found. Both inhibitory and excitatory responses were direct because they persisted in low-calcium saline. We conclude that whereas inhibitory effects from 5-HT cells may involve classic synaptic contacts close to the initial segment of Dep MNs, more central excitatory effects are most likely due to paracrine mechanisms.

MATERIALS AND METHODS

Experimental Animals

Experiments were performed on male form I adult crayfish (Procambarus clarkii; n = 47) weighing 25–30 g. The animals were collected locally (in the Réserve Naturelle de Bruges after prefectural authorization) and housed individually for 3 wk before experimentation. They were kept at 18–20°C on a 12:12-h light-dark cycle and fed once a week with shrimp pellets and carrots.

In Vitro Preparation

An in vitro preparation of the thoracic nervous system was used (El Manira and Clarac 1991; Sillar and Skorupski 1986). Before dissection, each animal was chilled in ice water for 30 min. Then it was decapitated, and the thorax and abdomen were pinned dorsal side up. A section of the ventral nerve cord containing the last three thoracic (T3–T5) and the first abdominal (A1) ganglia were dissected out with all of the nerves of the two proximal segments of the left fifth leg (Fig. 1).

The preparation was pinned dorsal side up on a Sylgard-lined petri dish (Dow Corning, Wiesbaden, Germany). The nervous system was continuously superfused with oxygenated control saline composed of (in mM) 195 NaCl, 5 KCl, 13 CaCl₂, 2 MgCl₂, and 3 HEPES...
MgCl₂, with the sodium concentration reduced accordingly to pre-
serve the osmolarity of the solution, was used to raise the spiking
level to superfuse the ganglia continuously with fresh Ringer or low-calcium
Ringer. Intracellular recordings from Dep motorneurons (MN) were performed
within the neuropil of the 5th thoracic ganglion.

Recordings and Electrical Stimulation

Extracellular recordings from the motor nerves innervating the Dep
and levator muscles were made using stainless steel pin electrodes
contacting the nerves and insulated with Vaseline. The differential
extracellular signals were amplified 2,000–10,000-fold and filtered
(high-pass 30 Hz, low-pass 30 kHz, 50-Hz notch filter) using Grass
Instruments AC preamplifiers. The bath solution was grounded using
a small silver plate that was chlorinated using chlorine bleach.
Stimulation of nerves was done with a programmable pulse generator
(Master-8; A.M.P.I.) and a stimulus isolation unit (A.M.P.I.). Intracellu-
lar recordings from Dep MNs were performed with glass mic-
pipettes (Clark Electromedical Instruments, Reading, United King-
dom) filled with either 3 M KCl (resistance 10–20 MΩ) or 5%
dextran-tetramethylrhodamine (3,000-Da mol mass; Life Sciences) in
0.2 M potassium acetate with the electrode shank filled with 2 M potas-
sium acetate. Neurons were injected for 1 h using +10-nA square-
wave pulses (500-ms duration at 1 Hz).

Ganglia were fixed overnight at 4°C in 4% paraformaldehyde in a
0.2 M phosphate buffer solution (pH 7.4) and rinsed for 4 h six times
in 50 ml of 0.2 M phosphate buffer solution. Preparations were then
incubated for 36 h at 4°C in a 1:2,500 dilution of anti-serotonin
antibody (Sigma-Aldrich) generated in rabbit against a formaldehyde
cross-linked serotonin-BSA conjugate. Following primary antibody
treatment, tissues were rinsed 4 h in phosphate buffer solution with
0.5% Triton X-100 and then postincubated for 36 h at 4°C with a
secondary antibody, which was goat anti-rabbit IgG labeled with
FITC diluted 1:40. Tissues were finally rinsed in 0.2 M phosphate
buffer for 6 h. Ganglia were then dehydrated in a series of ethanol
solutions of ascending strength (50, 70, and 90%, 10 min each; 95 and
100%, 2 times, 10 min each), cleared in methyl salicylate (Sigma-
Aldrich), and mounted in Eukitt (O. Kindler).

