

# Dynamic Interactions of Behavior and Amine Neurochemistry in Acquisition and Maintenance of Social Rank in Crayfish

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## Key Words

Neurochemistry • Aggression • Crustacea • Serotonin (5-HT) • Selective serotonin reuptake inhibitor (SSRI)

## Abstract

This review summarizes a set of experimental approaches with which we explore fighting behavior in crayfish and the importance of aminergic systems in its control. Our results illustrate that agonistic behavior in crustaceans can be characterized within a quantitative framework, that different types of behavioral plasticity in aggressive behavior are in need of physiological explanation, and that pharmacological intervention involving serotonergic systems produces characteristic changes in fighting. Moreover, we attempt to identify changes in neurochemistry during the acquisition of social status. Many of the studies presented here summarize ongoing work. Nonetheless, results to date complement and extend previous detailed physiological, morphological and biochemical studies exploring the roles of amines in aggression.

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## Introduction

The depths of northern, aquatic, cold-temperate environments are ruled by well-armed and well-armored crustaceans. Clawed decapods, such as crayfish, lobsters and

their close phylogenetic kin, are arguably the most successful predators and scavengers of benthic habitats, with weights from a few hundred grams (crayfish) up to 20+ kg (lobsters), and occurring in very high numbers under suitable conditions. For instance, *Orconectes rusticus*, the species of crayfish studied here, reach densities well in excess of 20 individuals/m<sup>2</sup> in Lake Ontario [Stewart and Haynes, 1994]. Even in lobsters, where numbers have declined in recent decades due to intensive fishing, historical records tell a story of plenty. Referred to as a ‘poor man’s dinner’ they were gathered in large numbers at low tide [Wood, 1635] and individuals weighing around 5 kg were common [Gould, 1841].

Despite their numbers in a given area, these animals lead a solitary existence, occupy shelters singly, and most meetings are agonistic in nature. So, aside from mating behavior in some species [Atema, 1986] and pair formation in others [Seibt, 1974], clawed decapods remain as originally characterized ‘a solitary, aggressive animal in its natural habitat’ [Herrick, 1909]. It is likely that the existence of many close neighbors plays an integral role in crayfish biology as individuals frequently encounter conspecifics during their daily forays – we regularly observe agonistic encounters as well as matings between individual *O. rusticus* who roam the stream bottom during the day at our collecting sites on the Portage River near Bowling Green, Ohio, USA.

With conspicuous, potentially lethal claws [Marden, 1973], the resolution of intraspecific conflict in this group demands a measured strategy. In close accordance with predictions of game theory models of fighting, behaviors

**Fig. 1.** Two male crayfish (*Orconectes rusticus*) face each other during an agonistic interaction in our 600 l seminatural aquarium. Individuals appear to test each other's ability for physical superiority in a series of stereotyped, behavioral maneuvers. Unrestrained use of the claws only occurs in rare occasions and is largely limited to the later stages of an encounter.



during agonistic encounters feature a number of different stereotyped patterns, proceed with an escalating sequence of intensities, and end when one of the combatants withdraws. Shelters play a central role in crayfish [Capelli and Hamilton, 1984; Ranta and Lindstrom, 1993] and lobster biology [Karnofsky et al., 1989; Figler et al., 1998], providing protection from predation as well as aiding in access to food and mates [Hyatt, 1983; Atema, 1986]. Protective shelter is of particular importance for early juveniles, where attacks by predators are common [Lavalli and Barshaw, 1986; Barshaw and Lavalli, 1988]. Fighting behavior in our seminatural observation aquaria (fig. 1) occurs during acquisition or defense of a burrow in many instances, but also ensues when individuals encounter each other during regular forays away from cover. Given an arena size and features designed using appropriate ethological considerations [Goessmann et al., 2000; Huber et al., 2001], encounters also readily occur with similar characteristics in a simplified scenario and lacking any obvious resource, suggesting an inherent disposition for agonism towards conspecific opponents.

In a typical scenario, intensity of fighting increases in step-wise fashion beginning with threat displays upon first contact, followed by phases of ritualized aggression, restrained use of claws, and in rare instances ending in brief periods of unbridled combat [Bruski and Dunham, 1987; Huber and Kravitz, 1995; Huber et al., 2001]. The presence of such a structured behavioral system, combined with an opportunity to bring the analysis to the level

of individual neurons, thus offers us unique opportunities for exploring fundamental issues of interactions between aggression, dominance, and amine neurochemistry in this group. Work in our laboratories has employed interdisciplinary approaches to explore a variety of issues in aggression, ranging from self-structuring properties in hierarchy formation to a search for its underlying neurochemical mechanisms. In close collaborations with the labs of Edward A. Kravitz (Harvard Medical School, Boston, Mass., USA) and Barbara Beltz (Wellesley College, Wellesley, Mass., USA) four objectives have guided our research: (1) we have developed a quantitative behavioral framework for fighting behavior in crayfish; (2) characterized behavioral sources of variation in need of physiological explanation; (3) examined the behavioral consequences of pharmacological amine manipulations, and (4) attempted to identify changes in neurochemistry that accompany behavioral differences of social status.

