THE JOURNAL OF COMPARATIVE NEUROLOGY 473:526–537 (2004)

A Crustacean Serotonin Receptor: Cloning and Distribution in the Thoracic Ganglia of Crayfish and Freshwater Prawn

MARÍA A. SOSA,[1,2*,†] NADJA SPITZER,[3,†] DONALD H. EDWARDS,[4] AND DEBORAH J. BARO[1,3,4]

[1]Institute of Neurobiology, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico 00901

[2]Department of Anatomy, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico 00936

[3]Department of Biology, Georgia State University, Atlanta, Georgia 30303

[4]Department of Biochemistry, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico 00936

ABSTRACT

Serotonin (5-HT) is involved in regulating important aspects of behavior and a variety of systemic physiological functions in both vertebrates and invertebrates. These functions are mediated through binding to 5-HT receptors, of which approximately 13 have been characterized in mammals. In crustaceans, important model systems for the study of the neural basis of behaviors, 5-HT is also linked with higher-order behaviors, associated with different 5-HT receptors that have been identified at the physiological and pharmacological levels. However, no crustacean 5-HT receptors have been identified at the molecular level. We have cloned a putative 5-HT_1 receptor (5-HT_{1crust}) from crayfish, prawn, and spiny lobster and have raised antibodies that recognize this protein in all three organisms. 5-HT_{1crust} immunoreactivity (5-HT_{1crust}ir) was observed surrounding the somata of specific groups of neurons and as punctate staining within the neuropil in all thoracic ganglia of crayfish and prawn. In the crayfish, 5-HT_{1crust}ir was also found in boutons surrounding the first and second nerves of each ganglion and on the 5-HT cells of T1–4. In the prawn, 5-HT_{1crust}ir was also found in axons that project across the ganglia and along the connectives. We found examples of colocalization of 5-HT_{1crust} with 5-HT, consistent with the short-term modulatory role of 5-HT, as well as cases of serotonergic staining in the absence of a 5-HT_{1crust} signal, which might imply that other 5-HT receptors are found at these locations. We also observed receptors that did not possess counterpart 5-HT staining, suggesting that these may also mediate long-term neurohormonal functions of serotonin. J. Comp. Neurol. 473:526–537, 2004.

Indexing terms: ***Macrobrachium rosenbergii; Panulirus interruptus; Procambarus clarkii*; serotonergic systems; 5-HT type 1 receptor; arthropod**

Grant sponsor: National Institutes of Health; Grant numbers: MH4819009, MBRS S06GM008224, RCMI G12RR03051, NCRR1S0RR13705, MH 62167, NS38770. Grant sponsor: National Science Foundation; Grant number: IBN9904017 and Puerto Rico Experimental Program to Stimulate Competitive Research. Grant sponsor: Natural Sciences and Engineering Research Council of Canada; Grant number: PGSA 253403-2002.

†M.A. Sosa and N. Spitzer contributed equally to this work.

*Correspondence to: María A. Sosa, Institute of Neurobiology, University of Puerto Rico, 201 Calle Norzagaray, San Juan, PR 00901. E-mail: masosa@neurobio.upr.clu.edu

Received 22 October 2003; Revised 19 December 2003; Accepted 6 January 2004

DOI 10.1002/cne.20092

Published online in Wiley InterScience (www.interscience.wiley.com).

The biogenic amine serotonin (5-hydroxytryptamine, 5-HT) is involved in regulating important aspects of behavior and a variety of systemic physiological functions. In the human central nervous system, 5-HT neurons in the brainstem, with projections widely distributed to the brain and spinal cord, are associated with the regulation of attention and other higher cognitive functions, including control of arousal and mood, satiety, and sexual behavior (Bethea et al., 2002). The diversity of functions associated with 5-HT are mediated through binding to a variety of 5-HT receptors. Approximately 13 mammalian receptors have been characterized and are grouped into seven classes on the basis of conserved structure and signaling mechanisms. Six classes (5-HT_1, 5-HT_2, 5-HT_4, 5-HT_5, 5-HT_6, 5-HT_7) are G-protein-coupled receptors (GPCR) and one class (5-HT_3) is a ligand-gated ion channel (reviewed in Kroeze et al., 2002).

In crustaceans, 5-HT is also linked with discrete circuits that control movements of the foregut (Ayali and Harris-Warrick, 1999), escape behavior (Yeh et al., 1996, 1997; Edwards et al., 1999), locomotion (Pearlstein et al., 1998), and posture (Harris-Warrick and Kravitz, 1984; Ma et al., 1992), as well as with higher-order behaviors such as aggression (Kravitz, 2000; Huber et al., 2001; Sosa and Baro, 1999, 2002). However, the behavioral findings are not as clear-cut with regard to the role of 5-HT in lobster aggression. Doernberg et al. (2001) showed that juveniles with reduced 5-HT increased the time spent fighting, which is in contrast to earlier postulations that increased 5-HT leads to increased aggression (Livingstone et al., 1980; Kravitz, 1988, 2000). In addition, Panksepp and Huber (2002) drug-ablated 5-HT neurons within the ventral nerve cord (except for the higher brain) and demonstrated that this did not elicit any quantifiable difference in aggressive behavior between crayfish. It may be that effector systems vary at different developmental stages and in different tissues. Indeed, receptors seem to vary even depending on the animal's social experience (Yeh et al., 1996). Thus, an understanding of the effectors that are present under the different conditions may be required to interpret these data.

Many of the aforementioned behaviors are associated with different 5-HT receptors that have been identified in crustaceans at the physiological and pharmacological levels (Zhang and Harris-Warrick, 1994; Yeh et al., 1997; Teshiba et al., 2001). However, no crustacean 5-HT receptors have been identified at the molecular level. In other invertebrates, counterparts of the mammalian 5-HT receptors have been cloned, but to date only three classes have been isolated corresponding to the mammalian classes 5-HT_1, 5-HT_2, and 5-HT_7 (reviewed in Tierney, 2001). For a given class, both the structure and signaling mechanisms are conserved across vertebrate/invertebrate lines. Thus, invertebrate 5-HT_1 receptor sequences are most homologous to the mammalian 5-HT_1 receptor class, and both vertebrate and invertebrate 5-HT_1 receptors couple to G_i/G_o proteins to mediate the classical inhibition of cAMP formation, as well as stimulation of phospholipase C (Saudou et al., 1992; Saudou and Hen, 1994; Tierney, 2001; Kroeze et al., 2002).

It is well documented that the serotonergic system is highly conserved among arthropods (Thompson et al., 1994; Beltz, 1999; Monastirioti, 1999; Harzsch, 2003). An important question is whether the conservation of the serotonergic system extends to effector molecules. As a first step toward a molecular appreciation of crustacean 5-HT receptors and their conservation across species, we have cloned a putative 5-HT_1 receptor from three different decapods (crayfish, prawns, and spiny lobsters). This putative receptor is referred to as 5-HT_{1crust}. We then raised antibodies that recognize this protein in all three organisms and used the antibody to determine the relationship between serotonergic processes and receptors in the thoracic ganglia, where the number of serotonergic neurons is small and the characteristic morphology of neurites is well defined (Beltz and Kravitz, 1983; Real and Czternasty, 1990; Harzsch and Waloszek, 2000). Here we show that this relationship can take a variety of forms, including close apposition between two profiles and receptors that appear far removed from 5-HT immunoreactive profiles.

MATERIALS AND METHODS

Experimental animals

Crayfish (*Procambarus clarkii*) measuring 3–4 cm in length were obtained from Atchafalaya Biological Supply (Raceland, LA). Animals were kept communally in 20-L tanks with continual filtration and aeration. Prawns (*Macrobrachium rosenbergii*) measuring 8–15 cm in length, from eyestalks to telson, were obtained from the Experimental Aquaculture Station of the University of Puerto Rico or from the Kentucky State University Aquaculture Program. They were kept in 30-gallon tanks whose water was continuously filtered and aerated (three prawns per tank, separated from one another with porous plastic dividers), or in a Z-plex stand-alone system (Aquatic Habitats, Apopka, FL), maintained at 26–28°C. Pacific spiny lobsters (*Panulirus interruptus*) were obtained from Don Tomlinson Commercial Fishing (San Diego, CA). Lobsters were maintained at 16°C in constantly aerated and filtered seawater. All animals were anesthetized by cooling on ice prior to experiments.

Cloning 5-HT_{1crust}

RNA was extracted from the nervous systems of all three species as previously described (Baro et al., 1994). First-strand cDNA was produced in a standard 20-μl reaction using Superscript II (Life Technologies, Gaithersburg, MD) and the reaction buffer and the directions provided with the enzyme: 1× first-strand buffer, 1–5 μg total RNA, 250 ng random hexamer, 500 μM dNTP, 10 mM DTT. Amino acid sequences from 5-HT type 1 receptors cloned previously in other species were aligned and conserved regions were chosen as templates for the synthesis of degenerate primers. A standard PCR was performed using 5 μl of first-strand cDNA and the following degenerate primers:

Forward: 5′ATICCIBYIGTYMGITAYTGGGC3′
Reverse: 5′CCRAAIARIATICKYTTRAAIGC3′

A 100-μl nested PCR was performed using 5 μl of the first PCR, the original forward primer, and the following nested reverse primer: 5′RTTIRMRTAICCIARCCANARRAA3′. The resulting PCR product was gel-isolated and cloned as previously described (Baro et al., 1994) and sequenced (Cornell BioResource Center, Ithaca, NY).