The ganglia were then imaged using a confocal microscope (Olympus
FluoView BX51), and the resulting digital images were analyzed using
FluoView software (Olympus). Close appositions between 5-HT-immu-
noreactive processes and Dep MNs were quantified as follows. Confocal
slices (0.2-μm thick) were obtained with ×40 objective (1.0 numerical
aperture; UPlanApo; Olympus). The occurrence of 5-HT (green) and Dep
MN neurite or axon (red) in the same voxels was indicative of colocal-
ization and appeared yellow (see Fig. 2). The maximum-intensity yellow

Local Pressure Application of 5-HT Puff

5-HT (1 mM) was applied locally by pressure ejection for 50 or 200
ms at 100- to 400-kPa pressure using a Picospritzer II (General Valve,
Fairfield, NJ) connected to two glass pipettes placed simultaneously in
the central region of the neuropil (c) and in a more peripheral site (p)
close to the initial segments of Dep MNs, respectively (see Fig. 3A).
The initial segment of Dep MNs corresponds to the SIZ. The main
neurite of crayfish walking MNs does not convey spikes, and only
electrotone propagation is observed in main neurite and branches
(Pearlstein et al. 1998). Spikes are produced on the border of the
neuripil in the SIZ, a site where the axon extends from the main
neurite. Anatomically, this transition is characterized by a marked
reduction in diameter. Pipettes were pulled using a Sutter P-80 puller.
The tips of the pipettes were then gently broken by touching the edge
of a soft piece of paper. The volume of ejection was estimated by
measuring under the microscope the diameter of the drop formed at
the tip of the pipette by the pressure pulse when the pipette was in air.
When too short a pressure pulse caused the fluid to move up along
the pipette tip, the duration of the pressure pulse (generally in the range
of 50–200 ms) was increased until a droplet with a diameter of ~20
μm was formed (~10 pl). The positioning of the ejection pipettes was
adjusted to obtain the maximum response in both ejection sites. A
washout period of at least 5 min for peripheral puffs and 20–40 min
for central puffs was included between subsequent recordings to take
into account recovery time after the effect of 5-HT.

Anatomic and Immunohistochemical Techniques

In the first experiments, Dep MNs were injected with 5% dextran-
tetramethylrhodamine (3,000-Da mol mass; Molecular Probes) in 0.2
M potassium acetate with the electrode shank filled with 2 M potas-
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voxels were counted as distinct close appositions when they were separated by an area of minimum intensity.

**Data Acquisition and Analysis**

Data were digitized and stored on a computer hard disk through an appropriate interface (1401plus) and software (Spike2) from Cambridge Electronic Design (Cambridge, United Kingdom). Statistical analyses were done with the Prism program (GraphPad Software, San Diego, CA). The results are given as mean values ± SE.

**RESULTS**

**Localization of Close Apposition Sites Between 5-HT-Immunoreactive Processes and Dep MNs**

We conducted a series of immunohistological experiments to determine the anatomic connectivity between the serotonergic inputs and the Dep MNs in isolated crayfish. In five experiments, the recorded Dep MN was intracellularly filled with dextran-tetramethylrhodamine (red color in Fig. 2). Each preparation was treated with 5-HT antibodies, and close apposition sites between the Dep MN and 5-HT-immunoreactive processes (stained with FITC, green color in Fig. 2) were analyzed with a confocal microscope. In the experiment presented in Fig. 2, close apposition sites were found in the posterolateral part of the neuropil in the region of MN initial segments (see Fig. 2A for a general view of the neuropil and Fig. 2B for enlarged view indicated by a rectangle in Fig. 2A). The close apposition sites are presented as a continuous series of confocal slices in Fig. 2C, 1–6. This series of confocal sections was taken with a ×40 objective, which corresponds to a z-axis interslice distance of 0.2 μm (the theoretical resolution limit with this objective being 0.250 μm). As can be seen in these sections, yellow color indicates that both fluorophores were in the same 0.2-μm deep voxels (close apposition). Note that, at these close apposition sites, the 5-HT-immunoreactive processes presented varicosities that could be indicative of a presynaptic site (Fig. 2D). By contrast, almost no close apposition sites were found in more central regions of the neuropil, and none was associated with varicosities (see Fig. 2E).
locations of close appositions accumulated from 5 experiments in Fig. 2E).