### **A Behavioral Framework for the Study of Crayfish Fighting**

Dyadic conflict among crayfish features behavior that is distinctly structured [Bovbjerg, 1953], utilizes a number of stereotyped behavior patterns, and progresses according to a strict set of rules [Huber and Kravitz, 1995; Huber and Delago, 1998]. The early stages of crayfish encounters commonly include threat displays and ritualized aggressive acts.

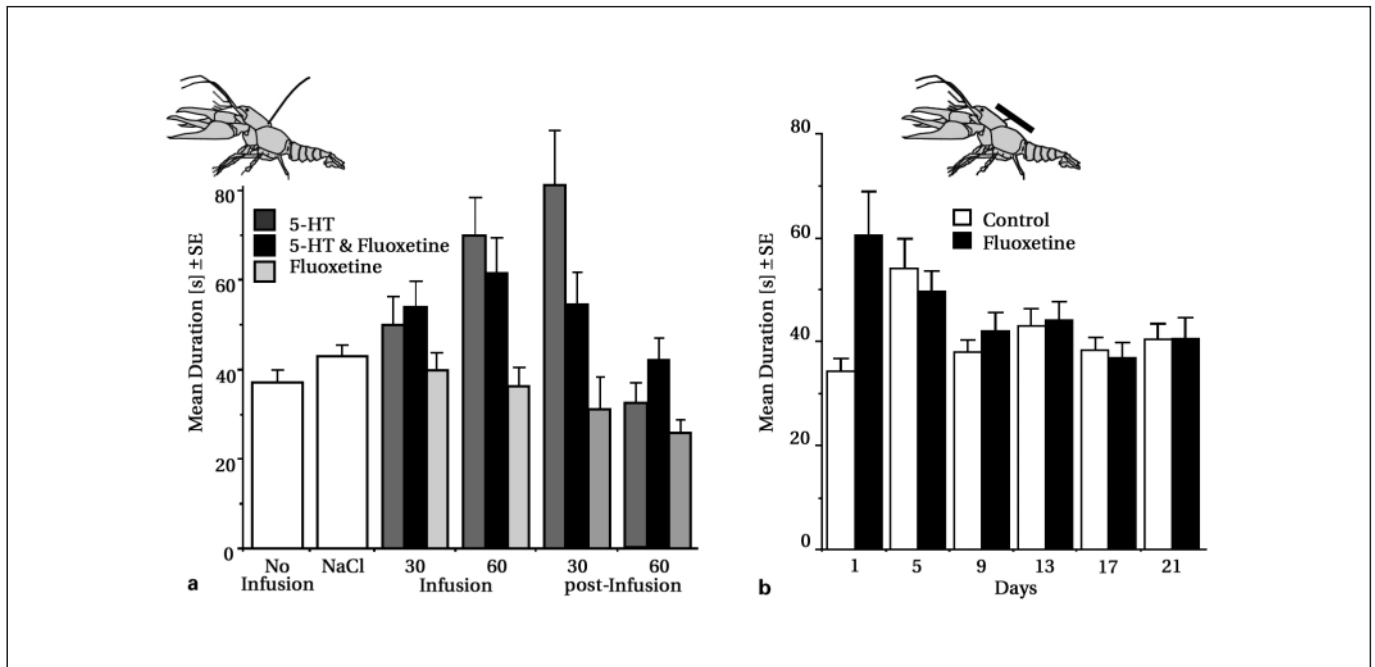
In a typical scenario encounters then continue with behaviors where claws are used in restrained fashion. If at that point fights have remained inconclusive (i.e. both individuals are still contesting the outcome), then the conflict may escalate to brief episodes of unrestrained use of the claws. The fight ends with the withdrawal of one opponent. The winner continues to initiate further bouts until the subordinate consistently retreats from the advances of the dominant. Behavioral ecological considerations provide a useful theoretical framework for an understanding of such behavior [Bovbjerg, 1956]. Game theory models of fighting [Parker and Rubenstein, 1981; Enquist and Leimar, 1983; Leimar and Enquist, 1984] predict that species equipped with damaging weapons will match characteristics predictive of eventual success in stepwise fashion and with escalating intensities. During this process individuals of similar strength acquire increasingly detailed information concerning the opponent's strength and fighting ability at a reduced risk [Dingle, 1983; Smith and Dunham, 1990, 1996; Rutherford et al., 1996]. By restricting injurious use of claws to cases of close matches in essential variables, this effectively reduces an individual's potential for sustaining physical damage. Assessment strategies thus predict that duration and progress of a fight is contingent upon a series of individual 'decisions', namely, whether an individual initiates an encounter, escalates to a higher intensity, retaliates if an opponent escalates, or retreats from further fighting. Consistent with its use in behavioral ecology, the term 'decision' is not meant to imply the operation of cognitive mechanisms. With success reinforced through evolutionary processes, it refers rather to the fitness advantage that a particular strategy confers upon its bearer. Using such concepts in a descriptive sense, decisions of individuals can thus be characterized as the probability with which a particular strategy will occur in a given context. In essence our work focuses to a lesser degree on what the animal does at any particular moment, but rather it attempts to describe in what way an animal approaches a given situation [Huber et al., 2001]. Ultimately this approach is used to provide an estimate of the individual's internal motivational state [Huber and Delago, 1998].