RACE to obtain 5-HT_{1Pan} termini

Panulirus mRNA was purified from nervous system total RNA with oligotex (Qiagen, Chatsworth, CA). 5′ and 3′

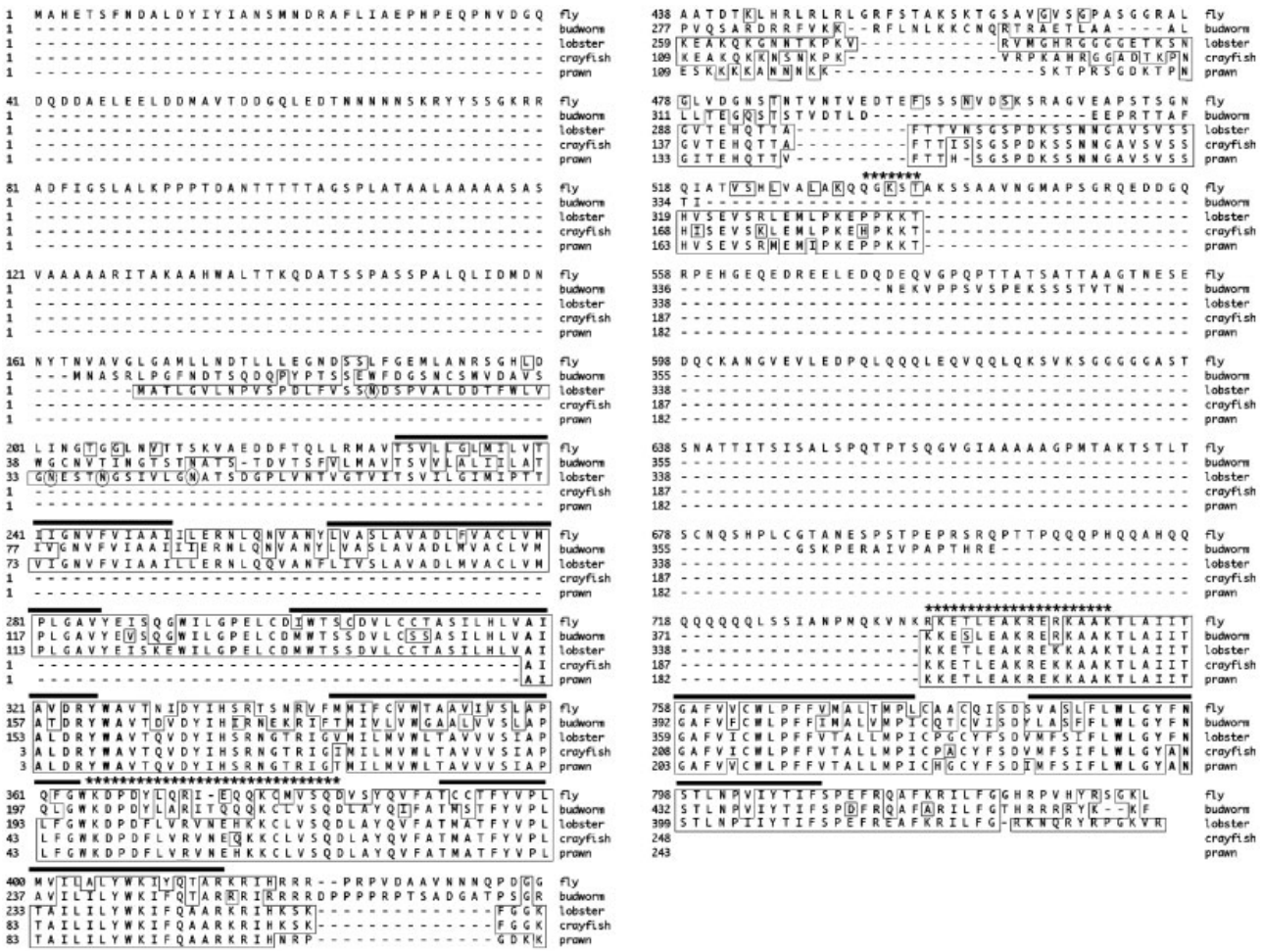

Fig. 1. Alignment of 5-HT$_{1crust}$. The amino acid sequences of 5-HT$_{1crust}$ are aligned with homologs in fly and budworm. Amino acids matching 5-HT$_{1Pan}$ are boxed. The degenerate primers used in cloning are shown in bold. Heavy lines above the sequence indicate transmembrane regions 1–7. Notice that not all transmembrane regions are represented in the crayfish and prawn clones, as they represent only partial cDNA sequences. Asterisks indicate the peptide sequences used for antibody production. The four putative N-linked glycosylation sites in the amino terminus of 5-HT$_{1Pan}$ are circled. Accession numbers are: prawn, AY528821; crayfish, AY528822; lobster, AY528823; budworm, CAA64863; fly, P28285.

RACE reactions were performed with the SMART RACE amplification kit (BD Biosciences, San Jose, CA) according to the manufacturer's instructions. Two separate RACE reactions were required to obtain the amino terminus. RACE products were cloned with TA or TURBO cloning kits (Invitrogen, La Jolla, CA) and sequenced at the Georgia State University Biotechnology Facility. Data analysis and alignments were performed with DNASTAR and Sequencher (Genecodes, Ann Arbor, MI). The primers used in the RACE were:

Front GSP: AGGATGAGGATGGCAGTGAGAGGCACGTAGAAGGT
Front NGSP: GGCTAGGTCTTGCGACACCAGGCACTTCTTGTG
Front GSP2: AACAGGTCCGGACTGACGGGGTTCAACACC
Front NGSP2: TCGAGTATGGTCGCCGATCCAGAGGTCAGAGG
Back GSP: AATGGGCCACAGAGGAGGAGGAGGAGAAACGAA
Back NGSP: CCAACGGCGTAACGGAACACCAGACAACAGC

Generation of affinity-purified anti-5-HT$_{1crust}$ antibodies

The amino acid sequences for all three species were aligned. Two conserved, highly charged, nontransmembrane regions were identified (Fig. 1):

Peptide A: KDPDFLVRVNEHKKCLVSQD
Peptide B: PKKTKKETLEAKREKKAAKT

Each peptide was used in a protein–protein blast (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) in order to determine if any known proteins contain a similar peptide sequence. In the case of peptide A, the only hit was to the 5-HT type 1 receptor cloned from the tobacco budworm

(accession number: CAA64863). In the case of peptide B, there were three hits against the 5-HT_{1A} receptors cloned from *Drosophila* (accession number: P28285), mosquito (accession number: EAA04158), and tobacco budworm (accession number: CAA64863). Based on these results, we concluded that antibodies against these sequences would specifically recognize 5-HT_{1crust}. Peptides representing these two regions were synthesized and each was used as an antigen in the production of an affinity-purified rabbit polyclonal antibody. For peptide B, a cysteine was included at the beginning of the sequence in order to conjugate it to the carrier, KLH. Peptide synthesis, conjugation to KLH, antibody production, and affinity purification were performed by Bethyl Laboratories (Montgomery, TX).

Protein extraction and Western blots

Nervous tissue was extracted from all three species and snap-frozen in liquid nitrogen. The tissue was then homogenized on ice in 3.7 volumes (w/v) of lysis buffer (10 mM Tris, pH 7.5 (Sigma, St. Louis, MO), 1 mM EDTA (Sigma), 10 μg/ml pepstatin (Roche, Nutley, NJ), 10 μg/ml leupeptin (Roche), 2 μg/ml aprotinin (Roche), 350 μg/ml benzamidine (Sigma), 1 mg/ml pefabloc SC and pefabloc protector (Roche), 50 μg/ml antipain (Roche), 2 mM phenanthroline (Sigma), 5 mM iodoacetamide (Sigma)). Triton X-100 (Sigma) was added to a final concentration of 2%, SDS (Sigma) was added to a final concentration of 0.5%, and the homogenate was shaken at 4°C for 1–2 hours and spun 20 minutes at maximum speed in a microfuge. The supernatant was transferred to a clean tube and stored at –70°C until use. Just prior to use, 20 μl of the crude protein preparation was mixed with 4 μl 6× loading buffer (5.4 ml 1 M Tris, pH 6.8, 1.54 g SDS, 7.2 ml glycerol, 1.4 g DTT, 75 μl 5% Bromophenol blue) and incubated at 99°C for 20 minutes. Then 7–10 μl were loaded in each lane of a 10% SDS-polyacrylamide gel and electrophoresed. The protein was transferred to a polyvinylidene difluoride membrane (MSI, Westboro, MA) using a semidry electroblotting apparatus (OWL) and Tobin's transfer buffer (25 mM Tris, 192 mM glycine (Sigma), 15% methanol), at 45 mA for 2 hours. The membrane was incubated 2 hours to overnight in blocking buffer (1× PBS (0.14 M NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4, pH 7.3) containing 4% powdered milk and 0.3% Tween 20). Primary antibody was added at a concentration of 0.25 μg/ml and the blot was shaken at room temperature overnight. The membrane was washed in three changes of PBS plus 0.3% Tween 20 for 10 minutes each and then transferred to a solution of blocking buffer containing a 1:3,000 dilution of the antirabbit secondary conjugate solution provided in the Immuno-Star goat antirabbit IgG detection kit (Bio-Rad, Hercules, CA). The blot was then washed and processed to detect a chemiluminescent signal according to the directions provided by the manufacturer. For preabsorption controls, a 1:1 mixture of peptide and antibody (w/w) was incubated at room temperature for 1–2 hours just prior to use.

