ELSEVIER

Comparative Biochemistry and Physiology Part B 127 (2000) 533–550

CBP

www.elsevier.com/locate/cbpb

The effects of 5-HT on sensory, central and motor neurons driving the abdominal superficial flexor muscles in the crayfish

J.R. Strawn [a], W.S. Neckameyer [b], R.L. Cooper [a,*]

[a] *101 Thomas Hunt Morgan School of Biological Sciences, University of Kentucky, Lexington, KY 40506-0225, USA*
[b] *Department of Pharmacology and Physiological Sciences, St Louis University, St Louis, MO 63104, USA*

Received 12 January 2000; received in revised form 1 August 2000; accepted 8 August 2000

Abstract

Serotonin (5-HT) induces a variety of physiological and behavioral effects in crustaceans. However, the mechanisms employed by 5-HT to effect behavorial changes are not fully understood. Among the mechanisms by which these changes might occur are alterations in synaptic drive and efficacy of sensory, interneurons and motor neurons, as well as direct effects on muscles. We investigated these aspects with the use of a defined sensory-motor system, which is entirely contained within a single abdominal segment and consists of a 'cuticular sensory neurons–segmental ganglia–abdominal superficial flexor motor neurons–muscles' circuit. Our studies address the role of 5-HT in altering (1) the activity of motor neurons induced by sensory stimulation; (2) the inherent excitability of superficial flexor motor neurons; (3) transmitter release properties of the motor nerve terminal and (4) input resistance of the muscle. Using en passant recordings from the motor nerve, with and without sensory stimulation, and intracellular recordings from the muscle, we show that 5-HT enhances sensory drive and output from the ventral nerve cord resulting in an increase in the firing frequency of the motor neurons. Also, 5-HT increases transmitter release at the neuromuscular junction, and alters input resistance of the muscle fibers

Keywords: Serotonin; Neurotransmission; Crayfish; Neuromuscular junction; Synapse; Quantal

1. Introduction

Neuromodulators are well recognized as important signaling molecules in all animals examined because of the wide variety of behavioral effects which they might elicit. They may serve as both neurotransmitters and paracrine factors in central and peripheral nervous systems (Guillemin, 1978; Jan and Jan, 1982; O'Shea and Schaffer, 1985). One neuromodulator in particular, serotonin (5-HT), has received much attention both in the news media and in the academic literature because it has been implicated in the behavioral expression of dominance, aggression, and assertiveness in many animals, including humans (Linnoilia and Virkkunen, 1982; Winberg et al., 1992; Cases et al., 1995; Coccaro, 1995; Yeh et al., 1996). The distribution of 5-HT in the peripheral nervous systems of invertebrates implies that the transmitter is not directed at a discrete target tissue but

* Corresponding author. Tel.: +1-859-2575950; fax: +1-859-2571717.
E-mail address: rlcoop1@pop.uky.edu (R.L. Cooper).

0305-0491/00/$ - see front matter
PII: S0305-0491(00)00287-X

over a widespread area, perhaps interacting with receptors on several different target cells. Consequently, this could result in relatively fast, as well as slow, long-lasting modifications of the efficacy in transmission at synapses where other transmitters are released (Kupfermann, 1979).

The crustacean nervous system is experimentally tractable, and previous studies have facilitated correlation of the physiology, from relatively few neurons and muscles, to particular behaviors. The advantage of neuromuscular systems in the crayfish is that entire muscles are innervated by one to no more than a dozen excitatory axons (LaFramboise et al., 2000; Sohn et al., 2000). Moreover, the drive of the motor neurons can readily be examined by stimulation of various known sensory neurons, which enables one to assess their role in altering motor neuron activity. In addition, the integration of sensory information in the ventral nerve cord (VNC) which effects the motor output is accessible for study. Lastly, the biophysical properties of muscle fibers that can effect the postsynaptic response are readily approachable. Thus, the crustacean nervous system enables one to investigate each component of a sensory–ganglia–motor neuron–muscle pathway which elicits that behavior.

In lobsters and crayfish, it has been shown that injections of serotonin (5-HT) into the circulatory system cause the animals to assume a semi-dominant posture (a raised extension of the chelipeds, although with a flexed state of their abdomen, Livingstone et al., 1980). The raised chelipeds is thought to be a visual, social-status signal. Injection of a different neuromodulator, octopamine, produces a submissive behavior, with lowering of the chelipeds but the same time with extension of the animal's extremities (Livingstone et al., 1980; Harris-Warrick and Kravitz, 1984). The actions of 5-HT in eliciting these behaviors were shown to be selective within the VNC to enhance excitation of excitatory flexor motor neurons and decrease excitation of the inhibitor flexor motor neurons. The converse is observed for the exciter and inhibitor motor neurons to the abdominal extensors. Since crayfish with higher levels of 5-HT have been shown to be more assertive (Huber et al., 1997; Huber and Delago, 1998), one might expect the more aggressive individuals to have flexed abdomens; however, this behavioral posture remains to be quantified during social interactions. 5-HT is excitatory at the presynaptic terminals of motor neurons (Dudel, 1965; Glusman and Kravitz, 1982; Livingstone et al., 1980; Griffis et al., 1999; Strawn et al., 1999), suggesting that the greater mass of flexor muscle on which 5-HT can act, relative to the extensor muscles and number of terminals, may account for the flexed state during injection of high concentrations of 5-HT. In addition, the motor nerves to the abdominal superficial flexor muscles are spontaneously active in the animal, as well as in isolated ventral nerve cords. Thus, if 5-HT enhances this activity, one would expect the animal to be in a flexed state. Previous investigations did not address whether sensory neuron activity could be altered by 5-HT, and whether the alterations in sensory neuron activity could have an effect on the activity of the superficial motor neurons. It is well established in the *Aplysia* nervous system that 5-HT sensitizes a gill-withdrawal reflex (Siegelbaum et al., 1982) and it appears from the results presented herein in the crayfish system that 5-HT can also enhance sensory drive.

The aims of this study were to examine the role of 5-HT on each component within a crayfish 'sensory–ganglia–motor nerve–muscle' consisting of specific, identified cells within the second abdominal segment. Experiments were designed to assess the role of 5-HT in altering (1) the activity of the sensory neurons which drive the motor neurons; (2) the activity of superficial flexor motor neurons; (3) vesicular release properties of the superficial motor nerve terminals and (4) direct input resistance of the muscle. Furthermore, we attempted to measure 5-HT levels in hemolymph and within the ventral nerve cord of crayfish. The few values of 5-HT reported in the literature for crustaceans vary considerably.

This study illustrates the importance of determining the sensory contribution to the intrinsic activity of motor neurons, and demonstrates that 5-HT modulates not only the motor neurons but also sensory input to the pathway. This abdominal superficial flexor preparation allows one to address issues fundamental to all circuits and chemical synapses.

Preliminary results of this study have been presented in abstract form (Strawn et al., 1999, 2000).

2. Methods

2.1. Animals

All experiments were performed using male freshwater crayfish, *Procambarus clarkii*, measuring 6–10 cm in body length (Atchafalaya Biological Supply, Raceland, LA). All animals were housed as individuals for at least 2 weeks and were fed dried fish food weekly.

2.2. Dissection

All experiments were carried out in a modified Van Harreveld's solution (205 mM NaCl; 5.3 mM KCl; 13.5 mM $CaCl_2 \cdot 2H_2O$; 2.45 mM $MgCl_2 \cdot 6H_2O$; 0.5 mM HEPES adjusted to pH 7.4. Cooper et al., 1998). The animals were sacrificed in less than 5 s by rapid decapitation followed by removal of the abdomen. The abdomen was separated from the thorax by cutting through the carapace between the thorax and abdomen. The VNC was transected between T5 and A1. The abdomen was then pinned ventral side up in a sylgard dish. Exposure of the VNC with the nerve roots and superficial flexor muscle entailed cutting along the midline of the ventral articular membrane (anterior to posterior) and then along the rib laterally from the midline. This section of articulating membrane was then reflected rostrally and removed so as to expose the superficial flexors, VNC and segmental ganglia to the bath solution.

For recording of synaptic currents from the neuromuscular junction, the dorsal carapace of the abdomen was cut away so as to provide access for the removal of the deep flexor. The third root was cut near the VNC and the VNC was removed. The preparation was repinned ventral side up and the superficial flexor and third root exposed as described above.

2.3. Intracellular and en passant recordings

All recordings were performed from the second abdominal segment. For intracellular recordings, a microelectrode (2 M potassium acetate, 20–30 MΩ) was inserted into a medial muscle fiber of the superficial flexor. Extracellular recordings of nerve activity in the third root were made by a suction electrode. All data were recorded on a VHS tape (Vetter) and to a Macintosh computer (Power Macintosh, 300 MHz G3) via MacLab/4s A/D converter (ADInstruments). For cuticular stimulations, a wooden dowel lined with several toothbrush bristles was placed on a mircomanipulator which was positioned along the lateral side of the second abdominal segment. The manipulator was then moved back and forth for a set distance of 1 cm along the cuticle of the segment in which the nerve root was being monitored. Subsequent experiments, using electrical stimulation (50 Hz, 300 ms) of the second root were performed to circumvent problems with standardizing the mechanical stimulation and adaptation of the sensory endings. The extracellular stimulation was applied to the proximal end of the second root by the use of a suction electrode.