Paired dominance relationships form when the outcome of agonistic bouts creates a lasting polarity and subsequent social interactions become increasingly predictable [Francis, 1988; Drews, 1993; Guiasu and Dunham, 1997]. In crayfish, subordinates rarely engage dominants either due to a decrease in aggressive state, individual recognition of a proven superior opponent [Vannini and Gherardi, 1981], or, most likely, the detection of an opponent's relative dominance status [Winston and Jacobson, 1978; Francis, 1988;

Zulandt-Schneider et al., 2000]. Even in group situations, fighting consists mainly of paired encounters and interactions involving three or more individuals are rare. Repeated dyadic conflicts thus create a web of social relationships among members of a group [Bovbjerg, 1956; Lowe, 1956]. A hierarchy forms with self-organizing properties in (near) linear fashion [Vannini and Sardini, 1971; Atema and Cobb, 1980; Copp, 1986; Issa et al., 1999] with future success contingent upon previous outcomes [Goessmann et al., 2000] through winner/loser effects [e.g. Burk, 1979; Chase et al., 1994; Hollis et al., 1995; Daws et al., 2001].

### **Characterization of Behavioral Plasticity in Need of Physiological Explanation**

Quantitative behavioral techniques summarized above allow us to characterize behavioral changes that result from a variety of contexts, such as winning or losing a single encounter, fighting at different levels of intensity, or occupying high social status for various lengths of time. When pairs or groups of individuals are placed into an aquarium, the number of agonistic challenges, their mean duration and maximum intensity reached, are initially high but then decreased steadily as a hierarchy develops among them [Copp, 1986; Issa et al., 1999]. Sex and relative differences in body- and claw size (i.e. asymmetries in excess of 10%) are important predictors of dominance [Evans and Shehadi-Moacdieh, 1988; Barki et al., 1992; Figler et al., 1995; Pavey and Fielder, 1996]. However, in meetings of similarly-sized individuals, early wins in one or several of these component agonistic bouts created a lasting polarity for future fighting [Issa et al., 1999]. Consistent with effects reported from a wide range of species [Beaugrand et al., 1991; Jackson, 1991; Dugatkin, 1997; Hsu and Wolf, 1999], crayfish who recently prevailed in an interaction subsequently demonstrated an increased likelihood to win again, even in encounters with unknown conspecifics. Similarly, crayfish who experienced a recent loss exhibited a decreased likelihood of acquiring further wins. Thus with a given social experience, crayfish can be conditioned towards dominant or subordinate status. For example, initial dominance relations can be reversed by repeatedly providing subordinates with wins against third parties and the previously dominant one with repeated losses [Daws et al., 2001]. In such 'winner' and 'loser' effects, the aggressive state of subordinates is lowered while that of dominant individuals increases [Francis, 1983; Beacham and Newman, 1987; Chase et al., 1994].

