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Biogenic Amines and Aggression: Experimental Approaches in Crustaceans

Key Words
Aggression
Crustacea
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Fluoxetine (Prozac)
Amine metabolites
Sulfate conjugation

Abstract
This review summarizes our experimental approaches attempting to link amines and their metabolites to aggression in crustaceans. The results demonstrate (i) that agonistic behavior in crustaceans can be quantified, (ii) that the amines themselves have telling and subtle effects on the fighting behavior of animals, (iii) that pharmacological interventions are possible that might allow a biochemical dissection of the underlying mechanisms involved in processes like decision making in these animals, and (iv) that selective metabolites of amines are excreted in the urine of lobsters where they may serve behavioral roles. Many of the studies presented here are preliminary. Nonetheless, we believe the results are provocative and nicely complement previous detailed physiological, morphological and biochemical studies exploring the roles of amines in aggression in crustaceans. We expect that the continued use of this invertebrate model system will allow us to gain considerable insight into, and understanding of, the role served by biogenic amines in a complex behavioral process like aggression.

Introduction

It is an honor for us to have been invited to present our work in this symposium dedicated to the memory of Walter Heiligenberg. Walter was an outstanding person, a superb scientist, an excellent neuroethologist, and a good friend. His untimely death was a great loss to all of us. We dedicate this article to Walter's family and to the memory of a wonderful person.

Our studies deal with aggression in crustaceans, and it seems fitting that some of Walter's earliest studies dealt with the same behavioral theme. Among these are his classic, much quoted studies showing that eye bar position is important in aggression in certain species of cichlid fishes [Heiligenberg et al., 1972]. In addition, Walter pioneered the use of quantitative procedures to analyze aggressive tendencies in animals, measured the probabilistic occurrence of certain behavioral actions, and demonstrated that the more a cichlid fish attacks conspecifics, the less likely it is to be disturbed by frightening stimuli, and that the converse is also true [Heiligenberg, 1965a, b; Heiligenberg and Kramer, 1972; Heiligenberg, 1973]. These were important

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and novel contributions to a complex field, and it is a tribute to Walter's foresight that the questions he began to address over 20 years ago remain unresolved in the literature. Moreover, such questions still are under active investigation in many laboratories at the present time: the studies we present here deal with many of the same themes.

Aggression is an important part of behavior, essential for the acquisition of food, shelter and mates in most species. Unbridled aggression, like the violence so prevalent in human society, is likely to be an exageration of the ritualistic events that symbolize normal agonistic encounters between conspecifics. We have very little understanding of the mechanisms underlying such aberrant human behavior. Since aggression is so fundamental a part of the behavioral repertoire of animals, however, it may be resonable to anticipate that certain of its underlying mechanisms would be highly conserved. If so, then valuable insights into human aggression may be attainable by studying animal model systems. Some of the best studied models of aggression examine dominance relationships [cf. Raleigh et al., 1991; Raleigh and McGuire, 1991]. With such systems one can ask: what are the rules that govern the establishment and maintenance of a dominance hierarchy; can we unravel the neuronal circuitries involved; and is it possible to define the physiological, cellular and molecular changes taking place in the nervous system mediated by the social interaction and associated with dominant or subordinate status?

Amines, such as serotonin and norepinephrine, have been suggested to serve important roles in aggression in most species of animals, including humans [cf. Kravitz, 1988; Coccaro, 1989; Raleigh et al., 1991; Raleigh and McGuire, 1991; Brunner et al., 1993; Miczek et al., 1994; Saudou et al., 1994]. For the last 15 years, we have been examining the possible role of two amines, serotonin and octopamine (the phenol analogue of norepinephrine), in agonistic (fighting) behavior of lobsters. These studies derive from an initial observation by Livingstone et al. [1980] that amines injected into the haemolymph of intact lobsters trigger postures resembling those seen in dominant (serotonin) and subordinate (octopamine) animals. In the intervening years studies from this laboratory have (i) defined the mechanism of the triggering of opposing postures by amine injection [Harris-Warrick and Kravitz, 1984]; (ii) mapped individual serotonin- [Beltz and Kravitz, 1983] and octopamine-containing [Schneider et al., 1993] neurons in the ventral nerve cord of lobsters (the lobster central nervous system); (iii) identified the sub-set of serotonin-containing neurosecretory cells concerned with postural regulation [Beltz and Kravitz, 1987]; (iv) mapped the distribution of the axonal arbors of these cells [Livingstone et al., 1981; Beltz and Kravitz, 1987]; (v) shown colocalization and the differential appearance during development of the peptide proctolin with serotonin in these cells [Beltz and Kravitz, 1987; Beltz et al., 1990]; (vi) demonstrated that the serotonin-containing neurosecretory neurons function as 'gain-setters' involved in posturally relevant neuronal circuitry [Ma et al., 1992]; (vii) begun to define the synaptic input to these neurons [Weiger and Ma, 1993], and (viii) demonstrated that the serotonin and octopamine phenotypes appear in individual neurons at very different times during the embryonic and larval development of lobsters [Beltz et al., 1992; Schneider et al., 1996].