Immunocytochemical procedures

Crayfish. Ventral nerve cords were exposed from the dorsal side and the sheath was removed from the dorsal side of each ganglion. The nerve cords were then removed and placed immediately in fresh 4% paraformaldehyde in saline (in mM): 202 NaCl; 5.37 KCl; 13.53 $CaCl_2$; 2.6 $MgCl_2$; 2.4 HEPES; pH 7.4, preparations were left overnight at 4°C with shaking. Nerve cords were washed in water and dehydrated through an ethanol series (10 minutes each: 30, 50, 70, 80, 90, 95, 100%), then rehydrated (100, 70, 50, 30% ethanol). Preparations were washed at 4°C in PBS twice, 1 hour each, and then in PBS + 0.25% Triton X-100 (PBTX) four times, 1 hour each. Nerve cords were then incubated 2 nights at 4°C with shaking in PBTX with 5% normal goat serum, 2 μg/ml 5-HT_{1crust}, and a 1:50 dilution of a mouse anti-serotonin monoclonal antibody (DAKO, Denmark). Nerve cords were washed 6 times, 1 hour each, at 4°C in PBTX, and then incubated in PBTX with a 1:50 dilution of Texas Red goat antimouse and Oregon Green goat antirabbit (Molecular Probes, Eugene, OR). After incubating overnight at 4°C, the preparations were washed 6 times, 1 hour each, in PBTX, dehydrated sequentially up to 100% ethanol, and mounted in methyl salicylate (Sigma). The specificity of the antibodies was tested by incubating with antiserum, preabsorbed with the peptides used to raise the antibodies. Slides were stored at –20°C until they were imaged on a Zeiss LSM 510 confocal microscope, analyzed, and reconstructed using Adobe PhotoShop 7.0 (San Jose, CA) on a Macintosh computer.

Prawn. Ventral nerve cords were removed in prawn Ringer (in mM): 220 NaCl; 5.5 KCl; 13.5 $CaCl_2$; 2.5 $MgCl_2$; 5 Tris; pH 7.4, immediately fixed by immersion in freshly prepared 4% paraformaldehyde in 0.1M PBS at room temperature for 1 hour with constant shaking, and rinsed overnight in PBS containing 2% Triton X-100 and 0.1% sodium azide (PTA). Preparations were then incubated in normal goat serum (1:20) at 4°C for 3–5 hours, followed by the primary antibody for 5-HT_{1crust}, at a concentration of 5 μg/ml, for 2–3 days. For double-labeling experiments, a mouse 5-HT monoclonal antibody (DAKO) was also added to the incubation mix in a 1:40 dilution. After washing 6 times, 30 minutes each, with PTA, tissues were incubated in the secondary antibody (goat antirabbit Alexa 488 and/or goat antimouse Alexa 594, Molecular Probes) in a 1:200 dilution at 4°C overnight. Preparations were washed in 0.1M PTA 6 times, 1 hour each, washed in PBS for 1 hour, and left overnight in 90% glycerol/PBS buffer. The next day they were mounted in Polyaquamount, coverslipped, and viewed with a Zeiss Axioskop using epifluorescent excitation and/or a Zeiss LSM Pascal confocal microscope. The specificity of the antibodies was tested by incubating with antiserum preabsorbed with the peptides used to raise the antibodies.

RESULTS

Cloning of a crustacean serotonergic receptor

Several 5-HT receptors have been cloned in invertebrates (Tierney, 2001). Accumulating data suggest that for a given subtype, proteins will be conserved at the level of the sequence and signaling pathways across vertebrate/invertebrate lines, although pharmacology may not. This was not always obvious, and as a result the first invertebrate receptors to be cloned were not named in a systematic fashion consistent with vertebrate nomenclature. Thus, the names of many invertebrate receptors have been revised, as indicated in Table 1. Throughout this article, we have adopted the invertebrate nomenclature

TABLE 1. *Drosophila* 5-HT Receptors

Protein accession number(s)	Subtype	References	Original names	Renamed
NP_524599	$5HT_7$	(Witz et al., 1990)	5HT-dro1	$5\text{-}HT_7$
NP_476802	$5\text{-}HT_1$	(Saudou et al., 1992)	5HT-dro2A	$5\text{-}HT_{1A}$
NP_523789	$5\text{-}HT_1$	(Saudou et al., 1992)	5HT-dro2B	$5\text{-}HT_{1B}$
NP_524223/NP_730859	$5\text{-}HT_2$	(Colas et al., 1995)	$5\text{-}HT_{2Dro}$	$5\text{-}HT_{2A}$

system first introduced by Colas (1995), and later by Tierney (2001). In this system, homology to the mammalian subtypes 1–7 (i.e., $5\text{-}HT_1$–$5\text{-}HT_7$) is indicated by a subscripted number immediately after 5-HT. When there is more than one known gene within a subtype, individuals are indicated by subscripted letters that follow the number (i.e., $5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$, etc.). However, this letter is not meant to imply orthology across vertebrate/invertebrate lines. Indeed, this is not the case, as it is generally accepted that the paralogs within a subtype (i.e., $5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$, etc.) evolved independently for mammals and invertebrates. That is to say, most major subtypes $5\text{-}HT_1$–$5\text{-}HT_7$ are thought to have evolved before the divergence of invertebrates and vertebrates, while the evolution of paralogs within a subtype (i.e., $5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$, etc.) occurred after the split.

We began our study by isolating a crustacean 5-HT type 1 receptor that had been previously cloned and well characterized in many other invertebrate species. This invertebrate receptor was first cloned from fly (Saudou et al., 1992) and later from snail (Sugamori et al., 1993), slug (Angers et al., 1998), nematode (Olde and McCombie, 1997), tobacco budworm and silk moth (von Nickisch-Rosenegk et al., 1996). At the sequence level, this receptor is most homologous to mammalian 5-HT type1 receptors and, as expected, it negatively couples to cAMP in every case where it has been examined.

Prawn, crayfish, and lobster nervous system cDNAs were used in degenerate PCRs as described in Materials and Methods. The PCR products were cloned and sequenced. These experiments resulted in a >736 basepair (bp) fragment for each species, corresponding to transmembrane regions III–VII of a GPCR (Fig. 1). A blast search with the consensus amino acid sequence suggested it was an ortholog of the $5\text{-}HT_1$ receptor. To reinforce this supposition, we performed 5′ and 3′ RACE to obtain the complete open reading frame (ORF) of the lobster receptor. Paired alignments (not shown) with arthropod 5-HT type 1 receptors revealed amino acid identity over the complete ORF ranging from 48–53% (accession numbers: Q25190, Q17239, P28285, P28286). In contrast, identity dropped when compared with other biogenic amine receptors from *Drosophila*: $5\text{-}HT_2$ (24%: CAA57429), dopamine type 1 (24–27%: U61264, U34383, X77234), dopamine type 2 (27%: AY150862), octopamine (25%: AF065443), and tyramine (30%: BAB71788). $5\text{-}HT_1$ orthology was further confirmed by a blast search of the complete database that returned e-values ranging from e-107 for invertebrate $5\text{-}HT_{1A}$, e-73 for invertebrate $5\text{-}HT_{1B}$, e-66 for mammalian $5\text{-}HT_{1A}$, and e-58 for invertebrate $5\text{-}HT_7$, while $5\text{-}HT_2$ receptors were not observed in the blast results. However, a pairwise blast with a *Drosophila* $5\text{-}HT_2$ receptor (accession CAA57429) yielded 7e-20. Following the nomenclature scheme described above, we have collectively named this receptor $5\text{-}HT_{1crust}$, while individual receptors are referred to as $5\text{-}HT_{1Pan}$, $5\text{-}HT_{1Pro}$, and $5\text{-}HT_{1Mac}$.

The crustacean $5\text{-}HT_1$ receptor has the typical GPCR transmembrane signature motifs in the second ($S(X)_3D(X)_6VMP$), third ($D(X)_6SI(X)_5I(X)_2DRY$), fifth (FXXP), sixth ($F(X)_3WXPFF$), and seventh ($WXGY(X)_2S(X)_2NP(X)_2Y$) hydrophobic domains (reviewed in Kroeze et al., 2002). The transmembrane regions participate in both ligand binding and receptor activation, and thus, many of these conserved residues contribute to those functions. As expected, the third intracellular loop (*i3*) displays the greatest divergence. Although unconserved, the carboxy terminus is characteristically short and highly charged. Like most vertebrate and invertebrate $5\text{-}HT_1$ receptors, it does not end in a PDZ domain (Songyang et al., 1997). Figure 1 illustrates that both the amino acid sequence and length of $5\text{-}HT_1$ amino termini are poorly conserved. The amino terminus of $5\text{-}HT_{1Pan}$ contains four putative N-linked glycosylation sites (Fig. 1).