**Excitatory and Inhibitory Responses are Triggered at Different Areas of Dep MNs**

To explore further the possibility that responses to 5-HT are triggered in the initial segment region of Dep MNs, very small volumes (5–10 pl; see MATERIALS AND METHODS) of 5-HT (10^{-3} M) were applied locally via pressure ejection, whereas a Dep MN was recorded intracellularly (Fig. 3). The duration of the pressure pulse was adjusted between 30 and 200 ms to get a marked effect without damage to the Dep MN recording. A surprising result was that the ejections at the initial segment region [peripheral site: (p) in Fig. 3A] exclusively produced hyperpolarizing responses (n = 7; Fig. 3B). A second 5-HT-containing pipette was placed in a more central region of the neuropil [see (c) in Fig. 3A]. At this central site, ejections exclusively evoked depolarizing responses (n = 9; Fig. 3C). It is important to note that, in a given experiment, both responses were evoked in the same Dep MN. Similar results were obtained from 11 Dep MNs (each from a different preparation).

In these experiments, the Dep motor nerve (Dep n) was also recorded. Peripheral ejections of 5-HT were clearly inhibitory as they blocked the tonic activity of other Dep MNs recorded from the Dep n (Fig. 3B). The inhibition of other Dep MNs was favored by the fact that all Dep MNs are very close to each other as are their initial segments (Hill and Cattaert 2008). In contrast, central ejections of 5-HT were clearly excitatory because they induced a concomitant discharge response of other Dep MNs recorded from the Dep n (Fig. 3C).

By comparison with experiments using bath application of 5-HT (Cattaert et al. 2010; Le Bon-Jego et al. 2004), the pressure-evoked responses of 5-HT were fast (hyperpolarizing responses started in <500 ms after the onset of the pressure pulse when the pipette was correctly placed, see Fig. 3B), whereas depolarizing responses were observed in <2 s, Fig. 3C).

**Analysis of Peripheral Responses to 5-HT in the Initial Segment Area**

Peripheral responses evoked by 5-HT local pressure ejection are direct. To check that the responses evoked in Dep MNs by pressure ejection of 5-HT were direct, at the end of experiments, a solution with low calcium and high magnesium was perfused for 2 h to block synaptic transmission (Dingledine and Somjen 1981; Sheriff and Mulloney 1996). When 5-HT was pressure-applied in the peripheral area of the neuropil in the vicinity of the initial segment of Dep MNs (see schema in Fig. 4A), the hyperpolarizing response persisted (Fig. 4B), and the Dep MN tonic discharge was blocked (Fig. 4B), as was the activity of other Dep MNs recorded from the Dep n, confirming that such responses were inhibitory. This result was obtained in four out of four experiments. The average tonic discharge rate of Dep MNs recorded from the Dep n dropped significantly from 22.12 ± 5.46 to 5.68 ± 1.19 Hz (paired t-test, P < 0.05). These inhibitory responses to 5-HT of the intracellularly recorded Dep MN and other Dep MNs of the Dep n persisted in the presence of the chloride channel blocker picrotoxin (PTX; 40 μM) applied in the bathing medium (Fig. 4C; n = 2 experiments), suggesting that chloride ions were not involved in these responses.

**Mechanisms of peripheral inhibitory responses evoked by 5-HT.** To analyze the mechanisms of hyperpolarizing responses evoked by 5-HT in the initial segment area of Dep MNs, the changes in input resistance (R_{in}) were monitored by current pulses (−1 nA) continuously injected in the Dep MN (2 Hz), whereas 5-HT was pressure-applied (Fig. 5A). In this experiment, 5-HT induced a −1.47-mV hyperpolarizing response in the intracellularly recorded Dep MN. For a better estimation of the hyperpolarizing response, a trace cleansed from current pulses is presented below the intracellular recording in Fig. 5A2. This cleansed trace was built using a homemade Spike2 script that measured the membrane potential at the end of each current pulse and linked the measurements.
In the experiment presented in Fig. 5A, the calculated $R_{in}$ of the Dep MN decreased from 8.89 ± 0.07 MΩ before 5-HT ejection to 8.20 ± 0.06 MΩ 10 s after the 5-HT ejection ($P < 0.0001$, unpaired $t$-test; $n = 20$ current pulses). Similar drops were observed in five experiments: the $R_{in}$ dropped from 5.98 ± 1.38 MΩ in control conditions to 4.70 ± 1.16 MΩ at the peak of the 5-HT hyperpolarizing response ($n = 5$; Fig. 5C). This average drop was significant ($P = 0.013$, paired $t$-test). The average time to reach the maximal response measured over these five experiments was 32.2 ± 14.8 s. Therefore, the rather fast hyperpolarizing response to 5-HT is associated with a decreased $R_{in}$, which indicates that ionic membrane channels had opened.