2.4. Analysis of synaptic currents

Focal macropatch recording was used to measure synaptic currents at the neuromuscular junctions. The synaptic currents were obtained using the loose patch technique by lightly placing a 10–20 μm diameter, fire-polished, glass electrode directly over a spatially isolated varicosity along a vital dye-visualized nerve terminal. To visualize the nerve terminals, living preparations were fluorescently stained for 2–5 min with 2–5 μM 4-[4-(diethylamino)styryl]-*N*-methylpyridinium iodide (4-di-2-Asp; Molecular Probes, Eugene, OR) in crayfish saline. The preparation was exposed to the dye solution for 2–5 min, followed by washing in crayfish saline. The synaptic transmission remained unaltered by this dye (Cooper et al., 1995b). The macropatch electrode is specific for the current recording within the region of the electrode lumen. The lumen of the patch electrode was filled with the same solution as the bathing medium. The electrode seal resistance was in the range from 100 KΩ to 1 MΩ. Since the seal could be easily lost if the muscle twitched under the electrode, stimulation was restricted to 1 Hz. Evoked excitatory postsynaptic currents (EPSCs) and miniature excitatory postsynaptic currents (mEPSCs) were recorded and analyzed to determine the mean quantal content (m), the number of release sites (n), and the probability of release at the sites (p) (Cooper et al., 1995c, 1996a,b; del Castillo and Katz, 1954). In each synaptic current recording, a trigger artifact and a nerve spike were visualized, indicating nerve stimulation. This was done to determine if a stimulation failed, or if

synaptic release failed, in spite of nerve terminal depolarization. Mean quantal content was determined by direct counts (m):

$$\text{direct counts } (m) = \left[\sum(0)(\#) + (1)(\#) + (2)(\#) \ldots\right] \Big/ \text{the total number of trials}$$

By direct counts of the evoked quantal events, estimates of n and p were obtained. In some cases, there were no evoked events that followed the nerve terminal spikes; such a failure in evoked release was counted as a zero. If only one single event occurred after the spike, it was counted as one; when double events occurred, they were referred to as two (Table 1). Quantal release over the time was monitored by examining the area of the evoked current (a measure of charge). The time of peak evoked events varied due to latency jitter, so that when multiple events occurred, the measurements of peak amplitude were not as reliable. The change in the charge measures before and after application of 5-HT (100 nM, Sigma) to the nerve terminals, and the frequency in occurence of charge of the evoked events were calculated. Additionally, the data sets were tested for a best-fit approximation based on assumptions discussed in earlier reports (Dudel and Kuffler, 1961; Cooper et al., 1995c). Binomial distributions represent the quantal nature of release in crayfish neuromuscular junctions (Wojtowicz et al., 1991). To test for non-uniform binomial distributions, the procedures described earlier were used (Wojtowicz et al., 1991). The chi-squared statistic (χ^2) and a modified Akaike information criterion (AIC) were used to estimate the distribution that best fits the observed distribution of events. A bootstrap method, as previously described (Cooper et al., 1995c), was employed to estimate the standard error of the estimated n and p values. The bootstrap method consisted of drawing 1000 random samples from the original data set based on an open scheme (Cooper et al., 1995c). Since exposure to 5-HT produced gradual changes in all of the quantal parameters, sample sets of data for every 200 events were used and were found to be sufficient to obtain statistically significant values for quantal predictions. The 1000 sampled grouped data and the 200 event-group sets were then compared (Table 1).

2.5. Immunofluorescence

Whole mount preparations of the isolated superficial flexor muscle were pinned to a Sylgard dish with the muscle in a stretched position. They were fixed with 2.5% (v/v) glutaraldehyde, 0.5% (v/v) formaldehyde dissolved in a PBS buffer (9.5 ml of $NaH_2PO_4 \cdot 2H_2O$ of 0.2 M stock, 40.5 ml $Na_2HPO_4 \cdot 7H_2O$ of 0.2 M stock, 4 g sucrose in a 100 ml volume) for 1 h with two changes of solution. The preparation was then placed into vials and washed in PBS buffer containing 0.2% (v/v) Triton X-100 and 1% (v/v) normal goat serum (Gibco/BRL, Grand Island, NY) for 1 h with three changes at room temperature. The tissue was then incubated with primary antibody to GABA (Sigma, 1:1000 in PBS buffer) on a shaker at 4°C for 12 h. The tissue was washed three times and incubated in secondary antibody (goat, anti-rabbit IgG conjugated with Texas Red, Sigma) diluted 1:200 with PBS buffer at room temperature for 2 h, followed by two washes in buffer. The synaptic locations were observed by immunocytochemistry as previously shown in nerve terminals (Cooper et al., 1995a; Msghina and Atwood, 1997; Cooper, 1998). Fluorescent images of the nerve terminals were viewed with a Leica DM IRBE inverted fluorescent microscope using a 63X (1.2 NA) water immersion objective with appropriate illumination. The composite images of Z-series were collected with a Leica TCS NT confocal microscope for illustration.

2.6. HPLC analysis

Quantification of 5-HT concentrations, both in the hemolymph and in the abdominal ganglia, was performed using high performance liquid chromatography with electrochemical detection (HPLC-ED). The animals were agitated and hemolymph was extracted using a chilled syringe and needle. For extraction of 5-HT from the ganglia, the VNC was quickly excised in cold crayfish saline and the T1–A1 as well as A2–A6 ganglia were removed and homogenized in 0.1 M perchloric acid. Both the hemolymph and homogenized ganglia were first deproteinated; the crude extract was then passed through a 0.22 μm filter (Ultra-Free, Millipore). The samples were injected into the HPLC system which consisted of an ESA pump (model 580), a manual injector (model 9125), and an HR-80 3 μm particle size column.

Table 1
Influence of 5-HT on quantal release parameters: mean quantal content (m), number of sites releasing (n), and probability of release at the sites (P)[a]

	Events	All Sweeps		1–200		201–400		401–600		601–800		801–1000	
		Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred
Prep. 1													
Saline	0	83	9	9	10	9	10	26	26	14	14	25	25
	1	904	904	189	187	186	186	173	174	184	184	172	177
	2	20	13	2	2	5	3	1	0	2	2	3	3
	m	0.93		0.97		0.98		0.87		0.94		0.89	
	n	2(0)		2 (0)		2 (0)		2 (0)		2 (0)		2 (0)	
	p	0.465(0.005)		0.483(0.008)		0.49(0.009)		0.438(0.012)		0.47(0.009)		0.445(0.124)	
5-HT	0	6	6	0	1	1	0	0	2	2	8	0	3
	1	335	335	66	66	61	15	67	67	65	66	76	76
	2	629	628	123	123	137	175	129	129	125	126	115	15
	3	29	31	10	10	1	10	2	2	10	0	6	6
	4	1	0	0	0	1	0	0	0	0	0	0	0
	m	1.68		1.71		1.71		1.65		1.72		1.62	
	n	4 (0)		3 (0)		4 (0)		3 (0)		3 (0)		3 (0)	
	p	0.421(0.004)		0.57 (0.013)		0.428 (0.009)		0.552 (0.012)		0.575 (0.01)		0.54 (0.013)	
Prep. 2													
Saline	0	181	181	23	23	38	38	76	78	44	44	0	0
	1	651	651	128	128	136	136	81	88	135	135	171	171
	2	167	167	48	45	26	26	43	40	21	21	29	29
	3	1	1	1	1	0	0	0	0	0	0	0	0
	m	0.34		0.5		0.26		0.43		0.21		0.29	
	n	3 (0)		3 (0)		2 (0)		2 (0)		2 (0)		2 (0)	
	p	0.329(0.006)		0.378(0.014)		0.47(0.002)		0.418(0.032)		0.443(0.02)		0.570(0.013)	
5-HT	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	3	0	0	0	0	0	0
	2	602	600	155	154	134	134	134	133	91	91	88	87
	3	357	339	40	42	63	63	58	60	98	98	98	84
	4	38	57	5	4	3	0	8	7	11	11	11	25
	5	2	4									2	3
	6	1	0									1	
	m	2.44		2.25		2.35		2.37		2.6		2.65	
	n	6 (0)		4 (0)		4 (0)		4 (0)		4 (0)		6 (0)	
	p	0.410 (0.0032)		0.562 (0.008)		0.592 (0.01)		0.592 (0.01)		0.65 (0.01)		0.442 (0.008)	

[a] A series of evoked events induced at 1 hz stimulation to the motor nerve before and after application of 5-HT (100 nM). The time series is divided into bins of 200 trials for assessment of quantal parameters: mean quantal content (m), the number of release sites (n), and the probability of release at the sites (p). The standard error of estimating n and p were obtained by a bootstrapping procedure (Cooper et al., 1995c) and are shown within the parentheses. The predicted (pred) distributions were obtained from fitting the observed (obs) distribution by a maximum likelihood estimation. Number of the discrete events indicated as: 0, failures; 1, ones; 2, twos; etc. are given.

Detection and quantifying were accomplished using a Coulochem detector (ESA model 5200A), an analytical cell (model 5011, channel 1 set to −50 mV and channel 2 set at 300 mV) and a guard cell (model 5020, set at 400 mV). The mobile phase consisted of 75 mM NaH_2PO_4, 1.5 mM SDS, 100 µl/l triethylamine, 15% acetonitrile, 12.5% methanol. Under these conditions, serotonin eluted at 12 minutes.

The average weight of the ganglia (T1–A1), 0.42 µg, was dissolved in 200 µl of perchloric acid. Of this amount, only 20 µl reaches the column. Considering the MW of 5-HT at 212.7 g/mol, for the 5-HT used in the calibration curves, this calculates to 94 nM 5-HT for the entire starting tissue.

3. Results

To record from the ventral surface of the superficial flexor muscles or from the superficial flexor motor nerve, the articular membrane of the segment was trimmed and removed to allow access to the entire ventral surface of the muscle (Fig. 1A). The connectives to adjoining ganglia and the 1st and 2nd roots were transected to determine their influence on the intrinsic activity measured from the superficial flexor motor nerve. Intracellular recordings from the medial fibers revealed the intrinsic activity associated with the motor neurons to the muscle.