Other investigators have carried out important experiments on the role of amines and amine neurons in the central nervous systems of crustaceans, utilizing both lobster and crayfish test systems for their studies [Glanzman and Krasne, 1983; Sandeman and Sandeman, 1994; Sandeman et al., 1995; Yeh et al., 1996, 1997]. Of these, the studies of most direct relevance to the experiments we present here were carried out recently by Yeh et al. [1996, 1997]. These studies show that associated with the establishment of dominance in crayfish, the serotonergic modulation of particular synaptic inputs to the terminal abdominal ganglion is changed. In animals kept in isolation serotonin facilitates transmission between sensory input to the ganglion and the lateral giant (LG) neurons (believed to be used in escape and possibly in fighting behavior in these animals). Over several days following the establishment of dominance, serotonin receptor properties change: serotonin depresses transmission between sensory input and the LG in subordinate animals; and serotonin facilitates the same synaptic contacts in dominant animals, but apparently by a different receptor mechanism than that seen in the isolated animals. Thus the relative importance of different receptor sub-types at these synaptic contacts changes depending on the social status of the animals. Such changes are very slow in onset and reversal, raising the possibility that they are due to changes at the level of gene expression of serotonin receptor subtypes.

The detailed studies that we and others have carried out shed light on important developmental and physiological roles served by amines in the ventral nerve cord of crustaceans. While they suggest that amines may be involved in important aspects of behavior in these animals, they do not directly demonstrate a role for these substances in crustacean fighting behavior. With the studies described in this review article, however, we begin to explore this far more difficult problem.

Experimental Results

Four sets of experiments are described here, all utilizing lobsters and crayfish as the test experimental organisms. I. Quantitative analysis of agonistic behavior in lobsters: Before pharmacological intervention was used to explore the roles of amines in behaving lobsters, we felt it necessary to establish the normal pattern of fighting behavior in these animals in quantitative terms. A summary of these recently published studies [Huber and Kravitz, 1995] is presented here. II. Serotonin injections in subordinate animals – influences on fighting behavior: In these experiments we inject or infuse serotonin into the haemolymph of intact lobsters and crayfish, wait for the static postural changes to decay away, and then observe the effects of serotonin injection on fighting behavior. III. Prozac blocks serotonin uptake in lobsters – its possible usefulness in behavioral studies: In these experiments we demonstrate that fluoxetine (Prozac) is an effective blocker of serotonin uptake in lobsters allowing the possible use of this drug in behavioral experiments. IV. The excretion of amine sulfate metabolites in the urine of lobsters: In these experiments we show that a metabolite of serotonin, serotonin-O-sulfate, is excreted in the urine of lobsters. This raises the possibility that substances of this type might be used in intraspecific communication. While many of the studies presented below are in preliminary form, we believe they suggest new routes towards a more precise definition of the roles of amines in fighting behavior in crustaceans.

I. Quantitative analysis of agonistic behavior in lobsters: These experiments utilize behaviorally naive juvenile lobsters *(Homarus americanus)* raised in isolation since the postlarval 4th stage when they begin their benthic existence. When closely matched in size, weight, and molt stage, juvenile animals readily engage in agonistic encounters (bouts) which vary in duration and intensity. Using video analyses of fights and a variety of statistical techniques: (1) an ethogram of agonistic behavior was constructed; (2) the temporal structure of the behavior was identified, and (3) the inter-individual variation in these patterns was evaluated [Huber and Kravitz, 1995].

The experiments demonstrate that resolution of intraspecific conflicts in juvenile lobsters proceeds according to strict rules of conduct. The agonistic encounters feature an escalating sequence of up to six stereotypical behavioral patterns, and they end when one opponent retreats from the encounter. A typical bout begins with extensive threat displays (meral spread), followed by highly ritualized aggressive acts (do-si-do) and restrained use of the claws (claw lock). If no decision is reached during these early stages, fights escalate to brief periods of unrestrained use of the large claws (tearing and ripping) which may result in serious physical damage to opponents. Fights end with the withdrawal of one of the animals, after which the winner initiates further bouts until the subordinate animal consistently retreats from the advance of the dominant animal. Predictions of game theory (i.e. assessment strategies) provide a useful theoretical framework for understanding fights