Averaged membrane voltage traces ($n = 20$) recorded during current pulses were fitted with exponential decay curves. The fitted curve was used to estimate time constant (see Fig. 5D). In this experiment, the time constant of the Dep MN membrane dropped from 19.7 ± 0.5 to 18.7 ± 0.3 ms. The drop in time constant was significant (paired $t$-test, $P = 0.028$) in five out of five Dep MNs (each recorded from a different animal): their average (and SE) fell from 16.87 ± 2.6 ms in control to 14.00 ± 2.42 ms after 5-HT ejection.

**Central responses evoked by 5-HT local pressure ejection are direct.** The same procedure as used for peripheral 5-HT application was followed for central 5-HT applications (see schema in Fig. 7A). In the presence of low-calcium saline, the neurons depolarized, and the intracellularly recorded Dep MN generally displayed a tonic discharge (Fig. 7B). Under these conditions, the intracellularly recorded Dep MN depolarized when 5-HT was pressure-applied in the central area of the neuropil (Fig. 7B), and its tonic discharge increased (as did other Dep MNs recorded from the Dep n). The average discharge frequency of the Dep n in low calcium increased from 17.77 ± 12.35 Hz in control to 42.26 ± 17.93 Hz after central puff application of 5-HT ($n = 4$ experiments on different animals). Note also that central application of 5-HT induced a tendency to rhythmic oscillations during the induced depolarizing response.
Mechanisms of depolarizing responses evoked by 5-HT in the central area. The changes in $R_{in}$ produced by 5-HT pressure application (duration 50 ms, frequency 2 Hz). In the experiment presented in Fig. 8, A1 and B, 5-HT induced a 3.85-mV depolarizing response in the intracellularly recorded Dep MN (for a better estimation of the depolarizing response, a trace cleansed from

Fig. 5. Mechanisms of hyperpolarizing response of Dep MNs to peripheral local 5-HT application. A: repetitive (2-Hz) −1-nA current pulses were injected in an intracellularly recorded Dep MN before, during, and after pressure-applied 5-HT (A1: raw data of the Dep MN intracellular recording; the thickness of the trace is due to current-pulse injection; A2: computer reconstruction of the time course of the Dep MN membrane potential). B: average trace of 5 experiments in which 5-HT was pressure-applied in the peripheral region of the ganglion close to the main root (traces were aligned to the potential of the Dep MNs just before 5-HT pressure application). C: average traces ($n = 15$) of the membrane potential deflection induced by current pulses applied in control conditions (black) and after 5-HT pressure ejection (gray). D: these traces were fitted to single-exponential decay curves used to estimate input resistance ($R_{in}$) and time constant of the intracellularly recorded Dep MN. E: change in the $R_{in}$ over time (same recording as in A). F: changes in $R_{in}$ of 5 Dep MNs produced by peripheral pressure application of 5-HT. The mean decrease is significant (paired $t$-test, $P = 0.013$). $*P < 0.05$.

Fig. 6. Reversal potentials of the hyperpolarizing responses of Dep MNs to 5-HT peripheral local application. A and B: estimation of the reversal potential of the hyperpolarizing 5-HT induced response. A: intracellular recordings from a Dep MN, the membrane potential of which was maintained at various membrane potentials by injection of a continuous current, whereas 5-HT was pressure-ejected in the periphery of the ganglion (in the Dep-MN initial segment area). B: plot of the amplitude of the hyperpolarizing responses against maintained membrane potential measured in 3 Dep MNs (each recorded from a different animal).
A central 5-HT Pressure ejection

B low Calcium (2h)

Fig. 7. Evidence that responses of Dep MNs to 5-HT central local application are direct. A: schematic representation of the disposition of the pipette used for pressure-applied 5-HT. B: depolarizing response of a Dep MN to local pressure application of 5-HT in the central area of the neuropil performed after 2 h in the presence of low calcium (to block synaptic transmission). In these conditions, the local application of 5-HT in central regions of the neuropil still produced a depolarization of the intracellularly recorded Dep MN and an increase of its spiking activity. Concomitantly, the discharge of other Dep MNs recorded from Dep n was also enhanced.

current pulses as described above is presented below the intracellular recording in Fig. 8A(2). This depolarizing response was consistently observed in seven Dep MNs (each recorded from a different animal). The average depolarization measured 5 min after the 5-HT puff was 2.59 ± 0.58 mV (n = 7; Fig. 8B). This depolarization from −74.14 ± 1.84 to −71.55 ± 2.12 mV was significant over all experiments (paired t-test, P < 0.01; n = 7).