Characterization of the $5\text{-}HT_{1crust}$ protein

To further characterize the size and distribution of the receptor, we generated antibodies against $5\text{-}HT_{1crust}$, as described in Materials and Methods. The published sizes of $5\text{-}HT_1$ orthologs vary with the species from 49–92 kD. This variation is largely due to differences in *i3* (343 vs. 106 amino acids, see Fig. 1), as well as variations in the lengths of the amino termini (230 vs. 60 amino acids). Figure 2 illustrates that $5\text{-}HT_{1crust}$ is similar to the other arthropod receptors and varies in size between ~40 and 80 kD. The Western blots for all three species shows a band at roughly 52–55 kD. The lobster signal is slightly larger than the predicted 48 kD and could indicate glycosylation or other posttranslational modifications. Overexposure of the lobster Western produces a very faint band at ~80 kD (not shown), consistent with the presence of a low abundance isoform, additional posttranslational modifications, and/or receptor dimerization. However, in crayfish and prawn, multiple bands are obvious without overexposure indicating a substantial amount of alternate splicing and/or posttranslational modification and/or dimerization. All signals were lost upon preabsorption with the peptide used to generate the antibody (not shown). Thus, we concluded that our antibody specifically recognizes the $5\text{-}HT_{1crust}$ receptor.

$5\text{-}HT_{1crust}$ staining in thoracic ganglia of prawn and crayfish

General structure and distribution of 5-HT neurons. Because much of what is known concerning the function of serotonin in the crustacean central nervous system is associated with thoracic neurons (Beltz and Kravitz, 1987; Pearlstein et al., 1998; Kravitz, 2000), we decided to initially focus our attention on the location and distribution of the $5\text{-}HT_{1crust}$ receptor in the thoracic ganglia, in relation to the location and distribution of serotonergic neurons and processes. The latter has been described for several species of Crustacea (Real and Czternasty, 1990; Harzsch and Waloszek, 2000), but not for prawn or spiny

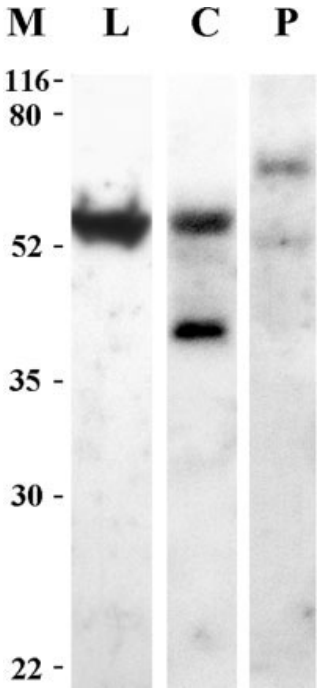

Fig. 2. Anti-5-HT_{1crust} specificity. Western blots containing protein extracts from the nervous system of lobster (L), crayfish (C), and prawn (P) were probed with anti-5-HT_{1crust} (antibody B in Materials and Methods). Molecular weight marker (M) in kD.

lobster. Here we describe thoracic serotonergic neurons in prawn, but not in lobster, and restrict our comparisons to prawn and crayfish.

The general organization of the thoracic region of the ventral nerve cord is similar in crayfish and prawn (Fig. 3), consisting of five ganglia (TG) joined by pairs of longitudinal connectives, and linked to the periphery of each hemisegment through three nerves or roots. In the crayfish, the paired connectives are more distinct from the adjacent ganglia and from each other than those in prawn, where the hemiconnectives are bundled together. The single exception is found between T3 and T4, where the connectives separate to allow passage of the sternal artery.

Points of similarity and difference also exist for the distribution and projections of serotonergic neurons in the thoracic ganglia of the two animals (Fig. 3). In the crayfish, each TG has a pair of 5-HT neurons that project anteriorly (Real and Czternasty, 1990). In T1–T4, the axons of these neurons cross to the contralateral side within each ganglion and then ascend in the connectives. In T5, as in the first abdominal ganglion, the axons of the 5-HT neurons form a characteristic loop as they ascend along the ipsilateral connectives. The connectives in the crayfish thoracic ventral nerve cord also contain 5-HT fibers arising from other areas of the cord, such as the abdominal ganglia. 5-HT fibers can also be seen exiting the cord through thoracic roots. These roots are surrounded by numerous boutons containing 5-HT that have been shown to be neurosecretory in the lobster (Beltz and Kravitz, 1987).

In the prawn, the first four TG each have two pairs of ascending 5-HT neurons with axons that travel along the lateral edge of the connectives. The axons of the medial pair of neurons ascend on the ipsilateral side, whereas those of the lateral pair cross the ganglion and ascend on the contralateral side. A third pair of 5-HT neurons is found towards the center of T1 and T2, with axons traveling ipsilaterally in the medial aspect of the connectives. All these 5-HT neurons are smaller than those found in the crayfish, except the medial pair of neurons in T4. The fifth thoracic ganglion has only one pair of 5-HT neurons, with axons that form a characteristic loop as they ascend on the medial aspect of the ipsilateral connectives, similar to the 5-HT neurons in T5 of the crayfish. The prawn's T5 5-HT neurons are similar in size to the large 5-HT neurons in T4. In contrast with what is observed in the crayfish, in the prawn no 5-HT boutons are observed on thoracic roots, and no 5-HT fibers are seen exiting the ganglia here.

5-HT_{1crust} staining. 5-HT_{1crust} immunoreactivity (5-HT_{1crust}ir) was observed surrounding the somata of specific groups of neurons and as punctate staining within the neuropil in all thoracic ganglia of both crayfish and prawn (Figs. 4–6). In the crayfish, but not the prawn, 5-HT_{1crust}ir was also found in boutons surrounding the first and second roots of each ganglion and on the 5-HT cells of T1–4. In the prawn, but not the crayfish, 5-HT_{1crust}ir was also found in axons that project across the ganglia and along the connectives. We also performed negative controls in which the primary antibody was omitted or preabsorbed with the peptide used in antigen production. In all cases the signal was lost, confirming the specificity of the signal for 5-HT_{1crust} in the wholemount preparation.

5-HT_{1crust}ir around somata and within the neuropil follows a similar pattern in all five TG in both crayfish and prawn; T3 (Fig. 4) and T4-T5 (Fig. 5) are shown here. On the ventral aspect, somata surrounded by 5-HT_{1crust}ir form a large cluster located at the midline of the ganglion along with four smaller clusters of similarly stained cells, arranged as the wings of a butterfly, located at the lateral edges of each ganglion (Figs. 4, 5B,D). The midline clusters are striking in prawn, where they consist of large numbers of small and large cell bodies arrayed across a broad portion of the midline of the ganglion (Figs. 4A, long arrow; 5B). The midline cluster is less prominent in crayfish, consisting of large numbers of small somata (Figs. 4B, long arrow; 5D). The "butterfly wing" clusters also differ between prawn and crayfish. In prawn, they consist of clumps of small, brightly stained somata arranged in the far anterior lateral and posterior lateral portion of the ganglion (Figs. 4A, short arrows; 5A). In crayfish, larger, distributed clusters of many smaller, well-stained cells are arranged in a similar pattern (Figs. 4B, short arrows; 5D). An additional far lateral posterior cluster of somata is also apparent in crayfish (Figs. 4B, arrowheads; 5D), but not in prawn (Fig. 4A, 5B). Clusters of somata are also seen on the dorsal aspects of thoracic ganglia of both animals (Fig. 5A,C); these somata are at the ganglionic margins and appear to be continuous with the tips of the "butterfly wing" clusters seen on the ventral aspect.

