J Comp Physiol A (2000) 186: 575–582

ORIGINAL PAPER

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Effects of exogenous serotonin on a motor behavior and shelter competition in juvenile lobsters (*Homarus americanus*)

Accepted: 14 April 2000

Abstract Three experiments were conducted to determine (1) the pharmacodynamics of 5-hydroxytryptamine in juvenile lobsters; (2) the effects of 5-hydroxytryptamine, using a range of dosages, on a motor behavior used to escape an aversive situation; and (3) the effect of doses that did and did not inhibit this motor behavior on measures of dominance and shelter competition. The fate of 5-hydroxytryptamine in hemolymph over a 60-min post-injection period showed that the concentration fell rapidly to a low plateau that was maintained for at least 1 h. Low doses of 5-hydroxytryptamine did not affect locomotor behavior, but higher doses inhibited it. Dominance and subsequent possession of a shelter were unaffected by a low dose of 5-hydroxytryptamine but a higher dose that inhibited locomotion resulted in lobsters that lost fights and did not secure or retain possession of the shelter. In the context of dominance and shelter competition, we were unable to demonstrate any advantage of the low dose of exogenous 5-hydroxytryptamine and a severe disadvantage with the higher dose. Previous reports of transient increases in aggression in 5-hydroxytryptamine-treated subordinate lobsters did not take into account motor inhibition as a possible critical variable in aggression.

Key words Serotonin · 5-Hydroxytryptamine · Motor behavior · Shelter competition · Aggression

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Abbreviation *5-HT* 5-hydroxytryptamine

Introduction

There is evidence that some forms of decapod crustacean aggression may be modulated by serotonin (5-hydroxytryptamine; 5-HT) (Antonsen and Paul 1997; Huber et al. 1997a, b; Huber and Delago 1998). Exogenously administered 5-HT usually results in American lobsters (*Homarus americanus*) and three species of crayfish (*Procambarus clarkii*, *Astacus astacus*, and *A. fluviatilis*) assuming a posture reminiscent of that of "dominant" lobster or crayfish in an aggressive encounter (Livingstone et al. 1980). In the same study it was demonstrated that exogenous octopamine usually results in "submissive" postures in the same species. However, in the crayfish, *Cherax destructor*, exogenous serotonin produces a submissive posture and octopamine produces an aggressive one (McRae 1996).

The effects of 5-HT and octopamine on postures and behavior, as well as the underlying mechanisms, clearly differ among crustacean species (Antonsen and Paul 1997). An example of a difference in the underlying mechanism can be found in Pasztor and MacMillan (1990).

These initial studies suggest an effect of these amines on the aggressive behavior of decapod crustaceans. There are, however, no reports of exogenous 5-HT influencing aggression in the context of the establishment of dominance relationships or territoriality in crustaceans as might be expected from these early findings, but there is some evidence of such effects in insects (Kostowsky and Tarchalska 1972). However, there is evidence that 5-HT administered to already subordinate lobsters and crayfish does result in a small increase in fighting (Huber et al. 1997a, b; Huber and Delago 1998) when they are reintroduced with a dominant adversary into a small aquarium with no known contestable resource. A more ecologically representative paradigm (i.e., one in which a vital resource is being contested) using unfamiliar

conspecifics in conjunction with multiple doses might reveal a 5-HT-mediated dominance effect.

In a study by Arnesen and Olivo (1988) it was demonstrated that 5-HT administered to a tethered crayfish (*P. clarkii*) partially immersed in water and resting on a floating foam rubber sphere reduced both locomotor movements and the optokinetic response to black and white lines on a revolving drum. Aggio et al. (1996) found that relatively high doses of 5-HT resulted in long postural stereotypy similar to that reported by Livingstone et al. (1980). Serotonin has a quite different effect in the green crab (*Carcinus maenas*). During daylight, when the control crab moves hardly at all, 5-HT-treated animals locomote more than controls but are somewhat uncoordinated, and there appears to be no pattern to their movements (McPhee and Wilkens 1989). These findings suggest a potentially confounding effect of 5-HT on specific interactional behaviors (such as "aggression" of various types) in decapods, i.e., the reduction in locomotion following administration of this amine.