During the depolarizing response to 5-HT, the R_{in} of the Dep MN was monitored by injection of −1 nA current pulses (Fig. 8C). A precise evaluation of R_{in} was obtained after fitting the average voltage-pulse responses (n = 20) with a single-exponential decay curve (Fig. 8D). After a 5-HT puff, R_{in} of the recorded Dep MN increased gradually and reached a maximum near 5 min after the 5-HT puff (Fig. 8E), at a similar time to when depolarization reaches its maximum (Fig. 8B). In the seven intracellularly recorded Dep MNs, the increase in R_{in} measured 5 min after the 5-HT puff was always highly significant compared with control. In these seven MNs, the R_{in} increased slowly to reach a plateau after 5–10 min (Fig. 10F). R_{in} increased significantly (P < 0.01, paired t-test) from 5.59 ± 1.93 MΩ in control conditions to 8.13 ± 2.39 MΩ at the peak of the depolarizing response (n = 7). Therefore, the rather slow depolarizing response to 5-HT is associated with an increased R_{in}, which indicates that membrane ion channels were closing. Note that the central effects of 5-HT on both depolarization and increased R_{in} were much slower than the opposite 5-HT effects observed in the periphery of the neuropil (see Fig. 5E). On washout of the 5-HT, R_{in} was recovered in five experiments (average recovery time: 16.6 ± 5.56 min; n = 5) but failed to occur in two experiments.

At the same time, we estimated the time constant of Dep MNs on averaged traces recorded during injection of −1-nA hyperpolarizing current pulses (Fig. 8C). In the experiment presented in Fig. 8A, the time constant increased from 18.38 ms (in control conditions) to 19.01 ms (5 min after 5-HT puff). These measurements were made in seven Dep MNs. The average time constant increased significantly from 19.76 ± 6.13 to 25.08 ± 8.29 ms (paired t-test, P = 0.033; n = 7).

Reversal potential of 5-HT central responses. To identify the ions involved in central depolarizing responses, a series of 5-HT puffs were delivered at various membrane potentials (at 15-min intervals to avoid desensitization of receptors). The various membrane potentials were tested in a random order and repeated at least two times each. The amplitude of the depolarizing response to 5-HT decreased as the membrane potential was more hyperpolarized, being almost suppressed at −80 mV (Fig. 9A), and then reversed to produce hyperpolarizing responses in two experiments when the membrane potential was adjusted to −85 mV by injection of a continuous hyperpolarizing current. Similar results were obtained in 4 experiments (i.e., 4 Dep MNs, each from a different animal). The plot of the amplitude of the response to 5-HT against the Dep MN membrane potential from these 4 experiments was fitted with a linear regression curve (r^2 = 0.88, P < 0.001). The reversal potential of the response is given by the regression line crossing the x-axis at V = −82.98 mV (Fig. 9B).

DISCUSSION

In a previous paper (Le Bon-Jego et al. 2004), it was shown that 5-HT modulates sensory-motor integration involved in postural control of crayfish walking legs via two main effects: altering R_{in} of the Dep MN (i.e., its excitability) and controlling the polysynaptic sensory-motor pathway (i.e., its sensory synaptic input). In such experiments carried out on communal animals, two types of effects were observed: in some animals, bath application of 5-HT induced an increase of Dep MN excitability, whereas in others, 5-HT induced a decrease of excitability (Le Bon-Jego et al. 2004). Here, in postural networks of isolated animals, we demonstrate that the increase and decrease of excitability involve different areas of the arbor of a given Dep MN.

Structure-Function Relationships Between 5-HT Processes and Dep MN in the Center and Periphery

Inhibitory responses were exclusively induced in the periphery of the neuropil close to the initial segment area, whereas excitatory responses were induced in central regions of the neuropil. The initial segment area almost exclusively contains close appositions and varicosities of 5-HT-immunoreactive processes that suggest classic synapses. In contrast, the central 5-HT-containing processes are more distant from Dep MN dendrites, and the few close appositions observed were never associated with varicosities of the 5-HT-immunoreactive process. In addition, the excitatory effects of 5-HT on Dep MNs are direct and not mediated by interneurons. Central Dep MN
dendrites may then be activated by paracrine transmission from more distant releasing sites, which could imply a slower and smaller rise in 5-HT concentration when the releasing sites are activated (Bunin and Wightman 1999).