The relationship between 5-HT_{1crust}ir and serotonergic neurons and profiles is seen in Figure 5. Serotonergic neurons are clustered near the midline of the ganglion in T4–5 in prawn, and 5-HT_{1crust}ir fibers pass

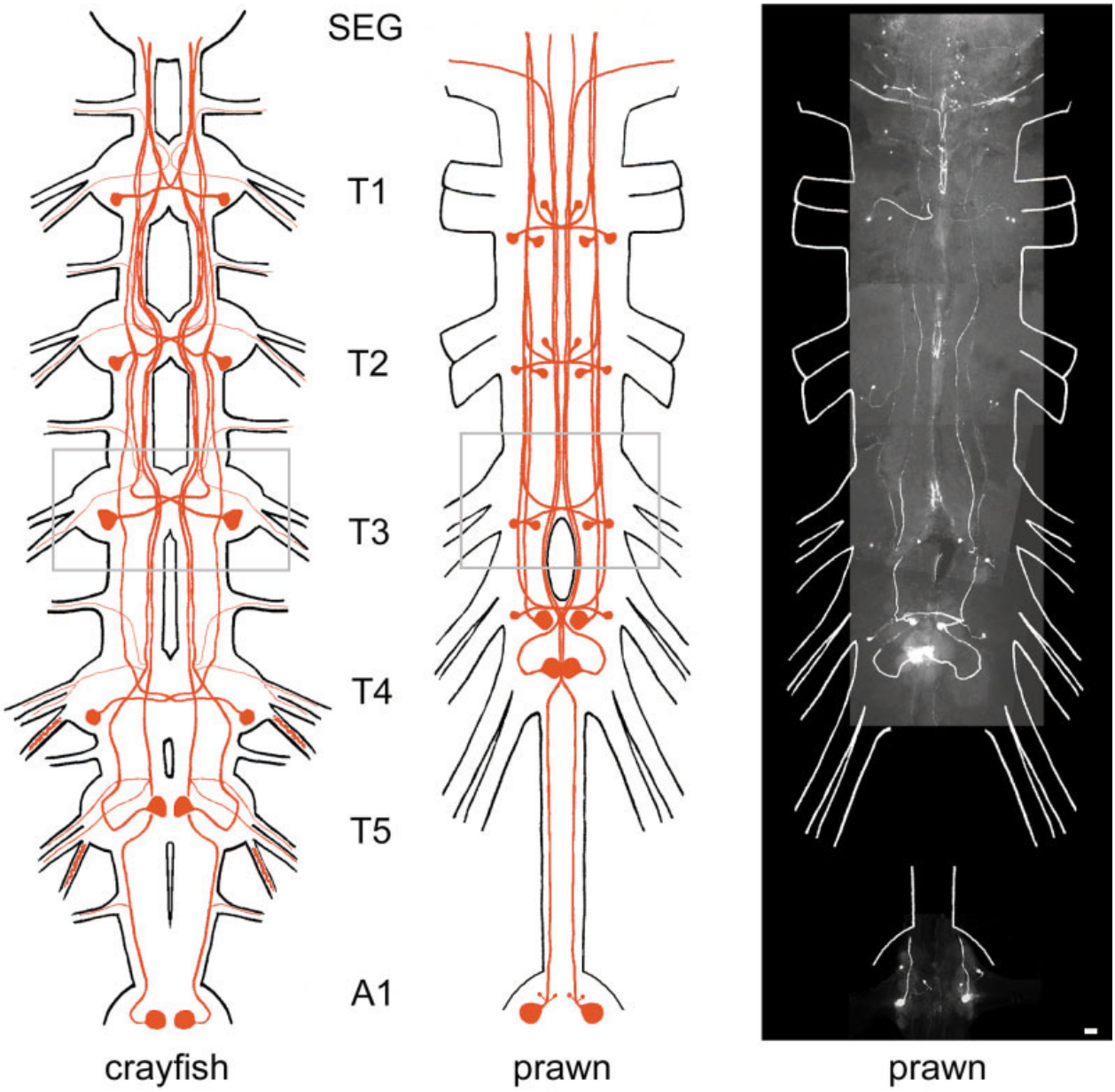

Fig. 3. **A:** Schematic of anatomical structure and 5-HT neurons in the thoracic ganglia of the crayfish and prawn. Black lines outline the five thoracic ganglia of crayfish and prawn, illustrating the similarities and differences between the two species. Shown in red are the major 5-HT neuronal cell bodies and fibers in the two species. Gray boxes: areas shown in Figure 4A (right box) and Figure 4B (left box). Schematic for the crayfish based in part on Real and Czternasty (1990). **B:** Montage of 5-HTir in the thoracic ganglia of the prawn (54 confocal Z-stack images, thickness of ~440 μm). Scale bar = 100 μm.

dorsal to them (Fig. 5A). It is not clear if the immunoreactivity is in the axolemma and/or the axoplasm, or whether it represents functioning receptors and/or receptors in transport. A few 5-HT_{1crust}ir smaller caliber fibers could also be observed branching horizontally from these central fibers toward each hemiganglion (Fig. 5A, arrows). Serotonergic neurons are located more laterally in crayfish, and no fibers appear labeled for the serotonin receptor (Fig. 5C). The fact that no filled 5-HT_{1crust}ir fibers are seen in the crayfish most probably means that the epitope recognized by the antibody is in a "hidden" conformation in transport vesicles and is only available for antigen–antibody interactions after the receptor is delivered to the plasma membrane. Both 5-HTir and 5-HT_{1crust}ir staining appear in the thoracic neuropil, where they are frequently seen to overlap (Fig. 5E, yellow profiles). In addition to these overlapping sites, 5-HTir and 5-HT_{1crust}ir profiles are often seen separately (Fig. 5E, red and green profiles). 5-HT_{1crust} receptors also appear far removed from

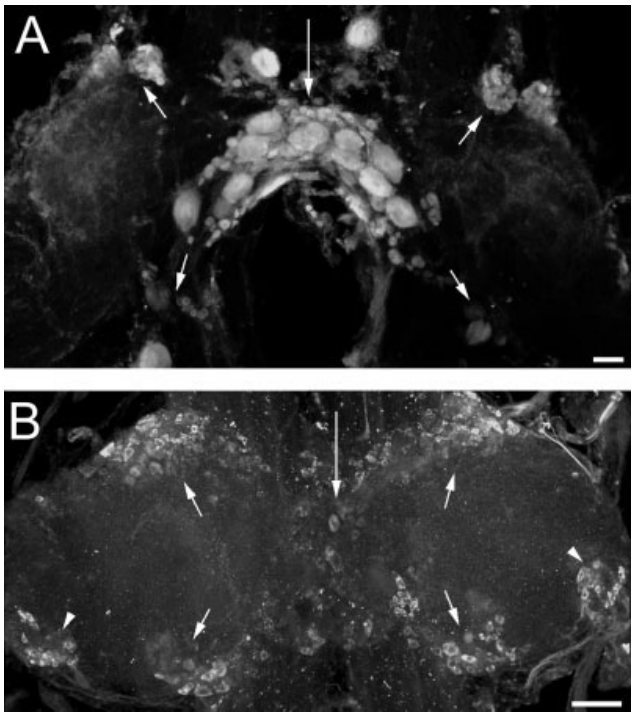

Fig. 4. 5-HT_{1crust}ir in the third thoracic ganglia of prawn (**A**) and crayfish (**B**). (**A**) The area boxed at right in Figure 3, a collage of two adjacent stacks, each ~230 μm deep, of 36 slices. (**B**) The corresponding area in the left box around T3 in Figure 3, a composite of 36 confocal Z-stack images, spanning a thickness of ~140 μm. Arrows point out the prawn's and crayfish's 5-HT_{1crust}ir midline (long arrows) and "butterfly wing" cell clusters (short arrows), as well as the crayfish's far lateral posterior cell clusters (arrowheads). Scale bars = 100 μm.

serotonin profiles on the cell somata. These receptors surround the cell somata in a punctate pattern (Fig. 6). This pattern is most clearly seen at the poles of these round cells (b, c, f, g in Fig. 6A). The labeling may be localized to the neural membranes or to glia surrounding the neurons, or both.

As we have observed thus far, the nature and distribution of 5-HT_{1crust}ir on cells and neuropil areas of the TG is very similar in the crayfish and prawn. However, there are some areas of the ventral nerve cord in which 5-HT_{1crust}ir is observed in one species and not the other. This is the case with 5-HT_{1crust}ir boutons of the first and second roots of the TG in the crayfish, and within a subset of axons traversing the TG in the prawn. Since serotonergic boutons are found on the first and second roots of the crayfish (Real and Czernasty, 1990), but not on any of the thoracic roots of the prawn, it is not surprising to find 5-HT_{1crust}ir in the former (Fig. 5F), but not the latter (not shown). Many of the serotonergic boutons showed overlapping staining for the 5-HT_{1crust}ir receptor (yellow), suggesting that many of the serotonergic release sites on these nerves are associated with activation of the 5-HT_{1crust} receptor specifically. These roots show many discrete areas, also in the shape of boutons, of 5-HT_{1crust}ir (green) that do not overlap with serotonergic boutons.

DISCUSSION

Serotonin plays many roles in crustaceans, ranging from neuromodulation of sensory neurons to regulation of aggressive behavior (Beltz, 1999). Serotonin also functions in the long-term development and maintenance of circuits (Sullivan et al., 2000; Beltz et al., 2001; Benton and Beltz, 2001; Koss et al., 2003; Richards et al., 2003). Comparative studies on the anatomy of the crustacean serotonergic system abound (reviewed in Harzsch and Waloszek, 2000); yet there is not one study to elucidate commonalities and differences in crustacean serotonergic effector systems. Thus, we have cloned a putative crustacean 5-HT receptor and compared its distribution with respect to 5-HT localization in two species of Crustacea.