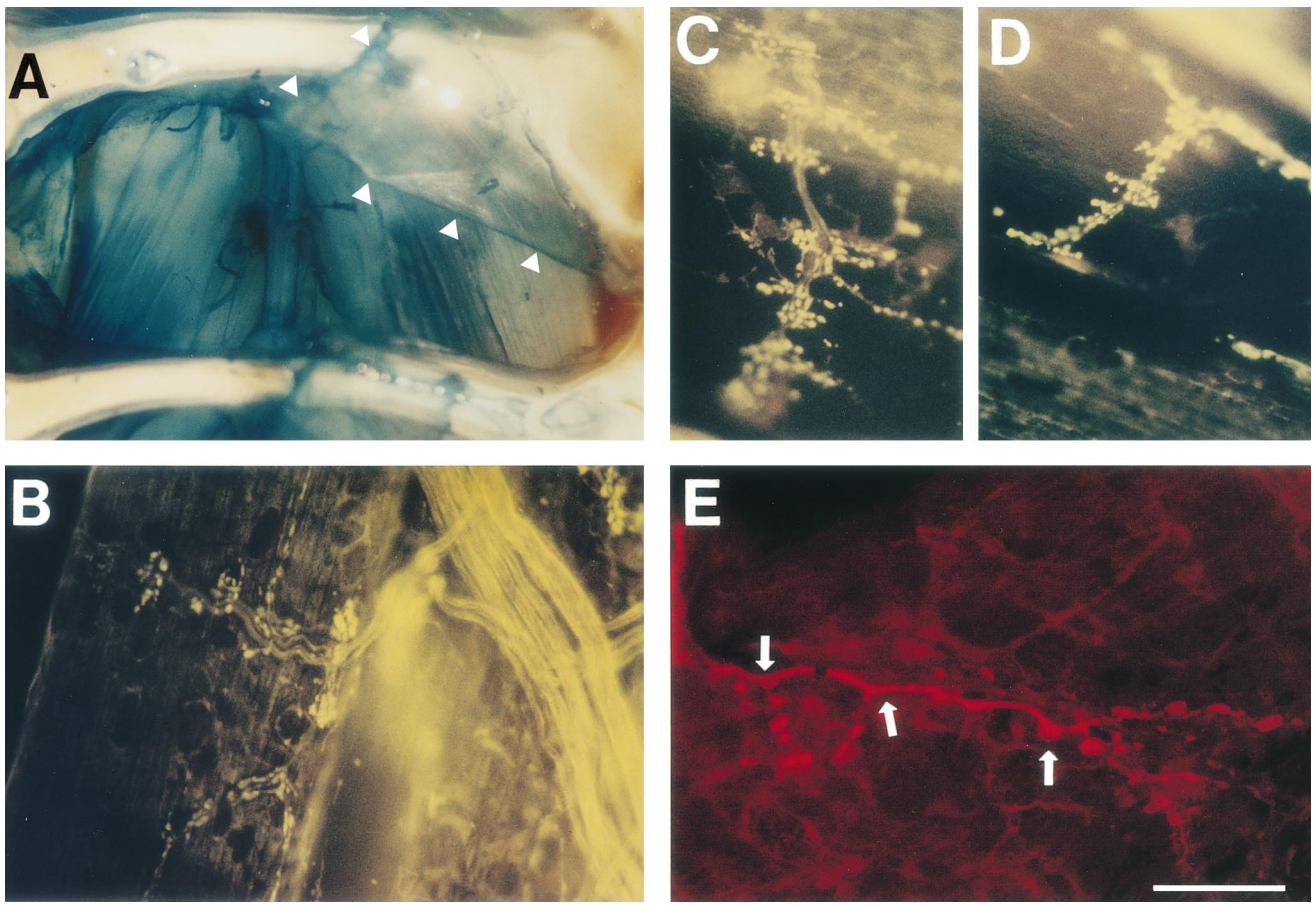

Fig. 1. (A) Ventral dissection of the 2nd abdominal segment stained with methylene blue. The articulating membrane (arrow heads) has been reflected so as to expose the slow superficial flexors (right) and the fast deep flexors are shown on the left. (B) The 3rd root with terminals and varicosities which have been stained with 4-Di-2-Asp. (C, D) Enlarged view of the clusters of varicosities on the muscle fiber surface of medial fibers. (E) GABA immunocytochemical analysis of the inhibitory axon terminal with varicosities. Scale bar: A, 3.6 cm; B–D, 135µm; E, 33 µm.

3.1. 5-HT increases firing frequency of the superficial flexor motor neurons

In each of the five preparations, application of 5-HT (100 nM) to the ventral nerve cord containing only segments A1–A3, with the 1st and 2nd roots severed, resulted in an increased firing frequency of the superficial flexor motor neurons as assessed by intracellular recordings from the medial superficial flexor muscle (Fig. 2A). The rate of change in the frequency of excitatory postsynaptic potentials (EPSPs) by exposure to 5-HT was determined by counting the number of events for 1 s every 10 s over the 10 min recording period. The change in firing frequency for a representative preparation is shown (Fig. 2B). Since there was substantial variation in the degree of basal activity among preparations, the mean frequency of the five preparations before and after exposure to 5-HT is shown to illustrate the range of activities. The mean frequency of 5-HT effects was determined after the maximum response occurred during the exposure to 5-HT (Fig. 2C). Preparations that had a high initial firing frequency showed a lower percent change as compared to preparations with a low basal rate (Fig. 2D). These rates are representative only of the activity on the individual fiber being monitored. Neighboring fibers exhibited lower or higher rates of basal activity depending on the number of excitatory motor neurons innervating the fiber.

The overall activity of the entire 3rd root was monitored after application of 5-HT by en passant recordings of the extracellular spikes (Fig. 3A). Variations in the rate of increasing activity amongst different preparations was also observed from the intracellular monitoring of EPSPs. A preparation which demonstrated a slow rise in activity while exposed to 5-HT is shown in Fig. 3B. The increase in firing frequency associated with the application of 5-HT was observed in five of the six preparations (Fig. 3C). The mean and standard error of the percentage change, 48.62 ± 15.16, clearly demonstrates an increase drive of the intrinsic spontaneous activity of these motor neurons. It became apparent that when sensory neurons were left intact, exposure to 5-HT resulted in a larger change to the frequency of the 3rd root motor neurons. Therefore, the influence of sensory neuron activity on the drive of the 3rd root, and the effects of 5-HT, were assessed.

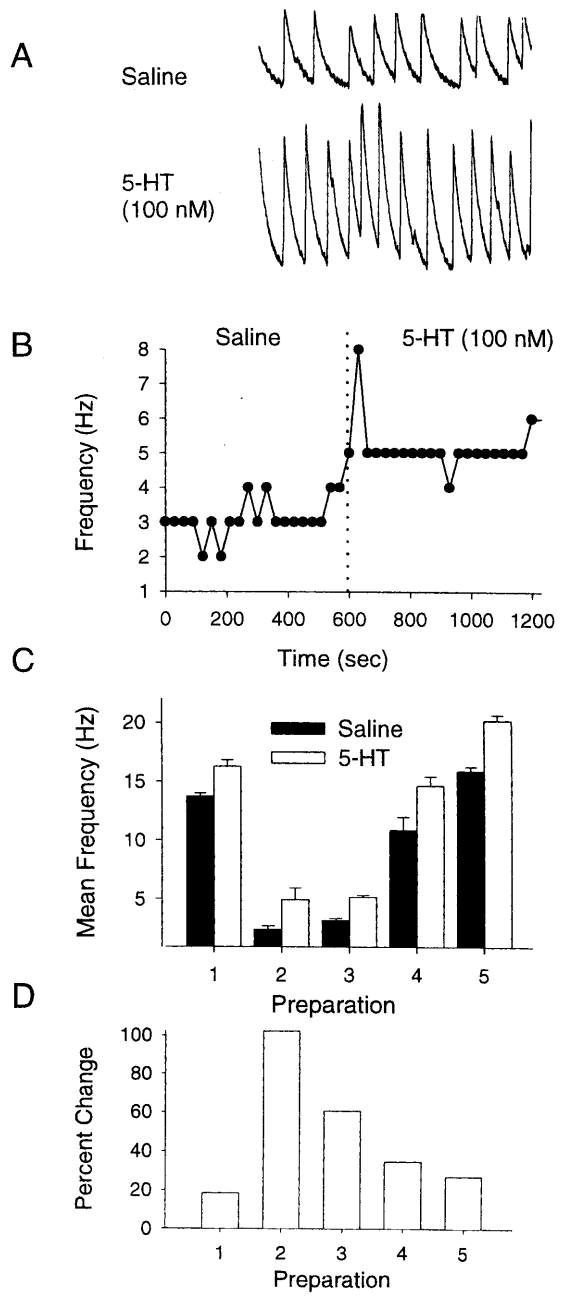

Fig. 2. (A) Effects of 5-HT on firing frequency of the motor neurons as recorded with an intracellular electrode within a medial muscle fiber to monitor excitatory postsynaptic potentials (EPSPs). (B), Representative preparation treated with 100 nM 5-HT which demonstrates the time frame of action and the persistence of 5-HT's effect in maintaining a higher frequency of EPSPs. 5-HT was added at the 600 s mark in the recording. (C) Mean frequency of EPSPs ($\pm$S.E.M.) in five animals with an intact CNS as well as the 1st and 2nd roots. (D) The percentage change for preparations in B are shown. Note the preparations that demonstrating the lowest initial activity increased the most upon exposure to 5-HT.