Previous work has reported, in great detail, the aggressive behavior between decapods in small observation aquaria (Huber and Kravitz 1995; Huber et al. 1997a, b; Huber and Delago 1998; R. Huber, personal communication). This behavioral paradigm, with its small arena, may be eliciting what Hediger termed a "critical reaction" (Hediger 1950; Lorenz 1966). A critical reaction occurs when antagonists are crowded together with no possibility of escape. Such crowding is not what usually occurs in the natural behavior of either lobsters or crayfish. The behavioral interactions observed in a critical reaction may not generalize to other aggressive behaviors found within functional contexts such as shelter defense (e.g., O'Neill and Cobb 1979; Karnofsky and Price 1989; Peeke et al. 1995; Figler et al. 1998), protection of a food source (Barki et al. 1997; Ranta and Lindström 1992), or protection of eggs during embryogenesis and development (Figler et al. 1995b, 1997a, b, 1998). The frequency, duration, and length of fight may well differ when one animal is defending a resource, and the other animal, if not victorious, has the opportunity to leave the field or at least move well along the gradient toward the boundary of the critical distance (Hediger 1950).

The aggression experiments reported here made use of larger experimental aquaria to avoid eliciting a critical reaction. The aquaria also contained a valuable resource (a shelter, which is of particular importance to juveniles (Atema and Voigt 1995; Spanier et al. 1998; also see Peeke et al. 1998), and a situation where the lobster that did not possess the shelter could escape visual, as well as physical, contact with the other pair member. Finally, rather than focusing primarily on the interactions between the antagonists, we used contest outcome and shelter possession as our main dependent variables. Shelter possession and defense are certainly forms of dominance, and dominance is asserted and maintained by aggression or the threat of aggression.

In experiment 1, we determined the time-course of exogenously administered 5-HT in the circulation of the lobster. In experiment 2, we evaluated the effects of five doses of 5-HT on a measure of motor behavior – escaping from a horizontal ledge in the water column to reach the substrate. A juvenile lobster placed on a narrow platform or ledge will, within a few seconds, move to an edge and dive to the substrate (G.S. Blank and H.V.S. Peeke, unpublished observations). We used this very reliable response as an indicator of motor behavior (i.e., does 5-HT affect the latency of response?). Based on the work of Arnesen and Olivo (1988) mentioned above and our own pilot work, we hypothesized that the effect of 5-HT would be to increase the latency of the lobster reaching the substrate. In experiment 3, we used the highest dose of 5-HT that did not affect the locomotor response and another that inhibited it in order to test effects of 5-HT on social dominance and shelter competition. We chose the two doses to evaluate the effects, if any, of this motor inhibition on 5-HT-modulated aggression. It may be that the increases in aggression that have previously been reported (Huber et al. 1997a, 1997b; Huber and Delago 1998) are mediated by motor inhibition, in that the subordinate animal is less able to withdraw/escape and hence is forced to fight, thus escalating aggression between the adversaries. A submissive pair member would appear to be less submissive if it did not move rapidly in an attempt to escape.

Materials and methods

Experiment 1: pharmacodynamics of 5-HT

Distribution and metabolism of 5-HT in the hemolymph

To determine the time-course of 5-HT in the circulation, six juvenile lobsters (*H. americanus*) were injected with ^{3}H-5-HT. The animals, full-sibling females that had been reared in the laboratory, had a mean ($\pm$SD) weight of 101.0 $\pm$ 16.0 g. The lobsters were in intermolt and were injected with a mixture of 0.1 μCi g^{-1} of ^{3}H-5-HT binoxalate (27.8 μCi mmol^{-1}; NEN Life Science Products) and either 0.3 mg kg^{-1} or 3.0 mg kg^{-1} of unlabeled 5-HT (Sigma) dissolved in lobster saline (Chang et al. 1999). Three lobsters were used for each concentration.

The lobsters were placed into aerated jars containing 1 l of ambient (13 °C) seawater. Temperature was maintained at 13 °C by placing the jars into a tank containing flowing ambient seawater. Injections of the labeled and unlabeled 5-HT were made intramuscularly into the first abdominal segment to one side of the ventral nerve cord. The needle was held in place for 10 s following the injection to minimize loss of the 5-HT via bleeding. Immediately thereafter (0 min time point), a 0.1-ml sample of hemolymph was obtained via a syringe from the base of a walking leg. The lobster was submerged in a jar containing 1.5 l of seawater and immediately a 0.5-ml sample of water was removed for counting. Hemolymph and water samples were similarly obtained from each animal after 5 min, 10 min, 20 min, 40 min, and 60 min. The samples were analyzed by means of scintillation spectrometry.

Metabolism of 5-HT

After 60 min, the three lobsters injected with 3.0 mg kg^{-1} of unlabeled 5-HT (along with the labeled 5-HT) were bled to exsanguination and the hemolymph was pooled. The hemolymph was frozen. Following thawing, the clot was broken up manually and 0.1 ml of the serum was removed and added to 0.3 ml of methanol.