5-HT_{1crust} receptor

Vertebrate and invertebrate monoamine receptors are conserved with regard to sequence and signaling pathways; however, their pharmacological profiles differ significantly (reviewed in Blenau and Baumann, 2001). Because of the sequence conservation, we were able to clone a putative 5-HT_{1crust} receptor that is highly homologous to other invertebrate 5-HT_1 orthologs. At the structural level, the receptor is well conserved across species, except at the amino terminus; but to date, no function has been assigned to the amino terminus (Kroeze et al., 2002). Western blots suggest that the 5-HT_{1crust} is alternately spliced and/or differentially modified posttranslationally in all three species. Although we do not yet know the effector mechanisms associated with the putative 5-HT_{1crust} receptor, the 5-HT_1 family signals through pertussis toxin-sensitive G_i/G_0 proteins to mediate a range of actions that include the ubiquitous inhibition of cAMP formation via $G_{\alpha i}$, as well as cell-specific $G_{\beta\gamma}$-mediated effects, including stimulation of phospholipase C_β, stimulation of MAP kinase, opening of K^+ channels, and closing of Ca^{2+} channels (Saudou et al., 1992; reviewed in Albert and Tiberi, 2001). The vertebrate 5-HT_1 receptor is expressed postsynaptically in many neurons, and it serves as the somatodendritic autoreceptor on serotonergic neurons of the raphe nucleus. Mice lacking the 5-HT_{1A} receptor display increased anxiety behavior and stress response. In *Drosophila* larvae, the 5-HT_{1A} receptor is expressed in a population of ventral cord (VUM) motoneurons in the abdominal and thoracic ganglia. These motoneurons innervate the body wall (Saudou et al., 1992). We examined the distribution of the putative crustacean ortholog in the ventral nerve cord of prawns and crayfish.

Thoracic serotonergic system in crayfish and prawns

While the serotonergic system is well conserved across arthropods (Beltz, 1999; Monastirioti, 1999; Harzsch, 2003), considerable segment- and species-specific variation in the number of serotonergic neurons and in the morphology of their neurites has been described for the crustacean thoracic segments (Harzsch and Waloszek, 2000). Here we demonstrate that in the prawn, serotonergic neurons maintain the repetitive segmental arrangement that is characteristic of all crustaceans and that is suggestive of serial homology (Fig. 7). However, unlike the crayfish, one or two additional pairs of cells are found in each ganglion and the neurons do not project out through thoracic nerve roots.

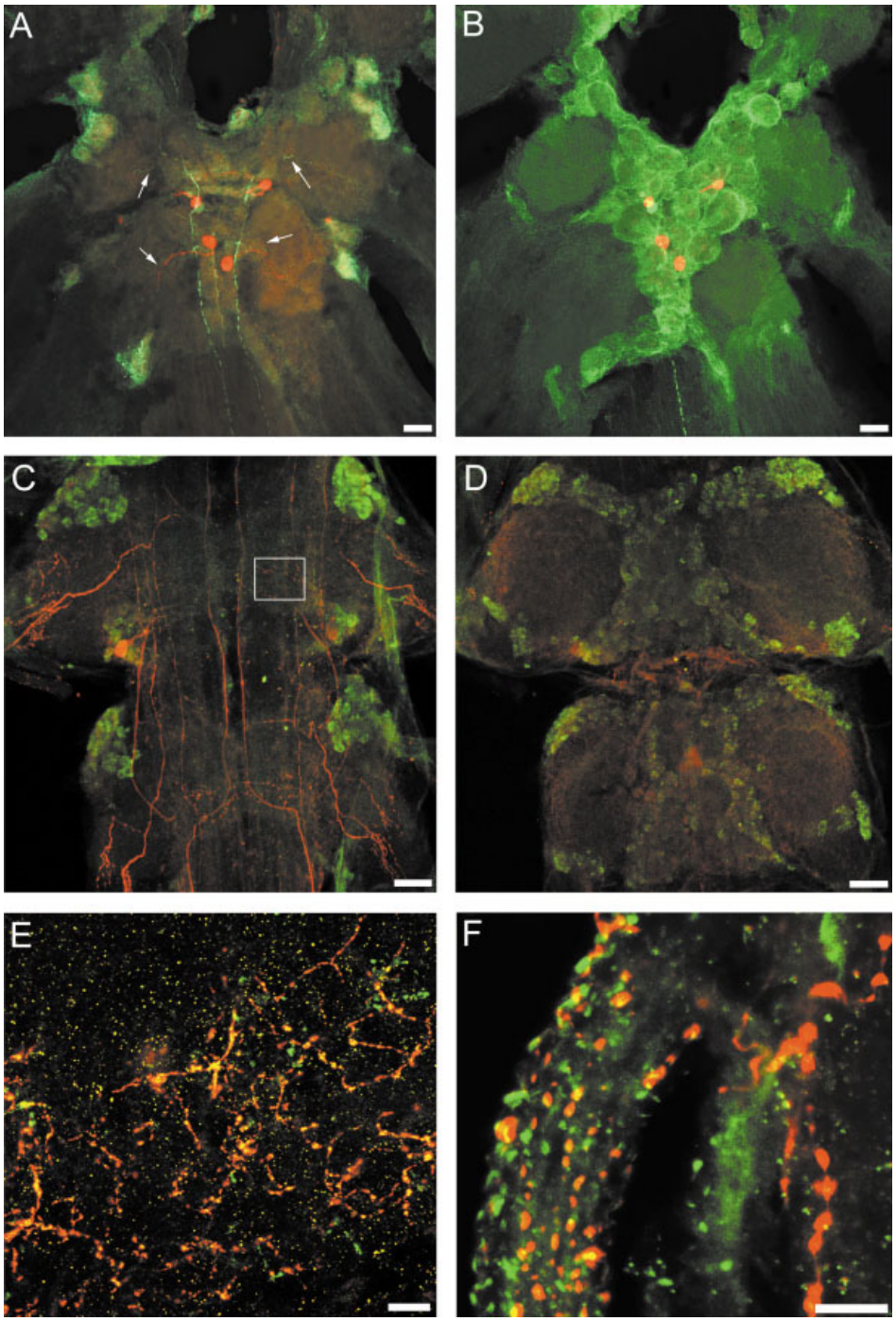

Fig. 5. 5-HT_{1crust}ir (green) and 5-HTir (red) in the fourth and fifth thoracic ganglia of prawn and crayfish. (**A**) and (**B**) show the dorsal and ventral aspects, respectively, of prawn T4–T5 ganglia. 5-$HT_{1crust1}$ir fibers were observed in the prawn, but not the crayfish. These fibers were seen traversing the ganglia and also extending towards the neuropil cores (arrows in A). Images are composites of 18–22 confocal Z-stack images, spanning a thickness of ~150 μm. Scale bar = 100 μm. (**C**) and (**D**) show the dorsal and ventral aspects, respectively, of crayfish T4–T5 ganglia. Images are projections of 13 confocal Z-stack images ~218 μm deep. On the ventral aspect, the four lateral clusters of cells become continuous with a centrally located cluster of 5-HT_{1crust}ir cells in both animals. Punctate 5-HT_{1crust}ir staining is also observed in the neuropil cores of the ganglia, an area where punctate 5-HTir staining can also be observed. **E:** A high-magnification image of the area boxed in C shows punctate labeling for 5-HT_{1crust} (green) and 5-HT (red) on processes in the neuropil; overlap of the transmitter and the receptor appears yellow. Single confocal optical slice 2.06 μm deep, scale bar = 10 μm. **F:** 5-HT_{1crust}ir (green) processes intertwine and partially colocalize (yellow) with the 5-HTir (red) plexus on roots 1 and 2 of crayfish ganglia T1–5.

In parallel, the effector systems of each species display similarities and differences. In all five TG of both prawn and crayfish, 5-HT_{1crust} receptors were found in the neuropil and around somata, where they formed a butterfly pattern that differed in detail between the two species. This butterfly pattern of somata is typical of cell body arrangement in crayfish thoracic ganglia (reviewed in Elson, 1996) and 5-HT_{1crust}ir is distributed among cell bodies within the previously defined ganglionic cell clusters. In addition, the second thoracic roots are thought to be neurohemal organs that release neurohormones into the hemolymph. In lobster and crayfish, there is a dense plexus of serotonergic terminals surrounding the roots in a pattern that is reminiscent of a dense plexus of GABAergic endings in abdominal nerve roots that modulate the excitability of motor neurons (Fraser and Heitler, 1993). In crayfish, 5-HT_{1crust} receptors colocalize with these serotonergic endings. If the receptors are on the serotonergic