3.2. 5-HT enhances sensory drive of superficial flexor motor neurons

While recording from the third root, the lateral cuticle of the animal was stimulated as illustrated in Fig. 4. Stimulation of cuticular sensory neurons increased the firing frequency of the motor neurons (Fig. 5A). The basal activity monitored in the 3rd root, with sensory neurons intact, also showed a increase upon exposure to 5-HT. The heightened effects of 5-HT on sensory drive of the superficial motor neurons was observed in all preparations examined ($n = 12$). The mean values reported are an average of five subsequent trials in saline and in 5-HT. The saline bath was exchanged with one containing 100 nM of 5-HT and incubated in the dark for 5 min before additional stimulation. In order to bypass the activation of the sensory endings to address the effects of 5-HT on sensory neuron drive, standardized stimulation was applied with an electrode placed on a severed distal end of the second root while recording from the third root in saline and in 5-HT. Again, 5-HT increased the baseline activity ($n = 7$, Fig. 5B) even in the absence of altered sensory drive. With the stimulation paradigm, the associated increases in activity were quantified by taking the response 1 s before stimulation (50 Hz, 300 ms), and 1 s after stimulation (Fig. 5B). This enhancement of the stimulated-associated drive of spontaneous activity was observed in seven out of seven preparations (Wilcoxon rank sum test, $P < 0.02$, Fig. 5C). Because the increases in activity recorded from the third root could be the result of the 5-HT's effects at the primary sensory afferent neurons themselves or effects within the VNC, we recorded directly from the second root in the presence and absence of cuticular stimulation. Although we did not measure the latency for the effect of 5-HT on the integration within the VNC, the diffusion of 5-HT across the ganglion sheath to act on the connections within the neuropil would likely take longer then the direct actions on the more readily exposed sensory neurons.

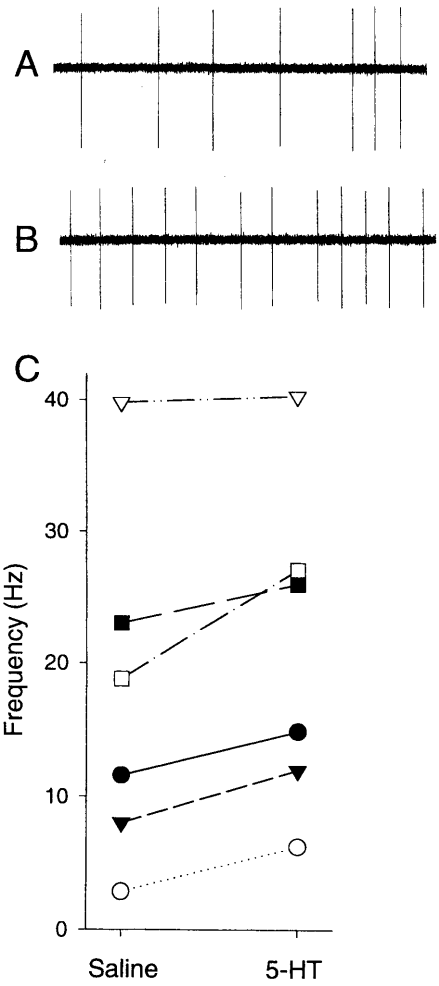

Fig. 3. Representative third root motor nerve activity as measured by the extracellular spikes before (A) and after (B) 5-HT (100 nM) exposure. (C) Mean frequency plot before and after application of 5-HT. Note the increase in frequency for each preparation ($n = 6$, $P < 0.05$, Wilcoxin rank sum test). The percentage changes among the preparations varied considerably.

The cuticular stimulation and direct second root stimulation, while monitoring the 3rd root activity, does not allow one to assess the role of 5-HT in altering the number of sensory neurons recruited or in altering a single neuron's ability to be activated or an effect of the sensory neuron's input within the VNC. Since the sensory axons are very small (1–10 μm) and difficult to monitor over time while changing the bathing medium, the entire distal 2nd root was monitored after it was transected from the ganglia. In the presence of 5-HT, there was an increase in the frequency of primary sensory neurons upon stimulation of the cuticle ($n = 6$, Fig. 6). This indicates that incoming information to the VNC is enhanced by 5-HT. For this paradigm, activity frequencies were quantified for 1s immediately before the simulation and for the first second of stimulation in both saline and 5-HT. In three of the six preparations there was an enhancement of sen-

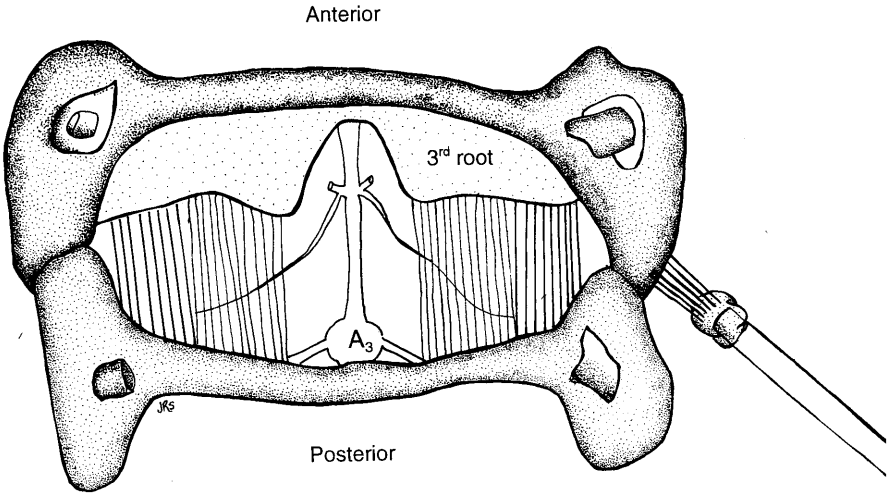

Fig. 4. Schematic of ventral view in a dissected second segment of the crayfish abdomen. The cuticular stimulation occurred along the lateral side of the segment. The mechanically stimulation was given at approximately 0.5 Hz (shown at top). The third ganglion (A_3) of the ventral nerve cord for the next caudal segment is shown.

sory neuron activity upon exposure to 5-HT without cuticular stimulation.

3.3. 5-HT alters synaptic transmission

5-HT is known to alter synaptic transmission by enhancing the EPSP amplitudes on a number of neuromuscular junctions in crayfish: opener muscle (Crider and Cooper 1999, 2000; Southard et al., 2000) leg extensor muscle (Shearer and Cooper, 2000) and abdominal extensors (Griffis et al., 1999). To assess the effects of 5-HT on synaptic transmission of the motor neuron terminals, the severed third root was stimulated at 1 Hz while recording from the superficial flexor. To ensure that only a single motor neuron was being recruited, a single sized postsynaptic event was monitored while the preparation was exposed to saline prior to the addition of 5-HT. Upon the addition of 5-HT, a rapid increase in the EPSP amplitude was observed (Fig. 7, $n = 5$, $P < 0.05$ Wilcoxon Rank sum test). However, the increase in EPSP amplitude could be due to several mechanisms. First, 5-HT could increase input resistance of the muscle which would increase the amplitude of the EPSP. 5-HT could also increase quantal release, which could also account for an increase in the EPSP amplitude. To determine the individual contributions of these mechanisms, both quantal release from the presynaptic terminal and input resistance of the postsynaptic muscle fiber were measured.

3.4. 5-HT increases quantal release

Presynaptic efficacy of synaptic transmission is readily assessed by counting the number of quantal events in evoked synaptic currents. 5-HT causes a direct effect on the presynaptic terminal by altering the number of evoked events. A focal macropatch electrode was used to record the synaptic currents before and after application of 5-HT (Fig. 8A, Table 1). To record from varicosities, the terminals were visualized by application of the vital dye (4-Di-2-ASP) and the lumen of the macropatch electrode was placed directly over a varicosity. The stained terminals on a medial muscle fiber along with five individual axons can easily be seen in Fig. 1B. Enlarged images of terminals illustrates the clusters of varicosities along the terminal (Fig. 1C, D). The distinct terminal morphology of varicosities along the terminal lengths is indicative of tonic motor nerve terminals as compared to phasic nerve terminals as has been described for other crustacean neuromuscular junctions (Bradacs et al., 1997). The inhibitory terminals can be visualized with immunocytochemistry (Fig. 1E). The arrangement of terminals and multiple varicosities within clusters hinders location of isolated varicosities uncontaminated by currents from neighboring varicosities.