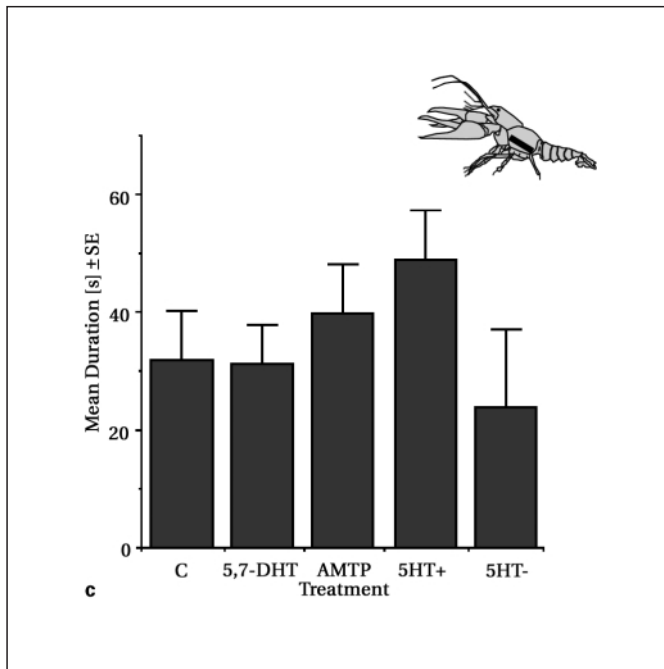


**Fig. 2.** Composite figure illustrates differences in fight duration resulting from pharmacological manipulations of crayfish serotonin systems. **a** Fine-bore fused silica capillaries were used to infuse serotonin, fluoxetine or both substances together into freely-moving, subordinate animals (all infused at 3  $\mu\text{g}/\text{min}$ ). Serotonin infusion resulted in longer fighting that persisted well after the infusion pump was turned off ( $F_{(5,2888)} = 14.777$ ,  $p < 0.001$ ). Multivariate statistical techniques (i.e. discriminant function analysis) revealed that longer bouts of fighting resulted from a decreased likelihood of retreat. Infusion of fluoxetine alone did not enhance aggression ( $F_{(5,2513)} = 1.130$ ,  $p = 0.342$ ), however, co-infused with serotonin resulted in a pronounced reduction of serotonin fight-enhancing effects ( $F_{(5,2941)} = 7.653$ ,  $p < 0.001$ ). **b** Chronic infusion of fluoxetine via osmotic minipumps increased duration of fighting during the early stages of treatment compared to animals receiving vehicle only ([treatment]  $F_{(1,2642)} = 22.160$ ,  $p < 0.001$ ; [day]  $F_{(5,2642)} = 2.9463$ ,  $p = 0.018$ ; [treatment\*day]  $F_{(5,2642)} = 3.724$ ,  $p = 0.002$ ). As with acute serotonin infusion, these differences in fighting were due to a decrease in the probability for retreat. **c** Duration of fighting in individuals which received chronic silastic implants containing either 5-HT synthesis inhibitors (5,7-dihydroxytryptamine or alpha-methyltryptamine) or serotonin at one of two different rates. No significant differences in fight duration existed among these groups ( $F_{(4,314)} = 1.06$ ,  $p = 0.374$ ).

In groups, linear hierarchies emerged which became increasingly stable over time. Winning influenced subsequent fighting behavior at two distinct time scales. In the short term, recent winners became less likely to retreat, even though other characteristics of fighting, such as chances for initiating, escalating or retaliating, remained essentially unchanged. Secondly, individuals who had occupied dominant positions for days became increasingly likely to escalate to higher intensities early in the encounter. Both effects biased the outcome of future interactions such that winning enhanced further success and losing decreased an individual's subsequent chances for dominance. Moreover, social conditioning as a result of previous wins or losses influenced both the dynamics of fighting behavior and the outcome of subsequent interactions on a timeframe of up to several days [Goessmann et al., 2000].

### Characterization of the Effects of Amine Manipulations

Numerous physiological mechanisms have been implicated in the control of variation in fighting behavior. Despite differences in the particulars, serotonin is strongly implicated in a neuromodulatory role for aggression across a wide variety of invertebrate and vertebrate taxa, including humans [Brunner et al., 1993a, b; Chen et al., 1994; Saudou et al., 1994; Nielsen et al., 1994, 1995; Kravitz, 2000]. Support has emerged from a broad range of experimental approaches including correlative evidence [Mehlman et al., 1994, 1995; Blumensohn et al., 1995; Reisner et al., 1996], studies of family histories [Brunner et al., 1993a, b; Coccaro et al., 1994; Virkkunen et al., 1995], dietary or neurotoxic amine depletion [Vergnes et al., 1988; Giammanco et al.,