The precipitated proteins were centrifuged and 50 µl of the supernatant were analyzed by HPLC. The system consisted of two Waters 501 pumps, a Waters 484 variable-wavelength absorbance detector, and a Vydac C_{18} reverse-phase column (0.45 cm × 22 cm). Flow rate was 1.0 ml min^{-1} with a linear gradient of 10–100% methanol in 10 mmol l^{-1} KH_2PO_4 over 20 min. The eluent was monitored for absorbance at 280 nm and 0.5-ml fractions were collected and analyzed via scintillation spectrometry. Radioactivity in the fractions was compared to the elution of external standard 5-HT.

Experiment 2: substrate-seeking behavior

Animals

Sixty intact, intermolt juvenile American lobsters were used in this experiment. Carapace lengths ranged from 47 mm to 63 mm, and weights ranged from 84 g to 210 g. The lobsters were hatched in kreisels and then individually housed in small opaque plastic containers with fiberglass mesh floors within a flow-through seawater system (Chang and Conklin 1993; Conklin and Chang 1993). As the young lobsters grew, the size of the plastic containers was increased. Water temperature ranged between 12 °C and 14 °C, close to the temperatures that lobsters seek in studies of behavioral thermoregulation (Crossin et al. 1998). Water level was maintained at 10 cm within the housing compartments. The compartments were covered with opaque plastic sheeting, except during experimental days (to permit the lobsters to adapt to the lighting of the laboratory) and for feeding, routine data collection, maintenance and cleaning. Lobsters were fed three times per week on a diet of frozen fish and shrimp. On experimental days feeding was postponed until tests were completed for that day. Room light/dark cycle was 14L:10D. Salinity was 32 mg ml^{-1}.

Apparatus

The experimental device (i.e., a horizontal ledge) was contained in a 50 cm × 30 cm × 132 cm (h × w × l) aquarium with a constant flow of filtered seawater (12–14 °C) that was drained by overflow. At each end of the aquaria a 19 cm × 19 cm × 40 cm (l × w × h) concrete block was placed in an upright position such that a 10 cm × 118 cm (w × l) opaque black Lexan (polycarbonate plastic) plank created a "walkway" between the two concrete blocks. The Lexan plank was placed such that the rough side was the top surface upon which the lobster was positioned. The walkway was 40 cm above the substrate that consisted of 9 cm of coarse sand substrate.

Procedures

Each lobster was randomly assigned to either the control group (CNTL) receiving a single injection of crustacean saline, or one of five experimental groups, each receiving a different dose of 5-HT. Serotonin and crustacean saline were pre-mixed (except in saline alone group), aliquoted into tubes, and then frozen. Tubes were thawed 30 min prior to use and kept on ice. The doses of 5-HT were as follows (mg kg^{-1}): 0.3, 0.45, 0.6, 1.2, and 3.0. These doses were chosen because preliminary findings in this laboratory indicated that doses greater than 3.0 mg kg^{-1} produced a chronic submissive posture, and lower doses produced little or no noticeable changes in lobster aggressive posturing as described above.

Once animals were assigned to treatment groups, individuals were then placed in a 12 cm × 20 cm × 5 cm (l × w × h) holding tray, covered with approximately 1 cm of seawater, and transferred to the experimental test room. This room was similar in characteristics to that of the holding facility (i.e., same light/dark cycle, overhead lighting, and water temperature as described above). Animals were removed from the holding tray and given the appropriate injection via a 28-guage needle in the abdominal muscle on either the left or right side avoiding the abdominal ganglia.

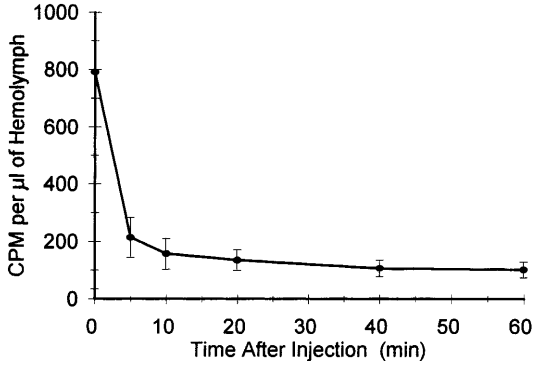

Fig. 1 Amount of radioactivity in the hemolymph of lobsters following injection of tritiated 5-hydroxytryptamine (^{3}H-5-HT) with either 0.3 mg kg^{-1} or 3.0 mg kg^{-1} of unlabeled 5-HT. Injection of 5-HT was made just prior to the first sampling at 0 min as described in the Materials and methods. Hemolymph was removed at subsequent time points and analyzed by scintillation spectrometry. Since the data for the two group of lobsters (0.3 mg kg^{-1} and 3.0 mg kg^{-1} of unlabeled 5-HT) were not significantly different, the data were combined ($n = 6$) for each point. Means ± SD are shown