In cases when multiple evoked events were obtained, discrete quantal events could not be quantified, although the entire synaptic event was still monitored and quantified by measuring the

charge (current x time) or the current area under the trace. We have demonstrated that 5-HT increases mean quantal content (m), the number of release sites (n), and the probability of release at the sites (p). To assess the rate of this modulator effect on the quantal parameters, every 200 and 1000 evoked events were grouped for quantal analysis (Table 1). To obtain estimates of the quantal parameters (n and p), each set of 1000 trials and the 200 trial subsets were examined and analyzed to determine the statistical distribution which would best describe its profile. To obtain the n and p values and the standard errors of the estimation, this analysis was followed by bootstrapping procedures. In addition, the standard errors (shown in parentheses in Table 1) for the estimations are relatively low, suggesting reasonable fits to the calculated quantal parameters. The results presented in Table 1, also indicate that applications of 100 nM 5-HT resulted in an increase in p and n during a 1 Hz stimulation. In the first preparation, the sites of release (i.e. n) generally increased from 2 sites to 3 or 4, where as in the second preparation, n increased from 2 or 3 to

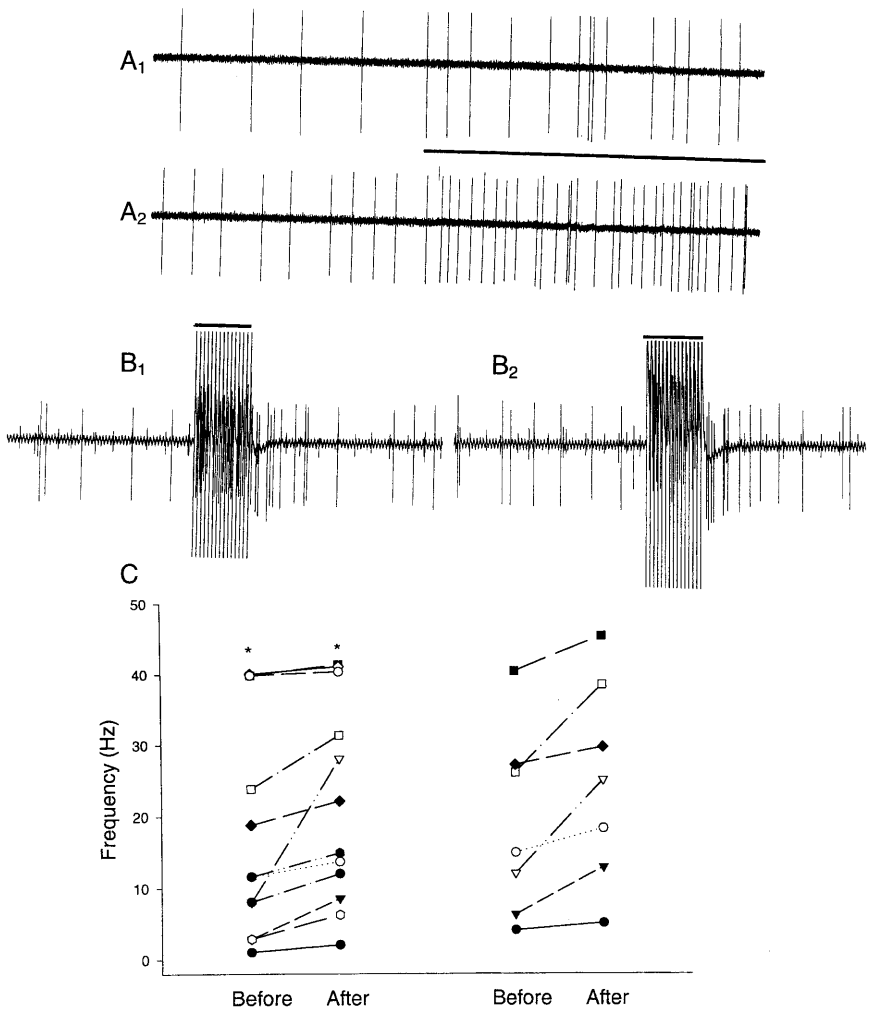

Fig. 5. Activity of the 3rd root before and during cuticular stimulation in saline (A_1) and in 100 nM 5-HT (A_2). The time during cuticle stimulation is indicated by the bar. Note the enhanced activity before and after stimulation when the preparation is bathed in 5-HT. Representative third root recording before and after electrical stimulation of the second root in saline (B_1) and during exposure to 100 nM 5-HT (B_2). (C) Mean frequencies are plotted for the 3rd root activity before and after electrical stimulation during saline and 5-HT exposure. The mean frequencies are also indicated for the activity resulting from the stimulations while exposed to saline or 5-HT. The asterisk (*) indicates the three preparations which demonstrate very similar firing frequencies.

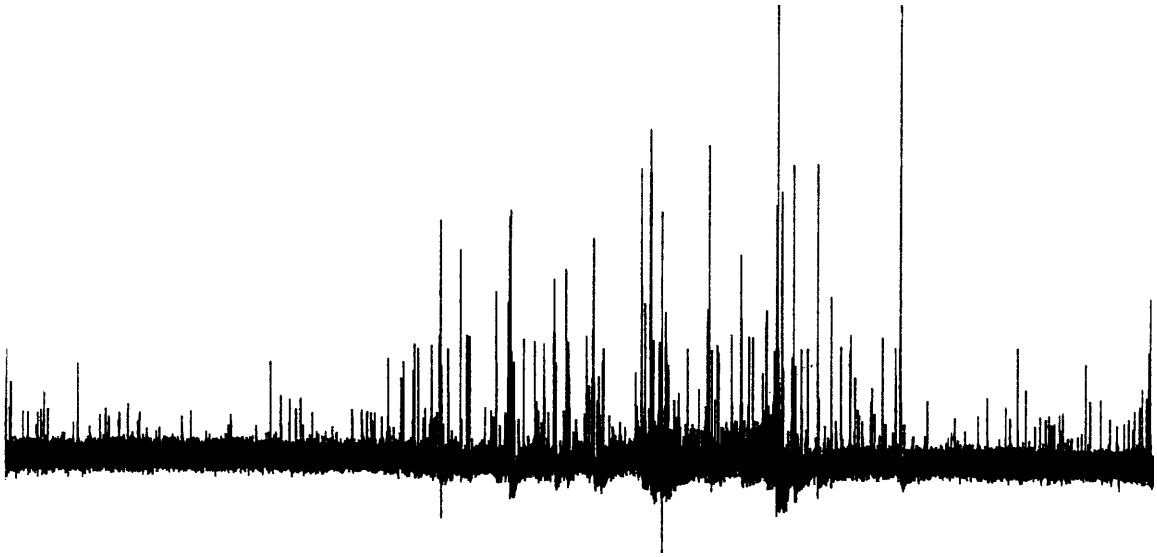

Fig. 6. (A) Recording from primary sensory afferents contained within the second root of the 2nd abdominal segment. Extracellular recording illustrates the activity before cuticular stimulation, but note the large increase in the number of different size units activated during the stimulation. (B) Plots of the mean activity before and during cuticular stimulation in six preparations while bathed in saline or 5-HT (100 nM) indicates that 5-HT enhances primary sensory activity when the neurons are stimulated. This is best observed in the overall frequency of activity during the stimulation in saline and 5-HT for the same preparations.

4 or larger. In both cases, the average probability of release at any one site (i.e. p) also increased, which is clearly evident in the subsets of 200 trials. When the data is grouped into the 1000 trials the p values are lower after 5-HT application because n has doubled. Overall, there is an increase in m in all cases after exposure of the terminals to 5-HT. The average of 1000 evoked events before and after the application of 5-HT show the overall effect on synaptic currents (Fig. 8B). To better examine the change, a scatter plot of the synaptic charge for one of the preparations is provided for a representative case in Fig. 8C. The larger currents are due to a greater number of events, indicating that 5-HT has a direct role in enhancing synaptic transmission by acting at the presynaptic terminal. This observation is supported by the direct quantal counts in Table 1. Note the increase in all the quantal parameters and also that the samples of every 200 events appear to be sufficient for obtaining statistically significant values for quantal predictions as well as a 1000 events. The larger EPSP amplitudes recorded with intracellular electrodes are not only due to an enhancement of vesicular release but also to a slight increase in input resistance of muscle fibers.

3.5. Input resistance is slightly increased

The effect of 5-HT on muscles input resistance caused a significant increase in the EPSP amplitude. The slight increase in the input resistance was observed in five out of five preparations (mean $\pm$ S.E.M: Saline, 29.02 ± 5.39; 5-HT, 38.48 ± 5.43, $P < 0.05$).

3.6. 5-HT quantification

High performance liquid chromatography with electrochemical detection was used to determine the concentration of 5-HT within the hemolymph and the ventral nerve cord. Since it has previously been reported that serotonergic neurons are localized in the thoracic ganglia and within the first abdominal ganglion (A1) for lobsters (Beltz and Kravitz, 1982), these six ganglia were removed for analysis. In two preparations we obtained 94 and 120 nM per combined ganglia. The additional abdominal ganglia (A2–A6) did not show any detectable 5-HT peaks. The hemolymph samples proved problematic since a very broad response was observed. Dilution of the sample, to eliminate the broad background, eliminated the peak at the time a standard of 5-HT would normally elute.

4. Discussion

Since the initial discovery of neuromodulators in crustaceans, an increasing number of neurohemoral modulators (buccalin, histamine, red pigment concentrating hormone, cholestokin, crustacean cardioactive peptide, and allatostatin) have been identified which regulate development, central pattern generators, and behavior (Christie et al., 1995; Kreissl et al., 1999). It has been suggested that the 5-HT and octopaminergic neurons may function as 'gain-setters' in altering the output of neuronal circuits (Ma et al., 1992; Schneider et al., 1996; Hörner et al., 1997). Much work remains to be done before we can fully understand the effects of neuromodulators on in-