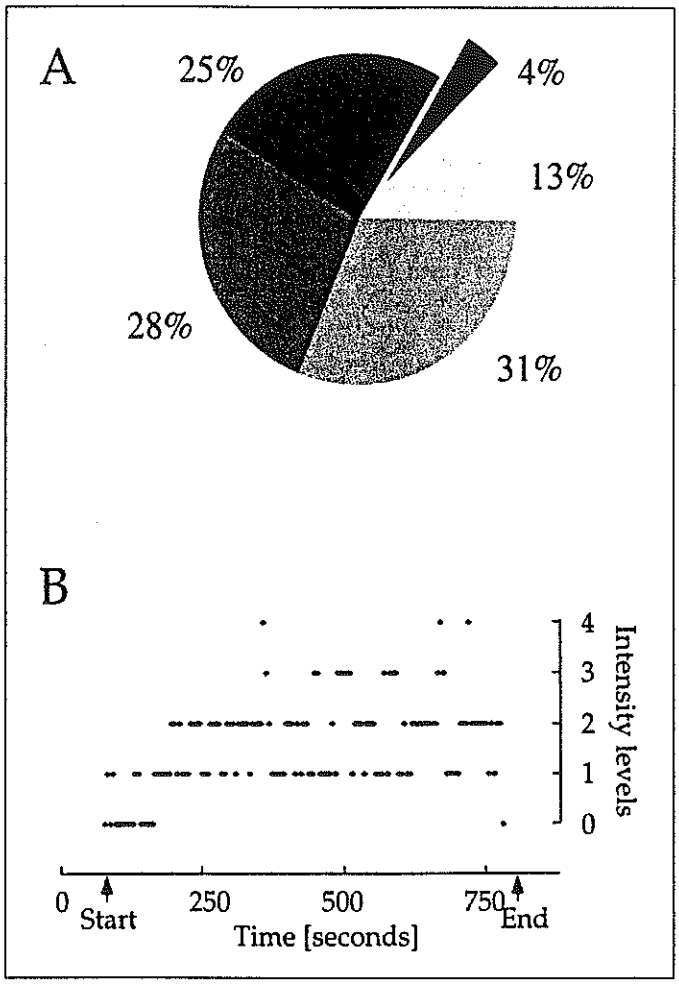

Fig. 1. Distribution of fighting intensities during agonistic interactions of juvenile lobsters. **A** The increasing grey scale levels indicate the increasing levels of intensity. Thus in a typical bout, animals spend 13% of the time without direct interaction (level 0), 31% of the time showing threat postures without physical contact (level 1), 28% of the time in physical contact but without using their claws (level 2), 25% of the time using claws to grab the opponent (level 3), and only 4% of the time in unrestrained use of their claws (level 4). **B** Demonstration of the temporal structure and gradual increase in intensity level in a single bout. The more violent aspects of a bout (level 4) tend to occur in brief periods late in the encounter.

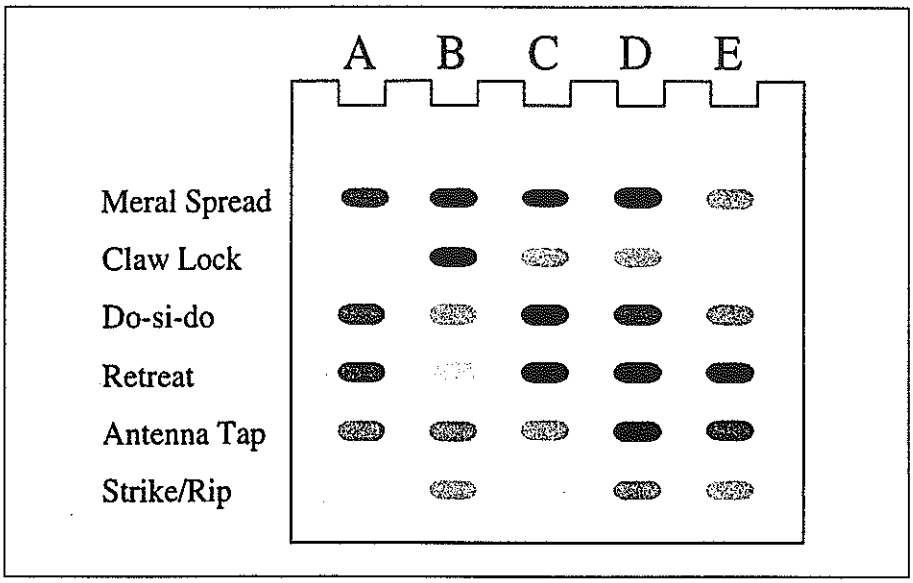

Fig. 2. Inter-individual differences in the patterns of fighting behavior for five individuals (**A–E**). This 'behavioral gel' depicts frequencies as the darkness of the corresponding bands.

in lobsters, and the system appears well designed to reduce the potential for damage.