After injection, the animal was replaced in the holding tray for 30 min to allow transient responses to the injection to dissipate and to use a period of time during which the levels of circulating 5-HT would remain somewhat constant (Fig. 1). The animal was then placed in the test aquarium on the walkway at one end facing the opposite end. Commencing with release of the animal, the latency to leave the walkway and reach the substrate was timed. If an animal failed to leave the walkway in 120 s it was removed and a default latency of 120 s was recorded. At the end of testing, the lobster was netted and returned to the main laboratory and replaced into its plastic cubicle.

Experiment 3: shelter competition and retention

Animals

Sixty-four intact, intermolt juvenile lobsters were used in this experiment. Carapace lengths ranged from 47 mm to 62 mm, and weights ranged from 83.7 g to 192.4 g. All animals were maintained in conditions identical to those in experiment 2. The environmental conditions, including lighting, water temperature, aeration and feeding regimes were also the same.

Apparatus

Three identical individual aquarium environments were used to observe shelter competition. Each aquarium was 76 cm × 30.5 cm × 48.2 cm (l × w × h). Filtered seawater flowed into each aquarium and was drained by overflow. Constant aeration was maintained by a single air stone. Coarse sand (9 cm) covered the bottom of each aquarium. Each aquarium contained a 20-cm square cement block with a tunnel through it. The cement block was placed such that one opening faced out into the aquarium and the other was placed against the aquarium end wall. Along both sides of each block, a 5 cm × 20 cm × 20 cm (w × l × h) cement block was placed to fill the entire end of the aquarium except for the tunnel (shelter), thus permitting a lobster to occupy only the shelter, the top of the cement blocks or remain in the open area of the aquarium. The shelter was 20 cm deep, 11 cm high at the sides, and 13.5 cm high in the middle, thus forming an arched ceiling through the length of the shelter. Three sides of the aquarium were covered with black plastic sheeting leaving only the side facing the observer uncovered.

Procedures

All lobsters were weighed, measured and paired for size and weight several days prior to testing. Dyads were composed of pair members of the same sex only, since we identified a significant advantage in shelter competition favoring males versus females of the same size (Peeke et al. 1998). Once pairs were formed they were randomly assigned to one of three treatment conditions consisting of a control (CNTL) and two experimental groups injected with 5-HT (0.3 mg kg^{-1} and 3.0 mg kg^{-1}; 5-HT 0.3 and 5-HT 3.0, respectively). For the CNTL group, both pair members were injected with lobster saline and for the experimental groups one pair member was injected with 5-HT and the other with lobster saline (Chang et al. 1999).

For all shelter competition pairings the procedure was as follows: on the day of pairings, lobsters were taken from their individual compartments and gently placed in 12 cm × 20 cm × 5 cm (l × w × h) holding trays filled with seawater to a level approximately 1 cm above the lobster's carapace and moved to the experimental test area. The test area was located in the same room as the holding area; therefore the light cycle, room temperature, water temperature and all other environmental factors were identical to the housing area. Once individual lobsters were brought to the experimental area they were removed from the holding tray and given their respective injection (i.e., lobster saline, or one of the two doses of 5-HT). All injections were administered as described in the procedure for experiment 1. After the injection, the animal was returned to the holding tray for 30 min. At the start of the behavioral observations, an observer, who was uninformed of the drug status of the members of the pair, was seated in front of the aquarium. One pair member was marked with a spot of typewriter correction fluid, by which only the experimenter, but not the observer, knew the status of the marked lobster.

Upon the expiration of the 30-min waiting period, paired animals were placed in the aquaria distally from the shelter cave. The animals were initially restrained behind a plastic T-shaped (as viewed from the top) barrier that permitted neither a view of the open aquarium area and shelter nor each other. When the two lobsters were both facing the shelter end simultaneously, the T partition was quickly removed providing access to the entire aquaria. At the same time a timer was activated.