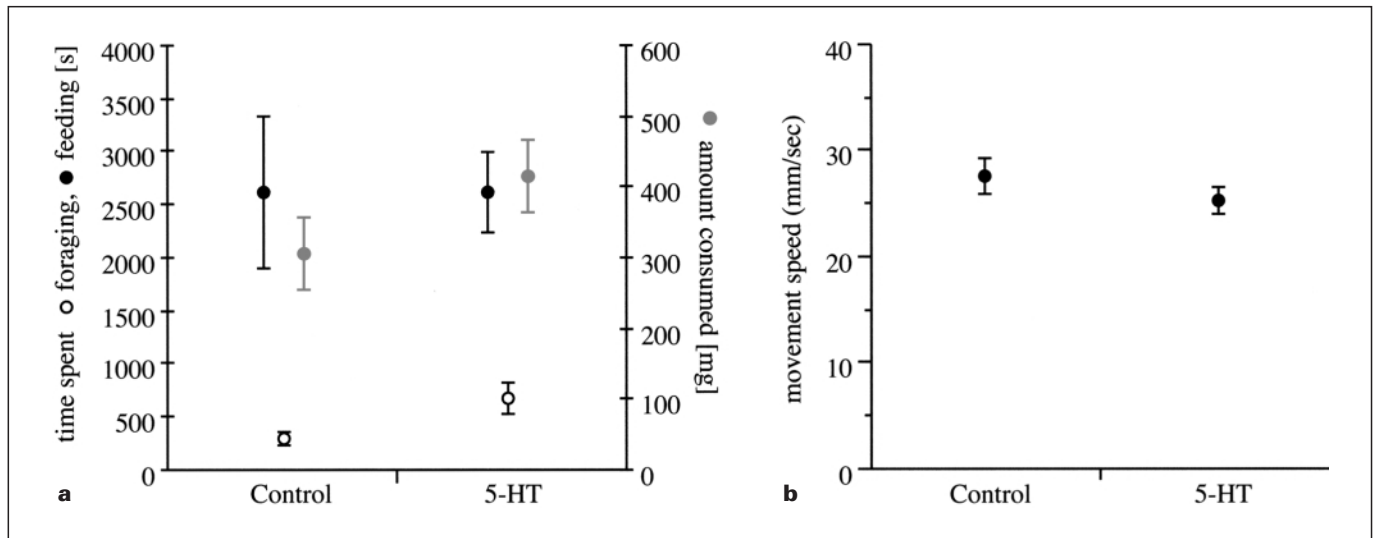


1990; Cleare and Bond, 1995; Doernberg et al., 2001], receptor pharmacology [Bell and Hobson, 1994; Mühlenkamp et al., 1995; Olivier et al., 1995], application of reuptake inhibitors [Olivier et al., 1989; Hilakivi-Clarke and Goldberg, 1993; Fuller, 1995, 1996], acute serotonin treatment [Pucowski et al., 1985; Maler and Ellis, 1987; Raleigh et al., 1991], and genetic knock-out studies [Saudou et al., 1994; Cases et al., 1995]. However, the nature of these relationships is not simple, and unraveling the precise role of the amine in aggressive behavior has proven particularly difficult. For example, disruption of monoamine oxidase A (MAOA), an enzyme involved in biogenic amine inactivation, leads to a marked increase in aggression and violence in rats [Cases et al., 1995] and humans [Brunner et al., 1993a, b], although our current understanding of serotonergic systems would have predicted the opposite. However, aside from serotonin, MAOA also metabolizes dopamine and other amines involved in affect, raising the potential for complex interactions between different neuromodulatory systems. A major caveat in studying amine systems is the inherent flexibility, dynamic nature, and feedback properties within different behavioral and physiological contexts [Yeh et al., 1997; Beltz et al., 1998; Bradbury, 2000].

In lobsters, crayfish, and other decapod crustaceans, activation of serotonin systems is closely associated with aggressive or dominant behavior [Antonsen and Paul, 1997; Edwards and Kravitz, 1997; Kravitz, 2000; Sneddon

et al., 2000; Tierney et al., 2000]. Such treatment altered decisions to retreat from opponents [Huber et al., 1997b; Huber and Delago, 1998], without changing the way fights were initiated, how they escalated, or their final outcome (fig. 2a). Treated subordinates continued to re-engage their opponents in extended bouts of fighting, even in situations that carry a substantial risk of injury. The most robust behavioral effects of serotonin emerged with continued infusion into freely-moving crayfish via a fine-bore canula and syringe pump. Aggressive state increased slowly with a peak after over one hour [Huber et al., 1997a]. Behavioral effects resulting from single or repeated injections of amine pulses proved somewhat less salient – possibly because such experimental treatments require that the individual is at the same time captured, restrained, pierced with a needle, and injected with a large bolus of serotonin. This may affect a variety of neurochemical (e.g. stress) systems and thereby confound any effects associated with the intended treatment itself. Moreover, serotonin-mediated effects appear to require a loading of pre-synaptic terminals (see below) with persisting augmentation of basal levels. Single pulses are metabolized rapidly [Huber et al., 1997a] and may thus be less efficient in producing behavioral effects. In addition to any natural, underlying complexities, methodological differences are thus likely to contribute to the diverse range of behavioral effects that have been reported for amine manipulations in decapods [e.g. Peeke et al., 2000]. Continuously infusing lower peak levels of serotonin [Huber et al., 1997a, b; Huber and Delago, 1998] may be preferable as it offers a less intrusive method of treatment, and may thus be less prone to artifacts. Moreover, while single injections may suffice to measure how in the short term a substance affects instances of behavior such as wins/loses, continuous amine-infusion may provide a better means for studying behavioral phenomena at longer time scales. Serotonin-associated changes in behavior accompanying such treatment closely resemble key characteristics observed in individuals who have recently won an encounter [Goessmann et al., 2000], suggesting the existence of a potential link.