Ethograms can be constructed from such analyses, and an illustration of the relative proportions of time that animals spend at different levels of fighting intensity during encounters is presented in figure 1A. An example from a single bout (fig. 1B) demonstrates the presence of a temporal structure to these fights, during which intensity levels increase gradually. The more violent aspects of a bout occur only for brief periods of time late in an encounter.

Although all animals tested adhere to these general rules, there is considerable variability in the percentage of time animals spend utilizing different behavioral patterns, and in the exact temporal sequence. A 'behavioral gel' (fig. 2) can be used to depict the percentage of time individual animals spend displaying each behavioral pattern during an agonistic encounter. It is readily seen that while all animals show similar patterns, the percentage of time they do so varies widely. This raises interesting questions of whether animals have preferred patterns of fighting (like people), or whether the patterns are variable from fight to fight and result from the unique social interaction between the two animals during that one encounter. Further experiments will be required to select between these possibilities. The ability to quantify fighting behavior, however, now allows us to carry out pharmacological or behavioral interventions with the animals, to ask whether important changes in behavior result. That is the direction we follow in the next set of experiments.

II. Serotonin injections in subordinate animals – influences on fighting behavior: In these experiments, crayfish *(Astacus astacus)* and lobsters *(Homarus americanus)* were used. In the lobster experiments, hierarchies were established between matched pairs of animals, the opponents were separated, and then a second control encounter was staged to confirm the dominance relationship. The subordinate animals were injected either with saline, or with saline containing serotonin at a final haemolymph concentration of 10^{-4} to 10^{-5} *M*. After a 30–45 minute recovery period, the postural changes produced by the serotonin injection disappeared. The injected subordinate animal was placed back in contact with the previously dominant individual, and the resulting agonistic interactions were videotaped for analysis. In the crayfish experiments, tips of indwelling fused silica fine bore cannulae were placed into the pericardial sinus of animals through a needle hole in the carapace and glued in place with cyano-acrylate (fig. 3). Pairs of animals differing in size by 30% were chosen and the cannulae were implanted in the smaller animals. In 10 pairs studied in this way, the larger animal assumed a dominant position within 30 minutes (pre-injection). During subsequent interactions, the subordinate animal was continually infused with saline for 30 minutes (control injection) using a syringe pump, and then with serotonin at 3 μg/min for the next 60 minutes (5-HT injection). The infusion pump then was turned off, but the behavioral interactions were monitored for another 60 minutes (post-injection).

The results of the crayfish and lobster experiments are in good agreement. Both demonstrate important increases in

the levels of fighting activity in subordinate animals following injections of serotonin. Based on data from eight saline-injected control and eight serotonin-injected subordinate lobsters, the fighting of the serotonin injected individuals greatly exceeded that of the animals injected with saline alone. After injection, and after a few initial withdrawals over the first five minutes, the serotonin injected subordinate animals begin to advance on the former dominants, and engage them in combat. The resulting encounters are usually prolonged and escalate through claw-locking to maneuvers involving unrestrained use of the claws. The rules of fighting appear indistinguishable from those seen in normal fighting, but the formerly subordinate animal now is more likely to initiate encounters and less likely to withdraw. In some cases this results in a reversal of the previous dominance relationship. The effect may last up to several hours, but more usually the winner of the first encounter is reestablished as the dominant animal within an hour. In the crayfish experiments, the smaller animal becomes subordinate within a few minutes during the pre-injection period and no longer resists the advances of the winner. A continuous infusion of saline during the control injection period results in no qualitative or quantitative changes in fight duration and intensity, but switching the infusion to serotonin (serotonin-injection period) produces a marked increase in fighting activity. This effect is slow in onset with a significant rise seen 35 min after the start of serotonin injection. When serotonin infusion was stopped (post-injection period), fighting slowly returned to the original levels, and the initial dominance hierarchy was re-established.

It is interesting to ask what has changed in the fighting behavior of a subordinate crayfish or lobster as a result of a serotonin injection. Subordinate animals ordinarily do not engage dominant animals in fights. This is particularly true if animals have fought for a prolonged period of time, and if the fights have escalated through multiple levels of intensity before a decision has been made. In the crayfish experiments we used multivariate statistical analyses to ask which variables in fighting behavior were altered as a result of a serotonin injection. By far the most important influence of serotonin injection on fighting was a decrease in the probability of the subordinate animal withdrawing from an escalating encounter.

Under normal circumstances, when crayfish and lobsters are faced with large asymmetries in body size, vigor, stamina, or fighting dexterity, they quickly retreat from advancing opponents. Serotonin-injected animals, however, repeatedly engage the opponent and continue fighting in the face of situations that ordinarily would result in withdrawal. It appears, therefore, that for a period of time, amine injection has altered the decision of an animal to back off and become subordinate. This raises the possibility that decision making in agonistic encounters may involve a balance between serotonin and some other substance or substances (octopamine?) at certain key sites in the brains of these animals and that we disturb that balance with the serotonin injection.