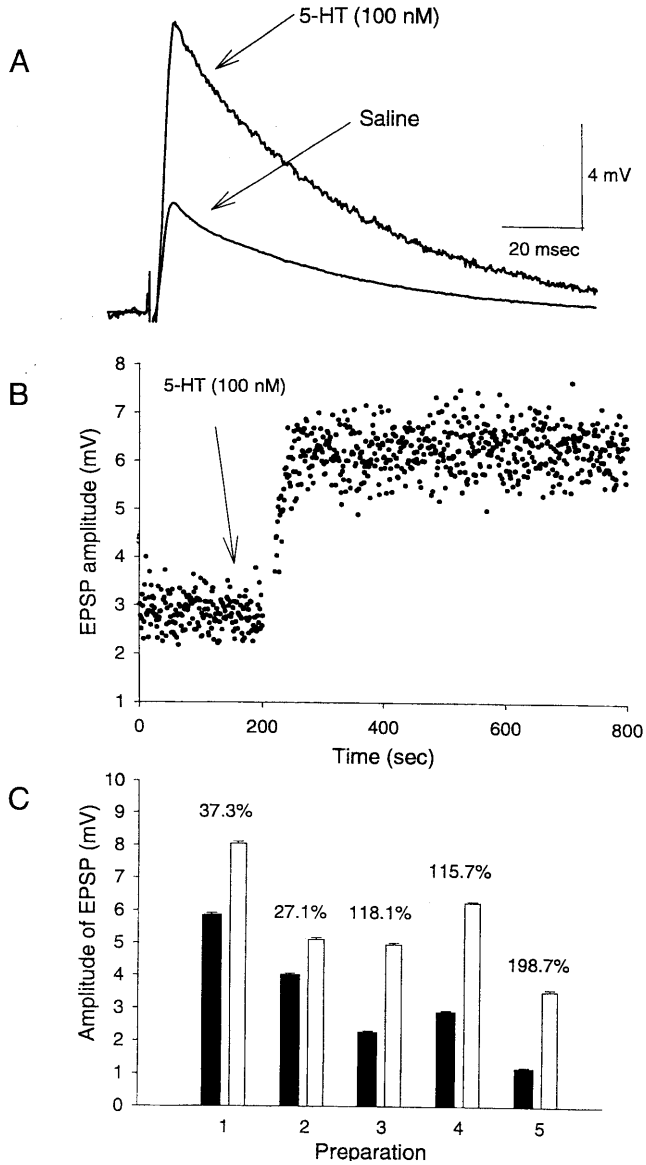

Fig. 7. (A) Average evoked post-synaptic potentials (EPSP) recorded in the superficial flexor muscle bathed in saline and after 5 min of exposure to 100 nM of 5-HT demonstrates the enhancement in the response. (B) A representative scatter plot of EPSP amplitudes evoked at 1 Hz stimulation rate before and during 5-HT exposure illustrates the rapid rise upon exposure to 5-HT. (C) Histograms showing the mean EPSP amplitudes before and after 5-HT treatment in each of five preparations (mean amplitudes $\pm$ S.E.M.). Note that there are very small deviations in the mean values within a preparation but significant differences among preparations. The percentage changes are indicated above each bar graph.

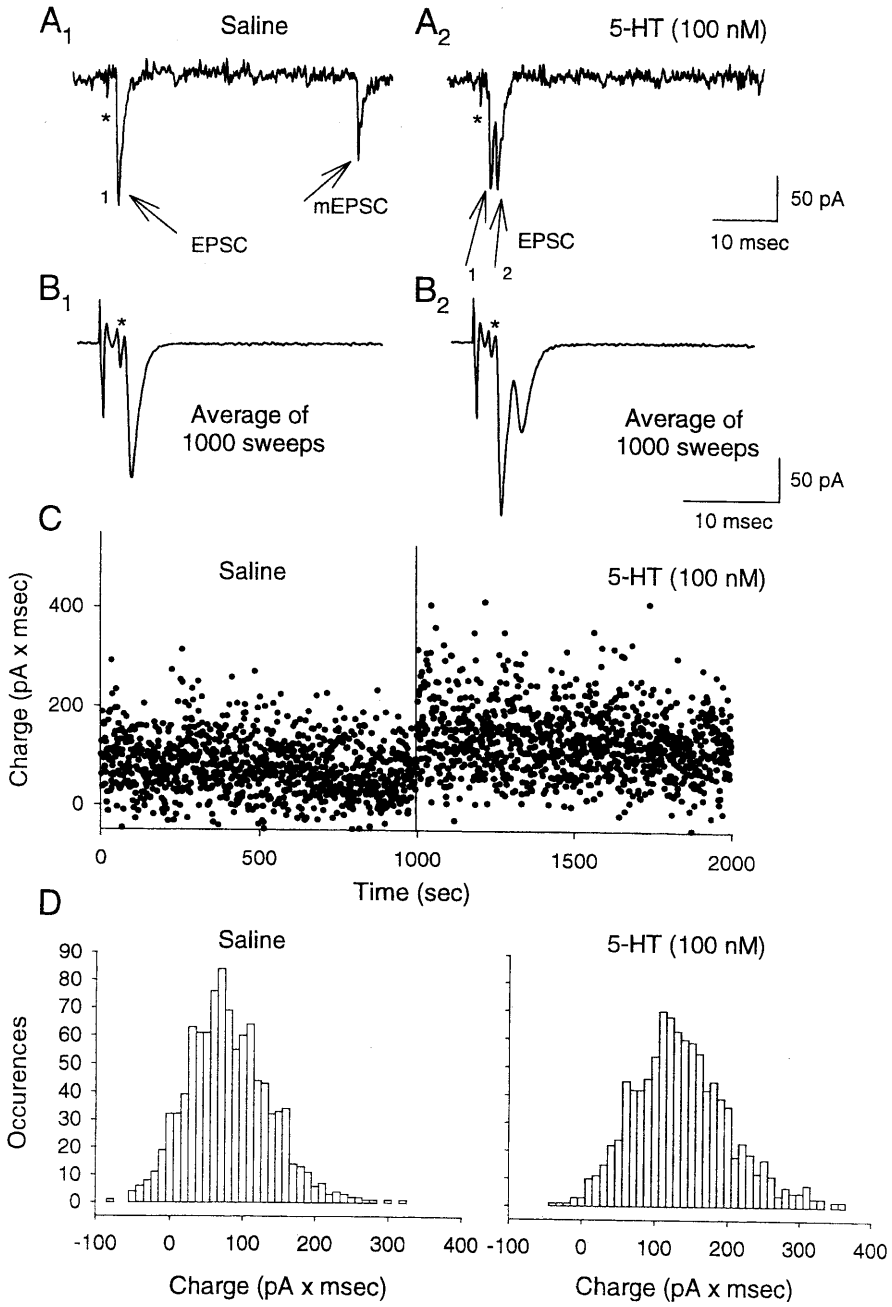

Fig. 8. Influence of 5-HT on synaptic currents as recorded with a focal macropatch electrode from a spatially isolated varicosity. Representative single traces in the presence of saline (A_1) and 100 nM 5-HT (A_2) are shown. Note in the evoked excitatory postsynaptic currents (EPSC's) individual quanta can be counted (arrows →). A miniature excitatory postsynaptic current (mEPSC) is shown in A_1. Also note that the asterisk (*) indicates the extracellular recording of an action potential (i.e. spike). Averaged responses for 1000 trials are shown during saline (B_1) and 5-HT exposure (B_2). (C) A representative scatter plot showing the influence of 5-HT on EPSC charge before and during the addition of 5-HT. (D) Histograms of the responses shown in the above scatter plot of charge (current × time) for saline and 5-HT treatment. Note the rightward shift in the histogram indicating larger evoked responses, likely due to the release of more vesicles as indicated with the direct quantal counting procedure.

dividual target cells. Given that different neuromodulators may work in concert with one another, analysis of their mixed action is an area for for future research. In addition, few studies, particularly in vertebrates, address the effects of neuromodulators on entire pathways which can regulate a specific behavior. In this study, we have used the well established crustacean superficial abdominal flexor preparation to examine the influence of both sensory input and 5-HT on the activity of the motor neurons.

Little is known about the direct effects of 5-HT application on crustacean neurons, with the exception of a few studies that have shown that 5-HT exerts its effects through an inositol triphosphate (IP_3) second-messenger system (Dixon and Atwood 1989a,b; Delaney et al., 1991). Few analyses to date have addressed the influence of known modulators directly at the sites of release from presynaptic neurons to quantify on a relative basis the sites of release (n) and the influence on the probability of vesicle release (p) at active synapses (Cooper and Ruffner, 1998; Crider and Cooper, 1999, 2000; He et al., 1999). The present work on the superficial flexor muscle and another recent study (Southard et al., 2000) support the hypothesis that silent synaptic sites are recruited in the presence of 5-HT, thus raising the quantal parameter, n. The probability of release at the initial sites of release also increase after application of 5-HT. Prior studies examining the effects of 5-HT have been accomplished by whole cell measures of muscle potentials arising from thousands of synapses (Grundfest and Reuben, 1961; Dudel, 1965; Kravitz et al., 1976; Wheal and Kerkut, 1976; Florey and Rathmayer 1978; Fisher and Florey, 1983). With the focal macropatch techniques, direct measurement of synaptic parameters in presynaptic motor nerve terminals that influence release can be implemented (Cooper et al., 1995b,c; Southard et al., 2000). In addition the direct actions of neuromodulators on sensory neurons, such as effects on transduction and spike frequency to the CNS, can be readily assessed in crayfish systems (Li et al., 1997).

A substantial amount of work on the neuromodulation of sensory-motor control has been conducted on the well-characterized serotoninergic sensory-motor circuit in *Aplysia*. This system has been shown to increase intracellular calcium levels in postsynaptic cells as well as effect a change in ionic conductances leading to spike broadening in rested synapses (Byrne and Kandel, 1996). Unlike crayfish, calcium entry is enhanced at the release sites in *Aplysia* presynaptic neurons in which facilitation is induced by 5-HT (Delaney et al., 1991; Eilot et al., 1993). From intracellular recordings in crayfish motor axons, there were no indications of spike broadening (Dixon and Atwood, 1985), although this may happen within the nerve terminals (Dudel, 1965). With the use of calcium sensitive indicators it was determined that 5-HT does not enhance an intracellular rise in calcium within the neuron (Delaney et al., 1991). Perhaps more sensitive temporal measures of calcium influx at active zones might better address this issue. In *Aplysia* sensory neurons, 5-HT can activate both PKA and PKC, which in part explains the temporal differences of the effects of a slow K^+ channel and an increase in Ca^{2+} influx (Braha et al., 1993). Pharmacological evidence indicates 5-HT_{1A} and 5-HT_2 receptors are present in the crayfish nervous system (Yeh et al., 1996). The use of a variety of receptor subtypes offers an advantage to the animal by utilizing alternate, intracellular biochemical pathways for modulating synaptic transmission.