Additional, distinct changes in behavior have been identified within the context of social interactions (see section II above) and are in need of physiological explanations. Injection of octopamine (i.e. the phenol analogue of norepinephrine) into squat lobsters was accompanied by an increase in escape behavior [Antonsen and Paul, 1997]. A linkage between steroid hormones and changes in the rate of escalation seems likely. Studies reporting such effects have focused on the (steroid controlled) molt cycle [Tamm and Cobb, 1978, 1980; Steger and Caldwell, 1983]; correlated



**Fig. 3.** Behavioral effects of acute treatment with serotonin. Measures of feeding and locomotion were obtained for crayfish (*Orconectes rusticus*) that had been injected with 3  $\mu\text{g}$  serotonin per gram body weight (1 mg serotonin:1 ml 125 mM saline) or with an equal volume of vehicle only. **a** Following treatment, individuals (weights: 6.2–22.2 g), which had not been fed for 14 days prior to the experiment were placed under a mesh cage (85 mm<sup>2</sup>) on one end of an observation tank (584  $\times$  305 mm). Consistent flow was provided by two water inlets located at the opposite end of the aquarium and a familiar food source (5 mm section of earthworm) was placed at the upstream end of the tank. Following a 30-min acclimation period the cage was removed allowing the animals to move freely around the aquarium. The amount of time to locate the food source (i.e. search time), time spent feeding once the food item had been found (i.e. feeding time) and the total amount consumed were quantified. One-way ANOVAs with  $\alpha$  adjusted for three comparisons using Dunn-Sedák ( $\alpha' = 0.017$ ) revealed no significant differences between groups in search time ( $F_{(1,19)} = 5.16$ ,  $p' = 0.036$ ), time spent feeding ( $F_{(1,19)} = 0.01$ ,  $p' = 0.996$ ) or the total amount consumed ( $F_{(1,19)} = 2.14$ ,  $p' = 0.161$ ). **b** Treated individuals (weights: 8.1–17.0 g) were acclimated for 30 min in a gravel lined, observation aquarium (400 mm<sup>2</sup>) by having a circular flowerpot (diameter = 100 mm) placed over them. The flowerpot was raised and the individual's movements were videotaped from above for 60 min. Locations (x and y coordinates) were obtained every 1.5 s from a series of digitized frames using video-tracking software (freely available at <http://caspar.bgsu.edu/~software/java/> based on Quicktime for Java libraries). The speed of locomotion was calculated as the average straight-line distance between consecutive captures for frames in which animals had moved. ANOVA revealed no significant main effect for treatment ( $F_{(1,64)} = 0.621$ ,  $p = 0.434$ ).

measures of aggressiveness with the profile of ecdysteroids such as 20-hydroxyecdysone [Baldaia et al., 1984; Graf and Delbecque, 1987; Snyder and Chang, 1991]; and increased aggression in stomatopods and lobsters by direct injection of ecdysone [Bollingbroke and Kass-Simon, 2001; Caldwell, pers. comm.].

In crayfish and lobsters serotonin is removed from the hemolymph within a few minutes, yet behavioral changes associated with amine infusions persist well beyond that. Observed changes in aggressive state may thus be due to one or several mechanisms [Huber et al., 1997a, b] including: (1) long lasting changes at post-synaptic sites located within decision-making centers of the nervous system; (2) increased release after 'extra' serotonin has accumulated in nerve terminals during the period when hemolymph serotonin levels were high; (3) the appearance of 'behaviorally

active' serotonin metabolites in the hemolymph; or (4) other neuronal mechanisms. We have begun to explore the possible role of these mechanisms in agonistic decision making using pharmacological manipulations with (1) application of fluoxetine [Huber et al., 1997a; Huber and Delago, 1998, Delago et al., in review]; (2) neurochemical disruption using the serotonin synthesis inhibitor 5,7-dihydroxytryptamine [Benton et al., 1997; Panksepp and Huber, in review]; and (3) chronic infusion of serotonin [Panksepp and Huber, in review].