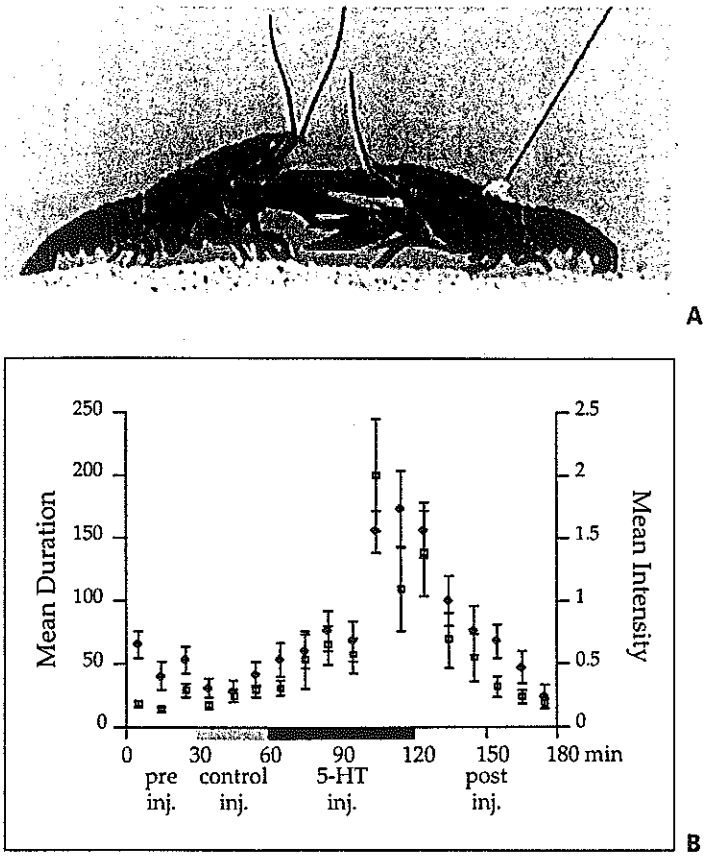

Fig. 3. The effects of serotonin injections on fighting behavior in subordinate crayfish. **A** A syringe pump is used to deliver test substances (8 µl/min) through fused silica fine bore cannulae into the pericardial sinus of freely moving crayfish during agonistic interactions. After 30 minutes without infusion (pre inj.), the pump is turned on, first with saline (control inj.) and then with serotonin (5-HT inj.). The behavioral interactions are monitored for a further 60 minutes after the syringe pump is turned off (post inj.). **B** The average duration of interactions in seconds (open squares) and the mean intensities (closed diamonds) for each 10-minute period of the experiment. The intensity levels ranging from 0 to 4 correspond to those described in figure 1.

In other species of animals, serotonin injections or treatment, or activation of serotonergic neurons, also can turn on or significantly influence important aspects of behavior. For examples, exposure to serotonin increases fighting behavior in species of ants [Kostowski and Tarchalska, 1972], induces swimming behavior in leeches [Willard, 1981;

Hossein-Hashemzadeh and Friesen, 1989], arouses feeding behavior in molluscs [Kupferman and Weiss, 1981; Yeoman et al., 1994], and enhances male mating, egg laying and other behaviors in nematodes [Loer and Kenyon, 1993].

III. Prozac blocks serotonin uptake in lobsters – its possible usefulness in behavioral studies: Any of several possible explanations could account for the behavioral results we obtain with serotonin injections into subordinate animals. The behavioral reversal could result from: (i) a postsynaptic mechanism in which relatively long lasting changes take place at synaptic sites someplace in decision-making centers within the lobster CNS; (ii) a presynaptic mechanism in which 'extra' serotonin, which can be released during behavioral encounters, is loaded into serotonergic nerve terminals during the period when high levels of serotonin exist in the haemolymph; (iii) the appearance of new behaviorally active metabolites of serotonin in the haemolymph, or (iv) other mechanisms. The half-time of removal of serotonin from haemolymph is about 10 minutes in lobsters. Therefore, by the time the previously subordinate animals are once again engaged in agonistic bouts (35–45 minutes), little of the originally injected serotonin remains in the haemolymph. We plan to utilize pharmacological reagents to attempt to distinguish between the various possibilities. Our first efforts in this direction are presented below, where we test whether the drug fluoxetine (Prozac) is an inhibitor of serotonin uptake in lobsters and ask whether uptake inhibitors might be useful tools in explaining the behavioral reversal.