Observations were focused on the dominance/subordinance interactions between pair members. A dominance sign (see Figler et al. 1995a) was defined as any approach, threat (e.g., chelae display), or attack (e.g., chelae strike, grabbing, lunging, pushing) by one pair member that was immediately followed by the opponent retreating (e.g., tail-flipping or scurrying backward) or displaying a submissive posture. A submissive posture involves holding its body flat against the substrate, with the pereiopods pointing forward and being held somewhat flattened (Scrivener 1971; Atema and Voigt 1995).

To be declared the winner of an encounter, one pair member had to accumulate six consecutive dominance signs, but these did not have to be identical behaviors (see above). Once a dominance sign had occurred, a 10-min time period commenced. If no further agonistic interactions occurred, the one with the most recent dominance sign was declared the victor. If, however, during this 10-min period, a dominance sign was followed by one from the other pair member (i.e., a contest reversal), the first pair member's score was returned to zero and the other animal was designated as having one dominance sign. A new 10-min time period then began. If, at any time, there was an aggressive interaction with no dominance sign being produced by either pair member (i.e., an aggressive standoff; Figler et al. 1995a), the dominance sign count for both pair members was returned to zero. A bout was terminated and no winner declared if no aggression occurred during the first 30 min of the encounter, or if no further aggression by either pair member occurred within 30 min after an aggressive standoff.

A tie was declared if the pair members shared the shelter without any aggressive interaction and neither one was in submissive posture. This shelter sharing had to be evident after a 30-min period following the initial commencement of the encounter, with no aggressive interaction during that period; at the end of the 30-min period after a standoff had occurred, with no further aggression; or if the 10-min period had elapsed since the last dominance sign with no further aggression.

Systems identical, or virtually identical, to this have been used in fish (de Boer and Heuts 1973; Figler et al. 1976), and invertebrates (crayfish: Figler et al. 1995a, b, 1997a, 1999; Blank and Figler 1996; lobsters: Figler et al. 1997b, 1998).

In addition to the observations made during the agonistic encounters, recordings were made of which animal was the first to enter the shelter, which animal possessed the shelter at the end of the encounter, and which animal possessed the shelter 24 h after the initial encounter.

Results

Pharmacodynamics of 5-HT

The data in Fig. 1 show that there is a rapid clearance of about 75% of the injected ^{3}H-5-HT from the hemolymph during the first 5 min following injection. This clearance is likely due to rapid distribution in the body and subsequent tissue uptake and/or binding as opposed to excretion or other loss to the medium. This pattern was the same for both of the 5-HT concentrations used and the data were therefore pooled. This explanation for the decline in hemolymph radioactivity (due to tissue distribution and not excretion) is confirmed by the data in Fig. 2 indicating that after an initial rise after 5 min, there is little appearance of additional radioactivity in the water following the injection. The total amount of radioactivity found in the water after 60 min accounts for approximately 10% of the initially injected ^{3}H-5-HT. Most of this radiolabel was probably due to leakage from the injection site. Much of this is attributable to the relatively high amount of label seen at 0 min point (670 ± 257 cpm ml^{-1}).

We next wanted to verify that 5-HT remained in the lobster during at least the first hour after injection.

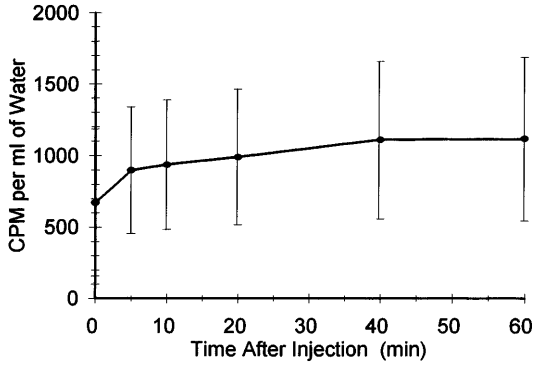

Fig. 2 Amount of radioactivity in the water in which the lobsters described in Fig. 1 were placed following injection of ^{3}H-5-HT (see Materials and methods). Samples (0.5 ml) were removed at the indicated time points and analyzed by scintillation spectrometry. Since the data for the two groups of lobsters were not significantly different, the data were combined ($n = 6$ for each time point). Means ± SD are shown