Acute fluoxetine alone did not affect fighting of subordinate animals, but fight-enhancing effects of serotonin were greatly reduced (fig. 2a) in its presence [Huber et al., 1997a, b; Huber and Delago, 1998]. Fluoxetine, the active ingredient of Prozac, is a highly effective antidepressant in humans, however, its pharmacological profile and precise mode of

action are in dispute. We used osmotic mini-pumps to deliver continuous infusions of fluoxetine to both crayfish and lobsters for 21 days (fig. 2b). During chronic fluoxetine exposure animals were randomly paired with opponents at intervals of 3–4 days. Such treatment produced subtle, aggression-enhancing effects, particularly during the initial days of treatment [Delago et al., in review]. With behavioral effects generally similar to those associated with acute infusions of serotonin, individuals were less likely to retreat from larger opponents, resulting in longer, more intense fighting.

As with any effect of pharmacological manipulation, specificity is always a concern. Namely, aside from those behaviors of primary focus, how many other aspects of behavior are altered as a function of the treatment [Peeke et al., 2000]? We have further explored the behavioral specificity of serotonin treatment in crayfish by examining its effects on foraging and feeding behavior, as well as on movement patterns and space usage in an open field paradigm. Much like agonistic behavior, the acts of locating and consuming food are organized into separate behavioral stages that can be readily distinguished and quantified. The behavioral sequence begins with the distal sensation of waterborne chemicals through the antennules. If motivated, orientation towards the food source follows. When in close proximity, the crayfish then switches to local search strategies for precise localization of the source [Moore and Grills, 1999]. Rapid approach at close range and retrieval of the food item are followed by consumption until satiety. Injections of serotonin did not alter the latency to find a food source, the time spent feeding, or the amount of food eaten (fig. 3a). Moreover, we explored the effects of serotonin treatment on general locomotor arousal by measuring a variety of spatial parameters describing the movements of individual crayfish in an arena (e.g. total distance traveled, speed of movement, space utilization). Single injections of serotonin did not alter movement patterns or the usage of space in the arena (fig. 3b).

The results from experiments with chronic fluoxetine treatment indicated the need for a more thorough examination of the time course of plasticity in amine function. We have since explored the effects of chronic amine manipulations by exposing crayfish to serotonin and 5,7-dihydroxytryptamine via silastic implants [Panksepp and Huber, in review]. Biochemical as well as behavioral changes accompanying such manipulations were counter-intuitive and suggested the action of compensatory mechanisms (fig. 2c). Treatment with 5,7-dihydroxytryptamine for 5–16 days greatly reduced serotonin levels in all sections of the nervous system except the brain. However, different rates of

chronic serotonin treatment did not similarly increase its levels in the central nervous system. Long-term enhancement of serotonin levels may have boosted inactivating processes, and it is possible that compensatory processes effectively counteracted our experimental interventions. Despite amine depletion, behavioral differences did not match our initial predictions of a decrease in aggressive state. Crayfish treated with 5,7-dihydroxytryptamine fought with behavioral characteristics that were indistinguishable from those of controls. Moreover, marked differences in the rate of escalation were observed in individuals that had received serotonin despite the treatment's inefficacy to raise absolute levels. Specifically, infusion at a slow rate increased aggressive state, while a faster infusion rate effectively reduced fighting.

### **Characterization of Changes in Amine Levels during Initiation, Establishment and Reinforcement of Dominance Relationships in Crayfish**

Studies from this lab and others suggest the existence of complex links between aggressive behavior and neurochemical processes. Although considerable attention has thus far been focused on the behavioral effects resulting from experimental manipulations of amine function, amine correlates of aggressive state and social status have received much less attention. It is likely that behavioral and physiological levels of organization exert feed-back effects onto each other. Differences in the concentration of neuromodulators as a function of social status have been reported in blood [Rose et al., 1971, 1975; Raleigh et al., 1984; Schuhr, 1987; Knoll and Egberink-Alink, 1989; Shively et al., 1991] and brain regions throughout a range of vertebrate taxa [Kemble et al., 1990; Summers et al., 1997; Øverli et al., 1998; Winberg and Lepage, 1998].

In crustaceans, social status influenced both concentrations of 5-HT in hemolymph [Sneddon et al., 2000] and the efficacy of modulators at identified synapses [Yeh et al., 1996, 1997; Krasne et al., 1997]. Neurons within local circuits controlling tail flip, a common behavior of retreat [Glanzman and Krasne, 1983, 1986; Bustamante and Krasne, 1991], exhibited reduced responsiveness in the presence of serotonin, as well as changes in their excitability [Krasne et al., 1997]. Pharmacological evidence suggests that differences in social status produce altered expression of serotonin receptor subtype populations [Yeh et al., 1996, 1997]. Our recent work has examined whether central nervous system amine levels reflect the acquisition of social