The drive to maintain the intrinsic spontaneous neural activity of the superficial flexor motor neurons has received much attention, but without much understanding as to the exact nature of the location of the input from within the ventral nerve cord (Eckert, 1961; Kennedy and Takeda 1965a,b). Although it was shown by Eckert (1961) that the abdominal stretch receptors of the abdomen (MROs) were not contributing to the intrinsic activity of the superficial flexor motor neurons, Kennedy and Takeda (1965a) did report that 'natural' stimuli to the carapace increased activity of the nerve, but they were not informative to the location of the stimulus and the type of stimulus. No reports previous to this study have described the influence of neuromodulators in altering sensory drive of the motor neurons. Since the activity still remains in isolated segmental ganglia it is assumed that it arises within the ganglion and can be driven to higher firing rates by sensory axon stimulation as well as by application of 5-HT. In an isolated ventral nerve cord with several ganglia intact, a higher rate of activity is common as well as an increase in occurences in activity of all six axons within the 3rd root to these muscles. It is well known that the six motor axons innervate these muscles and that their cell bodies reside in the segmental ganglia for both lobsters (Harris-Warrick and Kravitz, 1984) and crayfish (Kennedy and Takeda 1965a,b).

Since it has been postulated that 5-HT plays a role in regulating the behavioral state of the crayfish, lobsters, and crabs (Livingstone et al., 1980; Sneddon et al., 2000), several attempts have been made to determine its concentration in the VNC, the hemolymph, and in isolated ganglia of lobsters (Livingstone et al., 1980; Harris-Warrick and Kravitz 1984; Fadool et al., 1988). However, there has been considerable variation in the recorded measurements. Harris-Warrick and Kravitz (1984) utilized HPLC-ED to measure 5-HT concentrations only within the first two abdominal ganglia. Their findings, that 5-HT is present at 90 ± 70 fmol/mg wet weight in the first two abdominal ganglia, suggest that 5-HT is available for release from the VNC. Our measurements of 5-HT in the VNC indicated a range of 94–120 nM per combined T1–A1 ganglia. In addition, we found it extremely difficult to measure 5-HT concentrations in the hemolymph due to broad signal arising from unknown contaminants in the samples. Upon dilution of the hemolymph, the background problems were reduced but no signal could be detected at the elution time expected for 5-HT. Our inability to measure 5-HT levels in the hemolymph of the crayfish is likely a common technical problem since reports are not available in the literature for crayfish.

As pointed out in Livingstone, et al. (1980), the excitatory direct effects of 5-HT on muscle could result in flexion of their abdomen. This seems feasible, since the flexor muscles are much larger than the extensors. If a mass action on the muscles and neural systems were equal, then flexion would be the postural state the animal would assume. Later work by Harris-Warrick and Kravitz (1984) showed that 5-HT also has some direct effects in regulating the activity of the motor neuron cell bodies associated with flexion and extension. They reported that the cell bodies of motor neurons to the flexors were more active while the extensors were inhibited, resulting in flexion as the preferred state when exposed to 5-HT. We have now shown that 5-HT even enhanced the sensory input that drives the flexor motor neurons. In addition, we have demonstrated that 5-HT has a direct effect on the presynaptic nerve terminals of the flexor motor neurons. Even if there was no 5-HT-induced increase in activity in the VNC and the spontaneous intrinsic activity to the superficial flexor muscles remained the same, the transmitter release would be enhanced due to 5-HT's action at the presynaptic motor nerve terminals, thus promoting flexion of the abdomen.

The state of abdominal flexion in crayfish does not appear to be the posture that dominant crayfish, within a pair, exhibit during the social interactions or while maintaining a dominant hierarchical status. Submissive crayfish will even tuck their abdomens under themselves as they cower to an aggressor. These behaviors have been readily observed in the field and in laboratory settings (Bovbjerg, 1953, 1956; Bruski and Dunham, 1987; Li et al., 2000; Listerman et al., 2000). Interestingly, the behavioral postures noted in lobsters (Livingstone et al., 1980) are reversed for 5-HT and octopamine injections in the Australian crayfish, *Cherax destructor* (McRae, 1996). Possibly, entirely different responses would be observed in the superficial flexor preparation in the Australian crayfish. In addition, since dominance is generally size related among crayfish, one would expect a very plastic response system for rapidly altered social conditions. For instance, a dominant male in one dyad would likely change quickly to being submissive in another dyad pairing with the arrival of a much larger opponent. This suggests rapid modulation within an individual's social state, and if such states can be hormonally altered, this may in part regulate the crayfish's 'fight or flight' response. Quantification of abdominal positions during interactive behaviors have not been reported in the literature. The behaviors readily observed for dominant crayfish are meral spread of the chelipeds and abdominal extension of the solitary animal as well as during an interaction with another crayfish. It remains to be quantified if abdominal flexion is actually a dominant posture that can be linked to 5-HT levels in the hemolymph.

Acknowledgements

Funding was provided by NSF grants IBN-9808631 (RLC), NSF-ILI-DUE 9850907 (RLC) and NSF-IBN 9423616 (WSN) as well as an undergraduate training fellowship from HHMI and an NSF-REU to (JRS). Appreciation is given to Mr Joseph Shearer for editorial comments.

References

Beltz, B.S., Kravitz, E.A., 1982. Mapping of serotonin-like immunoreactivity in the lobster nervous system. J. Neurosci. 3, 585–602.

Bruski, C.A., Dunham, D.W., 1987. The importance of vision in agonistic communication of the crayfish *Orconectes rusticus*, I. an analysis of bout dynamics. Behaviour 63, 83–107.

Bovbjerg, R.V., 1953. Dominance order in the crayfish *Orconectes virilis* (Hagen). Physiol. Zool. 26, 173–178.

Bovbjerg, R.V., 1956. Some factors affecting aggressive behavior in crayfish. Physiol. Zool. 29, 127–136.

Bradacs, H., Cooper, R.L., Msghina, M., Atwood, H.L., 1997. Differential physiology and morphology of phasic and tonic motor axons in a crayfish limb extensor muscle. J. Exp. Biol. 200, 677–691.

Braha, O., Edmonds, B., Sacktor, T., Kandel, E.R., Klein, M., 1993. The contributions of protein kinase A and protein Kinase C to the actions of 5-HT on the L-type Ca^{2+} current of sensory neurons in *Aplysia*. J. Neurosci. 13, 1839–1851.

Byrne, J.H., Kandel, E.R., 1996. Presynaptic facilitation revisited: state and time dependence. J. Neurosci. 16, 425–435.

Cases, O., Seif, I., Grimsby, J., Gaspar, P., Chen, K., Pournin, S., Muller, U., Aguet, M., Babinet, C., Shih, J.C., De Maeyer, E., 1995. Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. Science 268, 1763–1766.

Christie, A.E., Skiebe, P., Marder, E., 1995. Matrix of neuromodulators in neurosecretory structures of the crab *Cancer borealis*. J. Exp. Biol. 198, 2431–2439.

Coccaro, E.F., 1995. Impulsive aggression and central serotonergic system function in humans: an example of a dimensional brain–behavior relationship. Int. Clin. Psychopharmacol. 7, 3–12.

Cooper, R.L., Hampson, D., Atwood, H.L., 1995a. Synaptotagmin-like expression in the motor nerve terminals of crayfish. Brain Res. 703, 214–216.

Cooper, R.L., Marin, L., Atwood, H.L., 1995b. Synaptic differentiation of a single motor neuron: conjoint definition of transmitter release, presynaptic calcium signals, and ultrastructure. J. Neurosci. 15, 4209–4222.

Cooper, R.L., Stewart, B.A., Wojtowicz, J.M., Wang, S., Atwood, H.L., 1995c. Quantal measurement and analysis methods compared for crayfish and *Drosophila* neuromuscular junctions and rat hippocampus. J. Neurosci. Meth. 61, 67–79.

Cooper, R.L., Harrington, C.C., Marin, L., Atwood, H.L., 1996a. Quantal release at visualized terminals of a crayfish motor axon: intraterminal and regional differences. J. Comp. Neurol. 375, 583–600.

Cooper, R.L., Winslow, J., Govind, C.K., Atwood, H.L., 1996b. Synaptic structural complexity as a factor enhancing probability of calcium-mediated transmitter release. J. Neurophysiol. 75, 2451–2466.

Cooper, R.L., 1998. Development of sensory processes during limb regeneration in adult crayfish. J. Exp. Biol. 201, 1745–1752.

Cooper, R.L., Ruffner, M.E., 1998. Depression of synaptic efficacy at intermolt in crayfish neuromuscular junctions by 20-Hydroxyecdysone, a molting hormone. J. Neurophysiol. 79, 1931–1941.

Cooper, R.L., Warren, W.M., Ashby, H.E., 1998. Activity of phasic motor neurons partially transforms the neuronal and muscle phenotype to a tonic-like state. Muscle Nerve 21, 921–931.

Crider, M.E., Cooper, R.L., 1999. The importance of the stimulation paradigm in determining facilitation and effects of neuromodulation. Brain Res. 842, 324–331.

Crider, M.E., Cooper, R.L., 2000. Differentially facilitation of high- and low-output nerve terminals from a single motor neuron. J. Appl. Physiol. 88, 987–996.