We hypothesize that there are two modes of action, classic synapses close to the initial segment and paracrine action in central dendrites (Fig. 10), which involve different 5-HT receptors with opposing mechanisms of action: central receptors...
mediate facilitation through a decreased potassium conductance, whereas initial segment receptors mediate inhibition through an increased potassium conductance. Centralized applied puffs of 5-HT were excitatory and appeared to act by decreasing a potassium conductance. The concentration of 5-HT at the synapses was unknown, but in other experiments, a low concentration (10 μM) of bath-applied 5-HT in dominant animals (Cattaert et al. 2010) or isolated animals (D. Cattaert, unpublished observations) was also excitatory. Therefore, it is likely that 5-HT receptors are present in central neurites of Dep MNs and produce excitation by reducing a persistent potassium conductance.

Two 5-HT receptors have been identified in crayfish nervous system, and one of those, 5-HT₁₉, may be present in the initial segments of the Dep MNs and the LG command neuron (Spitzer et al. 2005). Immunocytochemical staining has shown this receptor on efferent axons of the fifth thoracic ganglion (Spitzer et al. 2005) near the terminals of 5-HT neurons that innervate the Dep MNs (Fig. 2). The same receptors are also near serotonergic terminals adjacent to the initial segment of LG (Edwards and Spitzer 2006). This receptor downregulates cAMP, which is consistent with inhibition (Araki et al. 2005; Lee et al. 2008).

Functional Significance of the Spatial Separation of Excitatory and Inhibitory Responses to 5-HT onto Dep MNs

The initial segment of arthropod MNs is usually the site of the SIZ (Sandeman 1969) and is often contained within the motor nerve root, as it is for Dep MNs. Hyperpolarizing postsynaptic conductances produced at the SIZ by 5-HT or possibly another transmitter would likely exert a strong inhibition on spike initiation that would not readily be overcome by additional dendritic excitation (Vu and Krasne 1992). The depolarization and increased \( R_{in} \) produced in the Dep MNs by centrally acting 5-HT would act to increase EPSP amplitude and decrease the electrotonic size of the cell, thereby enabling the larger EPSPs to summate more effectively at the SIZ. The balance of effect of 5-HT on Dep MN responsiveness will depend on which site is more effectively activated by 5-HT, with peripheral inhibition at the initial segment having the advantage.

Such opposing effects of 5-HT on identified neurons have also been reported elsewhere in the crayfish nervous system, specifically in the synaptic responses of the LG escape command neuron to afferent input (Lee et al. 2008; Teshiba et al. 2001). Such opposing of synaptic effects of a single neuromodulator seem to represent a common situation (for a review, see Harris-Warrick 2011; Harris-Warrick and Johnson 2010). Not only 5-HT, but also dopamine were shown to produce opposite effects on the LP→PD synapse of the stomatogastric pyloric system (Kloppenburg et al. 2007). Four functional significances of such opposing effects of neuromodulators have been proposed (Harris-Warrick and Johnson 2010), three of which may apply here: 1) dual effects may provide for flexibility in the sign of the effect controlled by another mechanism acting differently on the two antagonistic effects; 2) opposition may only be apparent and indeed operate at different ranges (concentration, kinetic, or spatial); and 3) if 5-HT central and peripheral effects are not differentially regulated, they may serve to constrain the effects of 5-HT within a particular range (in this case, the peripheral inhibitory effects avoid overexcitation produced by central excitatory effects of 5-HT).

The application and region-specific modulatory effects of 5-HT described here and earlier (Antonsen and Edwards 2007; Lee et al. 2008) were seen in socially isolated animals; additional variations in the modulatory effects of 5-HT have been seen in socially dominant and subordinate animals (Le Jego et al. 2004; Yeh et al. 1996, 1997). At present, we do not know how the effects of application regime and application region will also depend on the animal’s social status, but it appears likely that they will add to this complex and interesting story.
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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

J.B.-C., F.A.I., and D.C. performed experiments; J.B.-C., F.A.I., and D.C. analyzed data; J.B.-C., F.A.I., and D.C. interpreted results of experiments; J.B.-C. and D.C. prepared figures; J.B.-C. and D.C. drafted manuscript; J.B.-C., F.A.I., D.H.E., and D.C. approved final version of manuscript; F.A.I., D.H.E., and D.C. conception and design of research.

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