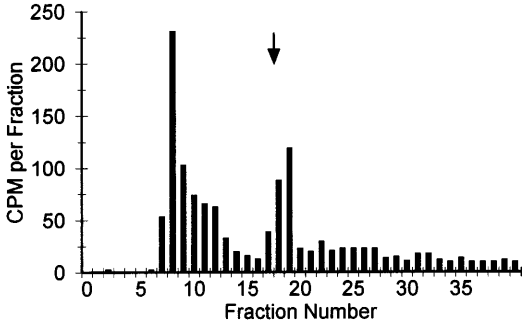

Fig. 3 Amount of radioactivity per fraction (0.5 ml) following HPLC of the hemolymph of lobsters 60 min after injection of ^{3}H-5-HT with 3.0 mg kg^{-1} of unlabeled 5-HT (see Materials and methods). The *arrow* indicates the elution position of an external 5-HT standard

Hemolymph was collected from the lobster used in Fig. 1 and extracted as described in Materials and methods. Following HPLC, we observed that 19.3% (fractions 17–19; Fig. 3) of the total recovered radiolabel co-migrated with an external 5-HT standard. Much of the injected neurotransmitter appeared to be metabolized to a more polar compound (41.4%; fractions 7–13; Fig. 3). The identity of this compound was not determined.

Substrate-seeking behavior

Statistical treatments were performed with non-parametric methods insofar as none of the measures used were normally distributed. A Kruskal-Wallis one-way ANOVA revealed a significant dose effect on latency to reach the substrate ($H(5) = 20.6$, $P = 0.001$; Fig. 4). Subsequent two-tailed Mann-Whitney Rank Sum Tests ($P < 0.05$) revealed that the three higher drug doses had reliably longer latencies than the CNTL: CNTL (median = 4.2 s) versus 0.6-mg kg^{-1} group (median = 19.7 s; $T = 66.5$, $P = 0.0041$), CNTL versus 1.2-mg kg^{-1} group (median = 41.3 s; $T = 70$, $P = 0.0091$), and CNTL versus 3.0-mg kg^{-1} group (median = 39.2 s; $T = 59.0$, $P = 0.0001$). No significant difference between the CNTL and the 0.3-mg kg^{-1} group (median = 4.8 s; $T = 91.5$, $P = 0.33$), nor between CNTL and the 0.45-mg kg^{-1} group (median = 9.4 s) was found ($T = 82.5$, $P = 0.096$).

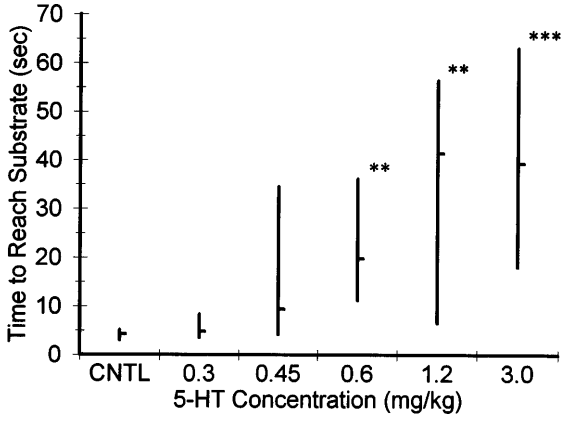

Fig. 4 Latencies for lobsters to leave an exposed ledge and gain the substrate 30 min after administration of one of five doses of serotonin or a saline control injection. Medians and the 25% and 75% quartiles are shown. *Asterisks* indicate significance at the $P < 0.01$ (**) and $P < 0.001$ (***) levels

Shelter competition and retention

Nine pairs of lobsters, matched for weight and carapace length, were observed in CNTL versus CNTL dyads. Of the nine encounters, one resulted in two animals sharing the shelter after the 1st day, but only one inhabiting it 24 h later. Of the eight remaining dyads, exactly half of those entering the shelter first had been determined to be the victor. Of those determined to be the victor by the dominance criteria, all eight possessed the shelter at the end of the bout ($P = 0.008$). Two-tailed binomial tests, $P < 0.05$ (Siegel 1956), were used for data analyses. When testing directional hypotheses based upon previous findings, one-tailed probabilities were used where noted. Peeke et al. (1998) have reported reliability of shelter possession across a 4-day period in juvenile *H. americanus*. In the present experiment, of those pair members that possessed the shelter at the end of the bout, only one was displaced by the opponent after 24 h, representing a significant shelter retention effect ($P = 0.035$, one-tailed).