A specific, high affinity Na^+-dependent serotonin uptake system exists in lobster tissues (Km=0.66 μM) along with a lower affinity, non-saturable uptake mechanism [Livingstone et al., 1981]. The high affinity uptake appears to be selective for serotonergic nerve terminals. In the original characterization of the uptake system we used second thoracic root neurosecretory sites to measure uptake. These contain a dense arbor of serotonergic nerve terminals derived from the A1 and T5 ganglia serotonergic neurons. In the present studies we utilized the same tissues to test whether fluoxetine inhibited serotonin uptake. Since there is great variability in the absolute amount of serotonin in each of the seven pairs of thoracic nerve roots containing neurosecretory sites, we used right and left roots from each segment as the experimental samples and their controls.

Our first experiments whowed no significant difference in the endogenous levels of serotonin or octopamine (as internal standard) when right and left roots from the same segment were compared (fig. 4A; serotonin – 815±77.7 pg/root; octopamine – 8,233±1,145 pg/root). Incubation of nerve roots with levels of serotonin (10^{-5} to 10^{-6} *M*) in the range of those producing behavioral effects on fighting leads to approximately a 60% increase in serotonin content (fig. 4B), but we have not yet demonstrated whether this 'extra' serotonin can be released with stimulation. With fluoxetine at 10^{-5} *M*, the uptake of serotonin is blocked (fig. 4B). Preliminary results suggest that the fluoxetine inhibition of serotonin uptake is competitive (fig. 4C, right side), but a more complete analysis is required to determine the kinetics of this inhibition. What may be of further interest is that while fluoxetine reduces and can completely block the uptake of serotonin, it shows little interference with the production of serotonin metabolites (fig. 4C, left side). Serotonin metabolites include a sulfate conjugate, a tentatively identified β-alanine conjugate, and a β-alanine, sulfate double conjugate [Kennedy, 1978]. The inhibitor results suggest that (i) the second, non-saturable uptake mechanism generates the pool of intracellular serotonin leading to the production of metabolites, and (ii) fluoxetine does not inhibit this second component of uptake. On the basis of previous studies [Livingstone et al., 1981], we anticipate that the second component is into non-serotonergic tissues, but further experiments will be necessary to confirm this.

In preliminary experiments fluoxetine was tested on intact animals. When injected alone at concentrations of 10^{-4} to 10^{-5} *M* it is not toxic to lobsters and has little or no effect on fighting behavior. In the immediate future we will test the effects of co-injection of serotonin and fluoxetine to see whether there is any interference with the behavioral reversal caused by serotonin. If the release of serotonin is important in agonistic encounters, it may be surprising that we see no acute effect with fluoxetine, since the block of serotonin uptake, and hence the increased availability of serotonin, should be immediate. On the other hand, when fluoxetine is used clinically in the treatment of depression, a similar absence of any acute beneficial effect is observed [Stokes, 1993]. It takes several weeks of daily drug therapy before any positive effects are seen from ingested fluoxetine. We plan to explore whether similar chronic effects are seen in lobster fighting behavior by utilizing a daily fluoxetine treatment regime coupled with regular tests of fighting ability.

IV. The excretion of amine sulfate metabolites in the urine of lobsters: In the final set of experiments described here, we explore the notion that status can be communicated between lobsters, and that this communication may be via the excretion of signalling molecules into the urine [Atema, 1986; Atema and Cowan, 1986; but see Snyder et al., 1993]. Here, we considered the possibility that amines,