Delaney, K., Tank, D.W., Zucker, R.S., 1991. Presynaptic calcium and serotonin-mediated enhancement of transmitter release at crayfish neuromuscular junction. J. Neurosci. 11, 2631–2643.

del Castillo, J., Katz, B., 1954. Quantal components of the end-plate potential. J. Physiol. 124, 560–573.

Dixon, D., Atwood, H.L., 1985. Crayfish motor nerve terminal's response to serotonin examined by intracellular microelectrode. J. Neurobiol. 16, 409–424.

Dixon, D., Atwood, H.L., 1989a. Adenyate cyclase system is essential for long-term facilitation at the crayfish neuromuscular junction. J. Neurosci. 9, 4246–4252.

Dixon, D., Atwood, H.L., 1989b. Conjoint action of phosphoinositol and adenylate cyclase systems in serotonin-induced facilitation at the crayfish neuromuscular junction. J. Neurophysiol. 62, 1251–1259.

Dudel, J., 1965. Potential changes in the crayfish motor nerve terminal during repetitive stimulation. Pfluegers Arch. 282, 323–337.

Dudel, J., Kuffler, S.W., 1961. The quantal nature of transmission and spontaneous miniature potentials at the crayfish neuromuscular junction. J. Physiol. 155, 514–529.

Eckert, R.O., 1961. Reflex relationships of the abdominal stretch receptors of the crayfish. J. Cell. Comp. Physiol. 57, 149–162.

Eilot, L.S., Kandel, E.R., Siegelbaum, S.A., Blumenfeld, H., 1993. Imaging terminals of Aplysia sensory neurons demonstrates role of enhanced Ca^{2+} influx in presynaptic facilitation. Nature 361, 634–637.

Fadool, D.A., Cobb, S.J., Kass-Simon, G., Brown, P.R., 1988. Liquid chromatographic procedures for the analysis of compounds in the serotonergic and octopamine pathways of lobster hemolymph. J. Chromatogr. 452, 491–501.

Florey, E., Rathmayer, M., 1978. The effects of octopamine and other amines on the heart and on the neuromuscular transmission in decapod crustaceans: further evidence for a role as a neurohormone. J. Comp. Biochem. Physiol. 61C, 229–237.

Fisher, L., Florey, E., 1983. Modulation of synaptic transmission and excitation-contraction coupling in the opener muscle of the crayfish, *Astacus leptodactylus*, by 5-hydroxytryptamine and octopamine. J. Exp. Biol. 102, 187–198.

Glusman, S., Kravitz, E.A., 1982. The action of serotonin on the excitatory nerve terminals in lobster nerve-muscle preparations. J. Physiol. 325, 223–241.

Griffis, B., Bonner, P.H., Cooper, R.L., 1999. Increased sensitivity of transformed (phasic to tonic-like) motoneurons to the neuromodulator 5-HT. Abst. Soc. Neurosci. 25, 792.13.

Grundfest, H., Reuben, J.P., 1961. Neuromuscular synaptic activity in lobster. In: Florey, E. (Ed.), Nervous Inhibition. Pergamon Press, Oxford, pp. 92–104.

Guillemin, R., 1978. Peptides in the brain. Science 202, 390–402.

He, P., Southard, R.C., Whiteheart, S.W., Cooper, R.L., 1999. Role of α-SNAP in promoting efficient neurotransmission at the crayfish neuromuscular junction. J. Neurophysiol. 82, 3406–3416.

Hörner, M., Weiger, W.A., Edwards, D.H., Kravitz, E.A., 1997. Excitation of identified serotonergic neurons by escape command neurons in lobsters. J. Exp. Biol. 200, 2017–2033.

Harris-Warrick, R.M., Kravitz, E.A., 1984. Cellular mechanisms for modulation of posture by octopamine and serotonin in the lobster. J. Neurosci. 4, 1976–1993.

Huber, R., Delago, A., 1998. Serotonin alters decisions to withdraw in fighting crayfish, *Astacus astacus*: the motivational concept revisited. J. Comp. Physiol. 182, 573–583.

Huber, R., Orzeszyna, M., Pokorny, N., Kravitz, E.A., 1997. Biogenic amines and aggression: experimental approaches in crustaceans. Brain Behav. Evol. 50, 60–68.

Jan, L.Y., Jan, Y.N., 1982. Peptidergic neurotransmission in the sympathetic ganglion of the frog. J. Physiol. 327, 219–246.

Kennedy, D., Takeda, K., 1965a. Reflex control of abdominal flexor muscles in the crayfish: the twitch system. J. Exp. Biol. 43, 211–227.

Kennedy, D., Takeda, K., 1965b. Reflex control of the abdominal flexor in the crayfish: the tonic system. J. Exp. Biol. 43, 229–246.

Kravitz, E.A., Batelle, B.A., Evans, P.D., Talamo, B.R., Wallace, B.G., 1976. Octopamine neurons in lobsters. Neurosci. Symp. 1, 67–81.

Kupfermann, I., 1979. Moduatory actions of neurotransmitters. Ann. Rev. Neurosci. 2, 447–465.

Kreissl, S., Weiss, T., Djokaj, S., Balezina, O., Rathmayer, W., 1999. Allatostatin modulates skeletal muscle performance in crustaceans through pre- and postsynaptic effects. Eur. J. Neurosci. 11, 2519–2530.

LaFramboise, W.A., Griffis, B., Bonner, P., Warren, W., Scalise, D., Guthrie, R.D., Cooper, R.L., 2000. Muscle type-specific myosin isoforms in crustacean muscles. J. Exp. Zool. 286, 36–48.

Li, H., Ward, E., Bradacs, H., Cooper, R.L., 1997. Neuromodulator effects on primary sensory neurons: rapidly and slowly adapting proprioceptors. Abst. Soc. Neurosci. 23, 313.8.

Li, H., Listerman, L.R., Doshi, D., Cooper, R.L., 2000. Heart rate measures in blind cave crayfish during environmental disturbances and social interactions. Comp. Biochem. Physiol. 127A, 55–70.

Linnoilia, V.M., Virkkunen, M., 1982. Aggression, suicidality, and serotonin. J. Clin. Psychiatry 53, 46–51.

Listerman, L., Deskins, J., Bradacs, H., Cooper, R.L., 2000. Measures of heart rate during social interactions in crayfish and effects of 5-HT. Comp. Biochem. Physiol. A 125, 251–263.

Livingstone, M.S., Harris-Warrick, R.M., Kravitz, E.A., 1980. Serotonin and octopamine produce opposite postures in lobsters. Science 208, 76–79.

Ma, P.M., Beltz, B.S., Kravitz, E.A., 1992. Serotonin-containing neurons in lobsters: their role as 'gain-setters' in postural control mechanisms. J. Neurophysiol. 68, 36–54.

McRae, T., 1996. On the postural effects induced in female *Cherax destructor* (Clark) by serotonin and octopamine. Freshwater Crayfish 11, 293–298.

Msghina, M., Atwood, H.L., 1997. Distribution and morphology of inhibitory innervation in crayfish (*Procambarus clarkii*) limb and abdominal muscles. Cell. Tissue Res. 290, 111–118.

O'Shea, M., Schaffer, M., 1985. Neuropeptide function: the invertebrate contribution. Ann. Rev. Neurosci. 8, 171–198.

Schneider, H., Budhiraja, P., Walter, I., Beltz, B.S., Peckol, E., Kravitz, E.A., 1996. Developmental expression of the octopamine phenotype in lobsters. J. Comp. Neurol. 371, 3–14.

Shearer, J., Cooper, R.L., 2000. The differential effects of 5-HT on tonic and phasic motor nerve terminals. Am. Zool. 39, 244A.

Siegelbaum, S.A., Camardo, J.S., Kandel, E.R., 1982. Serotonin and cyclic AMP close single K^+ channels in *Aplysia* sensory neurons. Nature 299, 413–417.

Sneddon, L.U., Taylor, A.C., Huntingford, F.A., Watson, D.G., 2000. Agonistic behavior and biogenic amines in shore crabs *Carcinus maenas*. J. exp. Biol. 203, 537–545.

Sohn, J., Mykles, D.L., Cooper, R.L., 2000. The anatomical, physiological and biochemical characterization of muscles associated with the articulating membrane in the dorsal surface of the crayfish abdomen. J. Exp. Zool. 287, 353–377.

Southard, R.C., Haggard, J., Crider, M.E., Whiteheart, S.W., Cooper, R.L., 2000. Influence of serotonin on the kinetics of vesicular release. Brain Res. 871, 16–28.

Strawn, J.R., Bonner, P.H., Cooper, R.L., 1999. Motor command and synaptic transmission: roles of CNS, sensory systems, and neuromodulation. Abst. Soc. Neurosci. 25, 792.12.

Strawn, J.R., Neckameyer, W.S., Cooper, R.L., 2000. The effects of 5-HT on sensory neurons, CNS command, and neuromuscular junctions of the crayfish abdominal superficial flexor. Am. Zool. 39, 245A.

Wheal, H.V., Kerkut, G.A., 1976. The pre-and post-synaptic actions of 5-HT in Crustacea. Comp. Biochem. Physiol. 54C, 67–70.

Winberg, S., Nisson, G.E., Olsen, K., 1992. Changes in brain serotonergic activity during hierarchic behavior in Arctic Charr (*Salvelinus alpinus* L.) are socially induced. J. Comp. Physiol. 170A, 93–100.

Wojtowicz, J.M., Smith, B.R., Atwood, H.L., 1991. Activity-dependent recruitment of silent synapses. Ann. NY Acad. Sci. 627, 169–179.

Yeh, S.-R., Fricke, R.A., Edwards, D.H., 1996. The effect of social experience on serotonergic modulation of the escape circuit of crayfish. Science 271, 366–369.