Twelve pairs of lobsters, composed as above, were observed in the CNTL versus 0.3-mg kg^{-1} group. Seven of the 12 bout victors were those pair members given 0.3 mg kg^{-1} of 5-HT. This difference in proportions did not reach statistical significance ($P > 0.05$). In terms of which pair member first found the shelter and entered it, 8 of the CNTL lobsters were first, and 4 of the 0.3-mg kg^{-1} group were first ($P > 0.05$). Interestingly, 6 of those that entered the shelter first did so while backing away from an antagonist with which it had a claw-lock on, or was claw-locked by, the antagonist (a claw-lock consists of a firm grip by at least one of the chelipeds of one member of the pair on the other pair member). The same 7 of the 12 lobsters possessing the shelter at the end of the bout were the pair members given 5-HT. Twenty-four hours later, 6 of the 0.3-mg kg^{-1} 5-HT group possessed the shelter, and 6 of the CNTL did. It is interesting that of the 12 animals that won the bout and held shelters at the end of the 1st day, 9 possessed them 24 h later. This represents a shelter retention effect that approaches significance ($P = 0.073$, one-tailed).

Twelve pairs of lobsters, matched by weight and carapace length, were used in the third treatment group in which one pair member received an injection of 3.0 mg kg^{-1} of 5-HT, and the control was injected with saline. One pair was discarded because the animal receiving the 5-HT injection assumed a defensive posture that lasted for over 30 min.

Ten of the 11 bout victors and those entering the shelter first were controls (non-5-HT-injected), both significant findings ($P = 0.012$). The same 5-HT-injected lobster that won the encounter and entered the shelter first, possessed it at the end of the bout and 24 h later. Overall, there were six reversals in shelter possession 24 h later, a non-significant effect ($P > 0.05$, one-tailed). However, 5 of the 6 pairs that showed reversals were from saline-injected to 5-HT-injected animals. It is unlikely that the 5-HT was still active in these animals 24 h after the first encounter.

The length of time from the removal of the separating T partition until dominance criteria were met by the victorious pair member significantly differed among the treatment groups (Kruskal-Wallis ANOVA, $H(2) = 7.59$, $P = 0.022$). Subsequent Mann-Whitney Rank Sum Tests (two-tailed, $P < 0.05$) revealed that the two 5-HT groups were significantly different from each other (5-HT 0.3 median = 65.5 min; 5-HT 3.0 median = 130.0 min; $T = 172$, $P = 0.015$). The CNTL group (median = 95.0 min) was significantly faster than the 5-HT 3.0 group ($T = 67.5$, $P = 0.044$). It is our impression that this prolongation of time to dominance criteria in the 5-HT 3.0 group was not due to a prolonged defense but rather to locomotor slowing, although we cannot quantify this impression. The CNTL and 5-HT 0.3 groups were not significantly different.

Discussion

The results of experiment 1 on the pharmacodynamics of 5-HT indicate that the injected 5-HT circulates rapidly throughout the hemolymph and that significant amounts of injected 5-HT remain in circulation after the injection and throughout the 30 min until the commencement of experiment 2. Most of the radioactive 5-HT was metabolized to a more polar product. The identification of this product is unknown. In addition, whether this metabolic product has any biological activity is presently unknown. Similar data were obtained in the crayfish *Oronectes limosus* (Hoeger 1990). In both our experiment and those of Hoeger (1990), it appears that in the limited number of decapod crustacean species examined, 5-HT metabolism does not involve the formation of 5-hydroxyindole acetic acid. This latter compound is the primary metabolite of 5-HT in vertebrates (Van de Kar 1991). Kennedy (1978) determined that one of the principal metabolites of 5-HT in the lobster is a sulfated conjugate. The actual structure of the metabolite, however, was not elucidated.

The results of experiment 2 on substrate-seeking behavior show that at doses of 5-HT higher than 0.3 mg kg^{-1}, there is an increase in escape time as measured by latency to dive from a ledge in the water column to find substrate. This substrate-seeking response may be a somewhat general characteristic in decapods as it has been observed in two other species (*Pacifastacus leniusculus* and *P. clarkii*; H.V.S. Peeke and J. Sippel, unpublished observations). It also appeared that the movements of lobsters injected with the higher doses of 5-HT were somewhat less coordinated than the low-dose and control animals. This is consistent with the report of McPhee and Wilkens (1989) on the locomotor activity and 5-HT in the green crab (*C. maenas*).

Based on the results of experiment 2, we chose two doses of 5-HT to use in experiment 3 on shelter competition and retention. We selected the maximum dose that was not different from controls on the latency of the substrate seeking response (0.3 mg kg^{-1}) and a higher dose (3.0 mg kg^{-1}) that did have an inhibitory effect. We chose 3.0 mg kg^{-1} because it was the highest dose we tried and it had no more adverse effect on the escape response than did several lower doses.