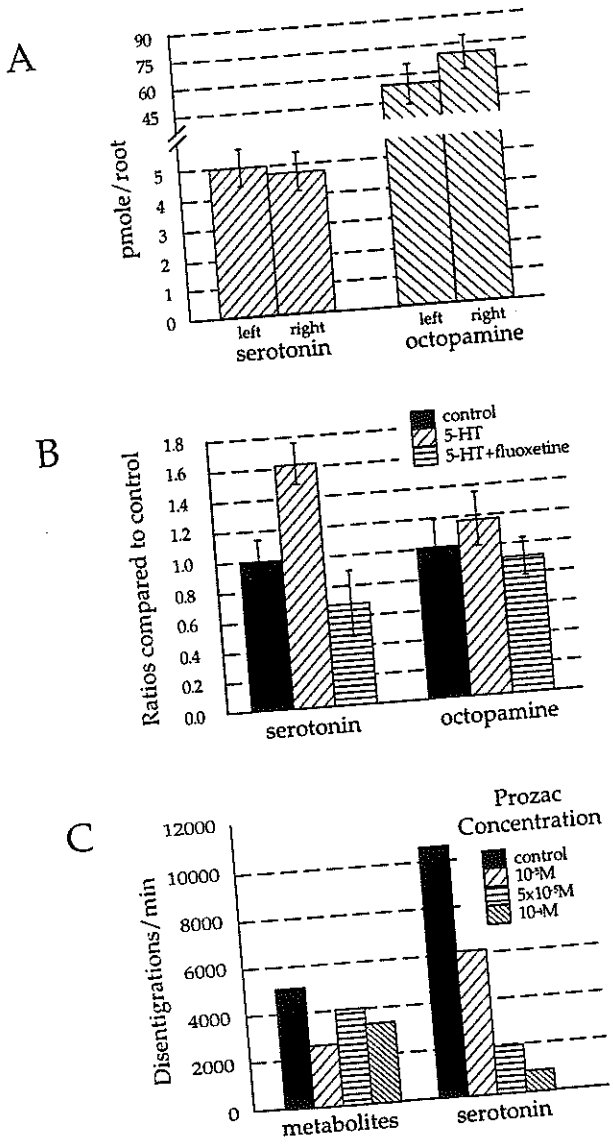

Fig. 4. Biochemical analyses of serotonin uptake in thoracic second nerve roots. Nerve cords were dissected and thoracic second roots isolated and incubated as described previously [Livingstone et al., 1981]. Serotonin, octopamine and amine metabolite levels were measured after incubation, extraction and separation of amines by HPLC. Tissues were homogenized in 0.1 *M* perchloric acid for extraction. Aliquots were run on an HPLC column (Ultrasphere 25×4.6 mm) in reverse phase, and amines and metabolites were measured using electrochemical and radiochemical detection methods. **A** Endogenous levels of amines were measured in paired left and right roots and found not to differ significantly in serotonin and octopamine contents. **B** The endogenous contents of serotonin and octopamine were measured in paired second nerve roots after incubation with serotonin (10^{-5} to 10^{-6} *M*) with or without added 10^{-5} *M* fluoxetine (Prozac). **C** Levels of radioactive serotonin and its metabolites found in tissues after incubation of tissues with ^{3}H-labelled serotonin (10^{-5} *M*) in the presence of different concentrations of fluoxetine.

or metabolites of amines might be components of a signalling machinery.

Lobsters were injected with radioactive serotonin, and urine was continuously collected in small bottles attached to the nephropores at the base of the antennules via plastic tubing (we thank Dan Wynstroem of the Atema laboratory for demonstrating this technology to us). The results showed that up to 30% of the radioactivity injected was excreted and collected in the urine over the next 30 hours (fig. 5A). Upon analysis by high performance liquid chromatography, none of the excreted radioactivity was present as serotonin; instead a single, unidentified, highly polar metabolite appeared to contain all of the excreted radioactivity (fig. 5B). As mentioned above there are three different metabolites of serotonin: serotonin-O-SO4; a β-alanine conjugate, and a β-alanine, sulfate double conjugate. These can be synthesized by many different lobster tissues. A separation of these conjugates generated from incubation of radioactive serotonin with lobster leg nerves is shown in figure 5C. The metabolite present in urine is identified as the O-sulfate conjugate by comparison with the synthetic metabolite (kindly provided by Dr. Richard A. Milius of the NIMH Chemical Synthesis program) and by demonstration of co-labelling of the metabolite with ^{3}H-labelled radioactive serotonin and ^{35}SO4 inorganic phosphate. To investigate the time course of inactivation of serotonin in the general circulation, lobsters were injected with tritiated serotonin, and the amounts of serotonin and its metabolites were measured in haemolymph samples taken at specific time intervals. The results showed that serotonin rapidly disappears from the haemolymph, while the sulfate conjugate, and to lesser extent the β-alanine conjugate, gradually accumulate (fig. 6). Earlier autoradiographic studies demonstrated that serotonin accumulates in serotonergic nerve terminals [Livingstone et al., 1981], while a second prominent site of uptake is into the cellular walls lining the haemolymph vessels [M.B. Kennedy, personal communication]. Presumably the latter site is where the amines are converted to sulfate metabolites which ultimately ar removed by excretory glands and excreted in the urin (fig. 6).

The excretion of sulfate conjugates of organic su stances in the urine in crustaceans have been reported other investigators [Schell and James, 1989; James et a 1991]. This is the first demonstration in these animals, ho ever, of the excretion of a biologically active amine a sulfate metabolite. In most animals indol- and catech amines are oxidized prior to excretion, a process that yi energy. The sulfate conjugate excreted here requires ATPs to form the sulfate bond and, therefore, is an ene