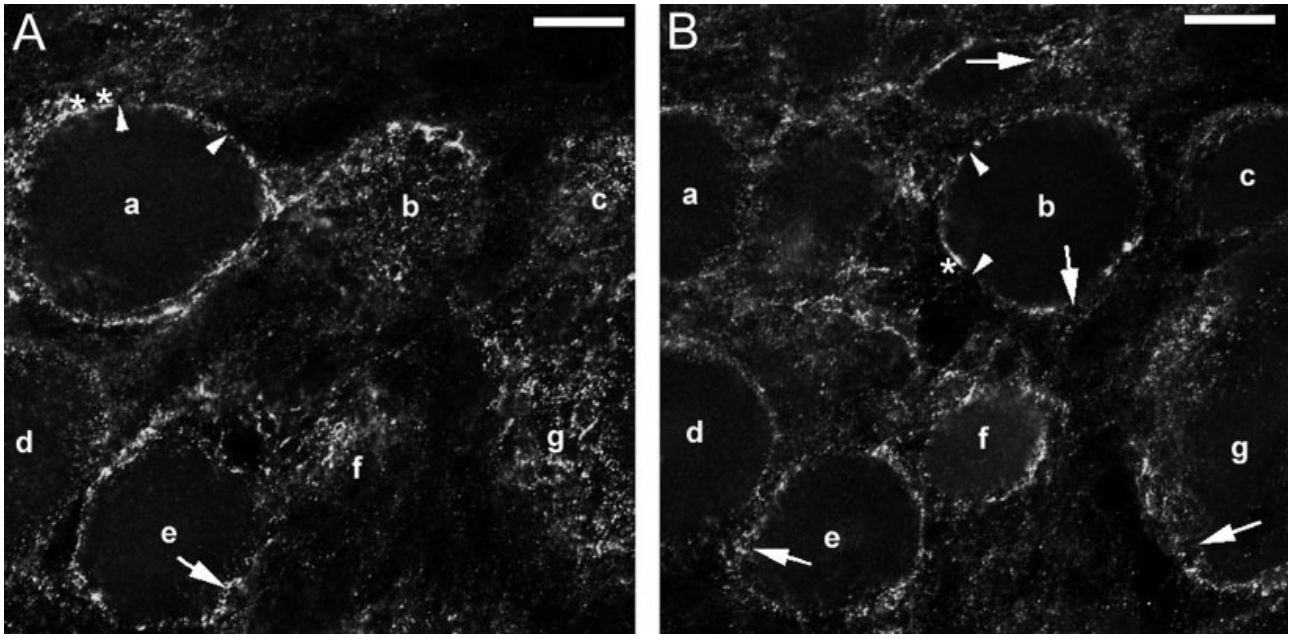

Fig. 6. 5-HT_{1crust} is found on the membrane of cluster cells. (**A**) and (**B**) show high-magnification confocal images of consecutive sections across the prawn's T4 central cluster of 5-HT_{1crust}ir cells (see Fig. 6). (B) is 16 μm deeper than (A). 5-HT_{1crust}ir is found on the outer edges of cells but not inside the cytoplasm. The punctate nature of the 5-HT_{1crust} is clearly observed. Lowercase letters indicate the location of corresponding cells on each image. Arrowheads indicate areas of the outer edge of the cell that have no staining. Asterisks indicate areas of the outer edge of the cell with continuous or solid staining. Arrows indicate stained circular profiles on the outer edge of the cell, which may correspond to glial cells. Scale bar = 50 μm.

endings themselves, then they may enable serotonin to modulate the neurohormonal release of serotonin. Alternatively, if they are on other targets, such as motor neurons that project out from these roots, they may modulate the excitability of these cells. In striking contrast, both the plexus and the receptors are absent in prawn, demonstrating that even in the midst of remarkable conservation, fundamental differences exist between species.

Relationship of thoracic 5-HT neurons and the 5-HT_{1crust} receptor

This is the first examination of the distribution of putative invertebrate serotonin receptors and 5-HT relative to each other. We found examples of colocalization of receptor with 5-HT, consistent with the short-term modulatory role of 5-HT. This occurred in the neuropil and in the thoracic first and second roots, as described above. There were also cases of serotonergic staining in the absence of an anti-5-HT_{1crust} signal. Since multiple 5-HT receptors exist (Zhang and Harris-Warrick, 1994; Yeh et al., 1996; Tierney, 2001), one interpretation of these data is that other 5-HT receptors reside at these locations. Alternatively, these might represent sites of paracrine release. Surprisingly, we also observed receptors that did not possess counterpart 5-HT staining, suggesting that these receptors may mediate the long-term and/or neurohormonal functions of serotonin. These receptors were located around the somata and in the second thoracic roots. While we cannot rule out the possibility that the somata label occurs in the glia that surround each cell body, it is intriguing to speculate that most receptors putatively functioning in long-term development and maintenance of neurons may be closer to the nucleus than the synapse. The receptor label found alone in thoracic roots may occur on motor neurons subject to hormonal modulation by serotonin.

Serotonin in the thorax

Serotonin is considered an important modulator of segmental reflexes in the crayfish walking system (Gill and Skorupski, 1996). Superfused serotonin reduced the inhibitory effect of glutamate on leg motor neurons and increased their input resistance. This action led to reduced glutamate-mediated mutual inhibition between elevator and depressor motor neurons, and so may function to reconfigure walking leg reflexes (Pearlstein et al., 1998). Similar effects are produced by direct stimulation of the A1 serotonergic interneurons (Issa, pers. commun.). Sneddon et al. (2000) have shown that exercise alone increases 5-HT along with other neuromodulators. Thus, the use of 5-HT to modulate the locomotive circuits in the thoracic ganglia are supportive of the current findings of 5-HT receptors within this same region. Other thoracic and abdominal sensorimotor circuits in crayfish are also subject to serotonergic modulation, including those producing swimmeret beating (Barthe et al., 1993) and tailflip escape (Yeh et al., 1997; Teshiba et al., 2001). The pericardial organs, a pair of neurosecretory organs that surround the crustacean heart and release neuromodulators into the hemolymph, are known to contain a wide array of peptide and amine modulators, including serotonin. At least some of the serotonin present in the pericardial organs projects there from the thoracic ganglia (Pulver and Marder, 2002).

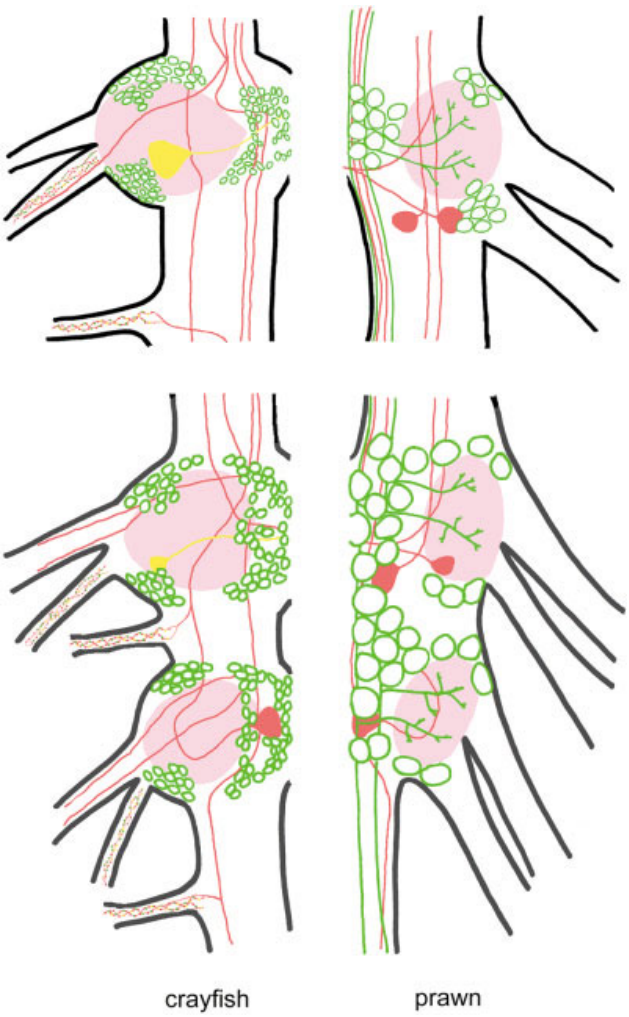

Fig. 7. Schematic representation of 5-HT_{1crust}ir in the crayfish and the prawn thoracic ganglia. The left half of the figure represents staining in the crayfish T3–T5 ganglia and the right half represents the corresponding staining in the prawn T3–T5 ganglia. Green indicates 5-HT_{1crust}ir staining, red indicates major 5-HT neurons and fibers, and yellow shows colocalization of 5-HT_{1crust}ir and 5-HTir. The staining pattern shown for these three ganglia is representative of that observed in T1 and T2.

It is unclear which, if any, of these actions are mediated by the 5-HT_{1crust} receptor described here; this will have to await identification of the thoracic neurons that display 5-HT_{1crust}ir and experiments that determine their sensitivity to serotonin when this receptor is present. However, the repeated pattern of staining of clusters of large and small neurons in each thoracic ganglion suggests that this receptor modulates segmentally repeated circuits in the thorax. The majority of these circuits are concerned with movement of the legs, suggesting that this receptor, and perhaps others, helps coordinate leg locomotor patterns.

LITERATURE CITED

Albert PR, Tiberi M. 2001. Receptor signaling and structure: insights from serotonin-1 receptors. Trends Endocrinol Metab 12:453–460.

Angers A, Storozhuk MV, Duchaine T, Castelluci VF, DesGroseillers L. 1998. Cloning and functional expression of an *Aplysia* 5-HT receptor negatively coupled to adenylate cyclase. J Neurosci 18:5586–5593.

Ayali A, Harris-Warrick RM. 1999. Monoamine control of the pacemaker kernel and cycle frequency in the lobster pyloric network. J Neurosci 19:6712–6722.

Baro DJ, Cole CL, Zarrin AR, Hughes S, Harris-Warrick RM. 1994. Shab gene expression in identified neurons of the pyloric network in the lobster stomatogastric ganglion. Receptors Channels 2:193–205.

Barthe JY, Bevengut M, Clarac F. 1993. In vitro proctolin and serotonin induced modulations of the abdominal motor system activities in crayfish. Brain Res 623:101–109.