Baseline data regarding social dominance and shelter competition in the test aquaria were obtained from nine size-matched pairs of juvenile lobsters that were each injected with lobster saline. It was demonstrated that the animal entering the shelter first did not necessarily win the bout nor capture the shelter. The animal that won the dominance contest was significantly more likely to retain the shelter at the end of the bout, and the animal that held the shelter at the end of day 1 also held the shelter when observed 24 h later. O'Neill and Cobb (1979) have previously found a direct relation between dominance and subsequent shelter possession in adult *H. americanus*. It is clear that the determination of social dominance by either dominance encounter outcome criteria or shelter possession provides a valid measure of this construct.

Twelve other pairs of lobsters were used to compare a saline injected animal with a pair mate that had received 0.3 mg kg^{-1} of 5-HT. This dose was found not to inhibit the substrate-seeking response (experiment 2). Exogenous 5-HT at this dose did not predict either the winner of the bout as measured by dominance criteria nor possession of the shelter. Although not quite reaching statistical significance, a majority of those pair members that held shelters after day 1 also did so 24 h later. This effect, being close to the findings for the non-drug pairings, reveals that this dose did not significantly influence social dominance, shelter possession or retention of the shelter.

The high-dose 5-HT condition involved 11 pairs, comparing a saline-injected lobster with a pair mate that had received 3.0 mg kg^{-1} of 5-HT. This dose resulted in only one 5-HT-treated animal winning the bout – the same animal that was the only one in possession of the shelter after day 1. After 24 h, when it would be expected that the 5-HT would have diminished, five lobsters from the 5-HT group overcame their opponents and possessed the shelter, and none reversed in the other direction, i.e., from 5-HT to saline. From the results of the high-dose pairs it is clear that 5-HT had a detrimental effect on the animal's ability to assert dominance and to capture and hold the shelter.

The combined results from experiments 2 and 3 suggest that if 5-HT does bring about an inhibition of adaptive motor responses (e.g., substrate seeking or subordinate retreat), then the results found by Huber et al. (1997b), in which a subordinate lobster appears to be more aggressive after being administered 5-HT, may be the result of a similar mechanism. Both motor responses, diving from the ledge to the substrate and moving away from a dominant adversary, involve retreating/escaping from an aversive stimulus. Furthermore, Yeh et al. (1996) found that 5-HT inhibited the lateral giant tail flip command neuron in subordinate animals which would be integrally involved in rapid retreat. The subordinate animal in the Huber et al. (1997b) study may have delayed performance of submissive behaviors, leading to a prolongation of fighting because the dominant lobster was confronted with a subordinate animal that could not rapidly retreat. The dominant animal, in turn, may have delayed and/or altered its behavior in other ways, perhaps appearing less dominant, and hence eliciting more aggression from the subordinate lobster. Therefore, by delaying or inhibiting normal motor behavior (i.e., retreat by a subordinate), 5-HT may indirectly, rather than directly, increase aggression between pair members in an already established dominance relationship. It is interesting to note that Huber et al. (1997b) and Huber and Delago (1998) did not find any dominance reversals with 5-HT administration to already subordinate pair members. In our experiments, of the six pairs that showed reversals in possession of the shelter 24 h later, five of them involved eviction of a saline-injected animal by a 5-HT-injected pair member, well after the effects of 5-HT would have diminished.

Whatever the mechanisms of the behavioral effects of exogenously increased 5-HT are on aggressive behavior, it is clear that they involve more than a direct increase in aggression of the subordinate lobster itself or a change in behavioral arousal (as suggested by Arnesen and Olivo 1988). The results of the present experiments revealed no effect on social dominance and shelter competition for the lower dose of 5-HT that did not affect the motor behavior; and the higher dose that did affect motor behavior reliably produced a subordinate animal. It would appear from these results that experiments involving 5-HT on aggression could be confounded by its effect on locomotion, particularly where the paradigm restricts the ability of the contestants to move apart during the encounter.

Acknowledgements We thank S.A. Chang and W.A. Hertz for technical assistance, Profs. E.A. Kravitz, R. Huber and D.H. Edwards for helpful input, and the reviewers who improved this manuscript. This paper is funded in part by a grant from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, US Department of Commerce, under Grant NA66RG0477, project number R/A-111A through the California Sea Grant College System, and in part by the California State Resources Agency (to E.S.C.). The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its sub-agencies. The US Government is authorized to reproduce and distribute for governmental purposes. These experiments comply with the "Principles of animal care," publication No. 86-23, revised 1985 of the National Institute of Health and the current laws of the USA.

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