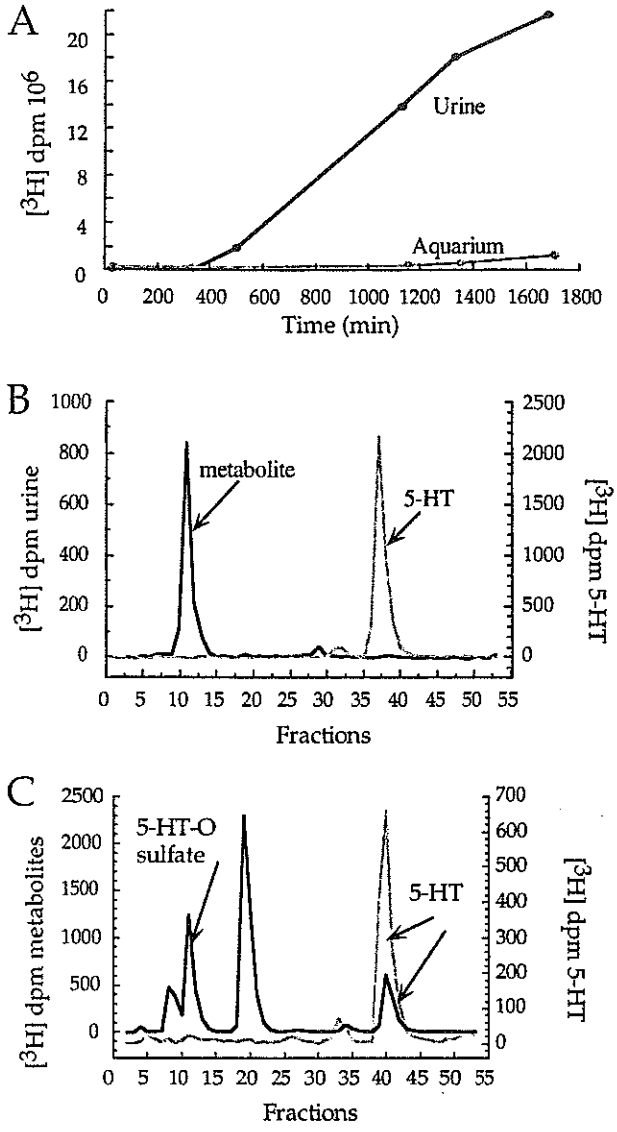

Fig. 5. Serotonin metabolites in urine and tissues of American lobsters. Animals with attached urinary pore catheters were injected with 150×10^6 dpm of ^{3}H-labelled high specific activity serotonin (**A** and **B**), or walking leg nerve tissues were incubated in radioactive serotonin and homogenized to extract metabolites (**C**). In **B** and **C** aliquots of the radioactive samples were separated by HPLC as above. **A** Cumulative amounts of radioactivity present in urine-collecting bottles and surrounding water. **B** HPLC chromatogram illustrating the location of the ^{3}H-labelled metabolite found in urine after haemolymph injections of serotonin. A second chromatogram run with approximately twice as much of the originally injected ^{3}H-labelled serotonin (5HT) is overlaid on the metabolite chromatogram. **C** Overlay of chromatograms of the ^{3}H-labelled serotonin precursor, and serotonin and its metabolites found in homogenized walking leg nerve tisue after incubation with ^{3}H-labelled serotonin.

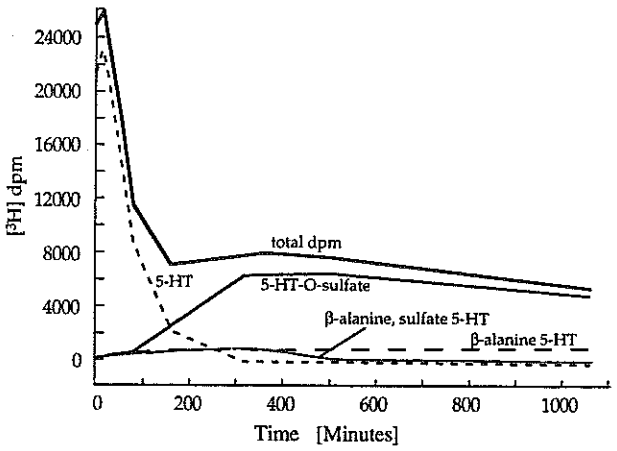

Fig. 6. Time course of serotonin disappearance and formation of metabolites after haemolymph injection of ^{3}H-labelled serotonin at 4 °C. Serotonin is removed rapidly from the haemolymph, taken up by tissues and converted into various metabolites which accumulate in the haemolymph. The principal metabolite is the O-sulfate conjugate, which ultimately is removed from the haemolymph by the green gland and excreted in the urine.

ically expensive metabolite to synthesize. This raises a possibility that these metabolites might be used for more interesting purposes than solely as excretory waste products. One such possibility is that amine metabolites are used in communication between lobsters. If so, and if lobsters can tell the difference between serotonin- and octopamine-sulfate conjugates (which we have not yet demonstrated to be excreted in urine), then the possibility exists that one animal has a way of knowing what is happening in the nervous system of another, by sampling the levels of amine metabolites in the urine. Future experiments will be required to explore such notions.

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