Beltz BS. 1999. Distribution and functional anatomy of amine-containing neurons in decapod crustaceans. Microsc Res Tech 44:105–120.

Beltz BS, Kravitz EA. 1983. Mapping of serotonin-like immunoreactivity in the lobster nervous system. J Neurosci 3:585–602.

Beltz BS, Kravitz EA. 1987. Physiological identification, morphological analysis, and development of identified serotonin-proctolin containing neurons in the lobster ventral nerve cord. J Neurosci 7:533–546.

Beltz BS, Benton JL, Sullivan JM. 2001. Transient uptake of serotonin by newborn olfactory projection neurons. Proc Natl Acad Sci USA 98: 12730–12735.

Benton J, Beltz BS. 2001. Effects of serotonin depletion on local interneurons in the developing olfactory pathway of lobsters. J Neurobiol 46: 193–205.

Bethea CL, Lu NZ, Gundlah C, Streicher JM. 2002. Diverse actions of ovarian steroids in the serotonin neural system. Front Neuroendocrinol 23:41–100.

Blenau W, Baumann A. 2001. Molecular and pharmacological properties of insect biogenic amine receptors: lessons from *Drosophila melanogaster* and *Apis mellifera*. Arch Insect Biochem Physiol 48:13–38.

Colas JF, Launay JM, Kellermann O, Rosay P, Maroteaux L. 1995. *Drosophila* 5-HT_2 serotonin receptor: coexpression with fushi-tarazu during segmentation. Proc Natl Acad Sci USA 92:5441–5445.

Doernberg SB, Cromarty SI, Heinrich R, Beltz BS, Kravitz EA. 2001. Agonistic behavior in naive juvenile lobsters depleted of serotonin by 5,7-dihydroxytryptamine. J Comp Physiol A Electronic publication DOI 1007/s003590000178.

Edwards DH, Heitler WJ, Krasne FB. 1999. Fifty years of a command neuron: the neurobiology of escape behavior in the crayfish. Trends Neurosci 22:153–161.

Elson RC. 1996. Neuroanatomy of a crayfish thoracic ganglion: sensory and motor roots of the walking-leg nerves and possible homologies with insects. J Comp Neurol 365:1–17.

Fraser K, Heitler WJ. 1993. Anatomical and physiological identification of inhibitors of the motor giant and segmental giant neurons in the crayfish. J Exp Biol 180:55–73.

Gill MD, Skorupski P. 1996. Modulation of spontaneous and reflex activity of crayfish leg motor neurons by octopamine and serotonin. J Neurophysiol 76:3535–3549.

Harris-Warrick RM, Kravitz EA. 1984. Cellular mechanisms for modulation of posture by octopamine and serotonin in the lobster. J Neurosci 4:1976–1993.

Harzsch S. 2003. Evolution of identified arthropod neurons: the serotonergic system in relation to *engrailed*-expressing cells in the embryonic ventral nerve cord of the American lobster *Homarus americanus* Milne Edwards, 1873 (malacostraca, pleocyemata, homarida). Dev Biol 258: 44–56.

Harzsch S, Waloszek D. 2000. Serotonin-immunoreactive neurons in the ventral nerve cord of Crustacea: a character to study aspects of arthropod phylogeny. Arthropod Struct Dev 29:307–322.

Huber R, Panksepp JB, Yue Z, Delago A, Moore P. 2001. Dynamic interactions of behavior and amine neurochemistry in acquisition and maintenance of social rank in crayfish. Brain Behav Evol 57:271–282.

Koss R, Diefenbach TJ, Kuang S, Doran SA, Goldberg JI. 2003. Coordinated development of identified serotonergic neurons and their target ciliary cells in *Helisoma trivolvis* embryos. J Comp Neurol 457:313–325.

Kravitz EA. 1988. Hormonal control of behavior: amines and the biasing of behavioral output in lobsters. Science 241:1775–1781.

Kravitz EA. 2000. Serotonin and aggression: insights gained from a lobster model system and speculations on the role of amine neurons in a complex behavior. J Comp Physiol [A] 186:221–238.

Kroeze WK, Kristiansen KK, Roth BL. 2002. Molecular biology of serotonin

receptors structure and function at the molecular level. Curr Top Med Chem 2:507–528.

Livingstone MS, Harris-Warrick RM, Kravitz EA. 1980. Serotonin and octopamine produce opposite postures in lobsters. Science 208:76–79.

Ma PM, Beltz BS, Kravitz EA. 1992. Serotonin-containing neurons in lobsters: their role as gain-setters in postural control mechanisms. J Neurophysiol 68:36–54.

Monastirioti M. 1999. Biogenic amine systems in the fruit fly *Drosophila melanogaster*. Microsc Res Tech 45:106–121.

Olde B, McCombie WR. 1997. Molecular cloning and functional expression of a serotonin receptor from *Caenorhabditis elegans*. J Mol Neurosci 8:53–62.

Panksepp JB, Huber R. 2002. Chronic alterations in serotonin function: dynamic neurochemical properties in agonistic behavior of the crayfish, *Orconectes rusticus*. J Neurobiol 50:276–290.

Pearlstein E, Clarac F, Cattaert D. 1998. Neuromodulation of reciprocal glutamatergic inhibition between antagonistic motoneurons by 5-hydroxytryptamine (5-HT) in crayfish walking system. Neurosci Lett 241:37–40.

Pulver SR, Marder E. 2002. Neuromodulatory complement of the pericardial organs in the embryonic lobster, *Homarus americanus*. J Comp Neurol 451:79–90.

Real D, Czternasty G. 1990. Mapping of serotonin-like immunoreactivity in the ventral nerve cord of crayfish. Brain Res 521:203–212.

Richards KS, Simon DJ, Pulver SR, Beltz BS, Marder E. 2003. Serotonin in the developing stomatogastric system of the lobster, *Homarus americanus*. J Neurobiol 54:380–392.

Saudou F, Hen R. 1994. 5-Hydroxytryptamine receptor subtypes in vertebrates and invertebrates. Neurochem Int 25:503–532.

Saudou F, Boschert U, Amlaiky N, Plassat J-L, Hen R. 1992. A family of *Drosophila* serotonin receptors with distinct intracellular signalling properties and expression patterns. EMBO J 11:7–17.

Sneddon LU, Taylor AC, Huntingford FA, Watson DG. 2000. Agonistic behaviour and biogenic amines in shore crabs *Carcinus maenas*. J Exp Biol 203:537–545.

Songyang Z, Fanning AS, Fu C, Xu J, Marfatia SM, Chishti AH, Crompton A, Chan AC, Anderson JM, Cantley LC. 1997. Recognition of unique carboxyl-terminal motifs by distinct PDZ domains. Science 275:73–77.

Sosa MA, Baro DJ. 1999. Comparison of 5-HT receptor mRNA expression in the ventral nerve cord of the male morphotypes of the giant tropical freshwater prawn. Soc Neurosci Abstr 25:1704.

Sosa M, Baro D. 2002. The role of amines and aminergic receptors in mediating dominance in the giant tropical freshwater prawn. In: Wiese K, Schmidt M, editors. Physiology of the crustacean nervous system. Berlin: Springer. p 143–155.

Sugamori KS, Sunahara RK, Guan HC, Bulloch AG, Tensen CP, Seeman P, Niznik HB, Van Tol HH. 1993. Serotonin receptor cDNA cloned from *Lymnaea stagnalis*. Proc Natl Acad Sci USA 90:11–15.

Sullivan JM, Benton JL, Beltz BS. 2000. Serotonin depletion in vivo inhibits the branching of olfactory projection neurons in the lobster deutocerebrum. J Neurosci 20:7716–7721.

Teshiba T, Shamsian A, Yashar B, Yeh SR, Edwards DH, Krasne FB. 2001. Dual and opposing modulatory effects of serotonin on crayfish lateral giant escape command neurons. J Neurosci 21:4523–4529.

Thompson KS, Zeidler MP, Bacon JP. 1994. Comparative anatomy of serotonin-like immunoreactive neurons in isopods: putative homologues in several species. J Comp Neurol 347:553–569.

Tierney AJ. 2001. Structure and function of invertebrate 5-HT receptors: a review. Comp Biochem Physiol A Mol Integr Physiol 128:791–804.

von Nickisch-Rosenegk E, Krieger J, Kubick S, Laage R, Strobel J, Strotmann J, Breer H. 1996. Cloning of biogenic amine receptors from moths (*Bombyx mori and Heliothis virescens*). Insect Biochem Mol Biol 26: 817–827.

Witz P, Amlaiky N, Plassat JL, Maroteaux L, Borrelli E, Hen R. 1990. Cloning and characterization of a *Drosophila* serotonin receptor that activates adenylate cyclase. Proc Natl Acad Sci USA 87:8940–8944.

Yeh SR, Fricke RA, Edwards DH. 1996. The effect of social experience on serotonergic modulation of the escape circuit of crayfish. Science 271: 366–369.

Yeh SR, Musolf B, Edwards DH. 1997. Neuronal adaptations to changes in the social dominance status of crayfish. J Neurosci 17:697–708.

Zhang B, Harris-Warrick RM. 1994. Multiple receptors mediate the modulatory effects of serotonergic neurons in a small neural network. J Exp Biol 190:55–77.