status in the short-term, as well as long-term increases in body size during adult growth. Using HPLC with electrochemical detection we have explored such relationships for individual segments of the crayfish nervous system. Experimental conditions were selected to maximize differences in behavior between dominants and subordinates [Yue, 2001, Yue and Huber, in prep.]. Towards this goal, two crayfish were housed together and able to interact for 24 h. A single shelter was introduced after the initial determination of dominance. In this study no changes in amines accompanied an almost doubling of adult body weights from 5.8–10.3 grams. Amine levels thus do not increase in proportion with the rest of the body, and it seems that adult crayfish continue to grow around central aminergic systems. Allometric growth patterns in CNS structures are not unusual, having been reported in other systems [Hochner and Spira, 1987; Bloomfield and Hitchcock, 1991; Hill et al., 1994], and may be accompanied by surprising physiological properties [Edwards et al., 1994a, b]. Furthermore, results indicate that after 24 h no significant differences in the CNS levels of DA and 5HT were detected among dominant, subordinate, and control individuals [Yue, 2001; Yue and Huber, in prep.]. Absolute levels of CNS are determined by the collective action of activating, transporting, releasing and inactivating processes which are also under circadian control [Castanon-Cervantes et al., 1999]. This work thus allows us to view only one small part of the picture, and interpreting a lack of change in absolute levels is difficult; 5-HT and DA systems may well respond to social experience at other time frames [Summers and Greenberg, 1995], involve increased production that is matched by increased turnover [Blanchard et al., 1991; Winberg et al., 1991; Winberg and Nilsson, 1993; Summers et al., 1997], or act via post-synaptic processes [Yeh et al., 1997]. Moreover, a view of amine neuromodulatory systems as dynamic entities [Hebert and Gerhardt, 1998] rather than long-term, static characteristics is also consistent with results from the chronic amine manipulations reported here.

## Conclusions

Discussions of aggression in animal behavior have, at least in recent decades, focused predominantly on the evolutionary significance of conflict [Caldwell and Dingle, 1975; Fry, 1980; Schulz, 1986; Ratnieks, 1991], the ultimate consequences associated with different behavioral strategies [Parker, 1974; Maynard-Smith, 1979], or individual properties which may confer an advantage to the owner [Lamprecht, 1986; Matsuzawa and Shiraishi, 1992; Fair-

banks, 1994]. Although there has been significant progress in research concerned with the ultimate consequences of behavior, other areas have yielded results more slowly. This is particularly true with regard to the physiological roles played by neuromodulatory substances for motivational elements [Tinbergen, 1951], behavioral states [Bryson, 1971; Blanchard and Blanchard, 1989], and dispositions for fighting [Lorenz, 1966]. The rediscovery of neurochemical factors has thus recently led to renewed inclusion of proximate mechanisms and physiological variables into mainstream ethology [van Staaden, 1998]. Due to their special characteristics, the rediscovery of interdisciplinary approaches for understanding behavioral causation has proven particularly fruitful in the case of neuromodulators. An increasingly dynamic view of such systems closely matches ideas that have previously emerged from work on the behavioral effects of steroid hormones [Arnold and Schlinger, 1993; Henrich and Brown, 1995; Ottinger and Abdelnabi, 1997], the network properties of amines/peptides [Johnson and Harris-Warrick, 1990; Katz and Frost, 1997; Stevenson and Meuser, 1997; Hoffman et al., 1998], and the functional characteristics of amine/peptide systems in specific social contexts [Fox et al., 1997; Yeh et al., 1997]. Such neuromodulatory substances do not 'produce' behavior per se but rather fine tune, or modulate, ongoing activity. In addition, serotonergic systems are likely influenced by the behavioral and experiential background of an individual through feedback mechanisms [Raleigh et al., 1984; Winberg et al., 1997]. Thus, in order to examine the particular roles of neuromodulators, it is essential that we combine studies of physiology, biochemistry, and molecular genetics, with quantitative behavioral analyses.

This review has summarized the experimental approaches with which we are attempting to link amines and their metabolites to aggression in crustaceans. Our work has (1) characterized the behavioral rules governing fighting in clawed decapod crustaceans, (2) estimated the contribution of learned plasticity for such behavior, (3) evaluated decisions during fighting in terms of game theory models, (4) identified the number and characteristics of individual sources of behavioral heterogeneity, (5) supported the significance of serotonin function for decisions involving retreat, (6) illustrated how the outcome of encounters influences fighting behavior in subsequent encounters, and (7) how the repeated application of this type of social conditioning leads to the formation of linear hierarchies through self-structuring.

Changes in serotonergic function appear to be an important physiological determinant of aggression in decapods. Raising levels of serotonin, either directly by infusion or by



blocking its re-uptake, resulted in enhanced fighting. Important questions remain concerning the particular neurons responsible for such observed changes in decision-making. We have recently shown that serotonergic input from a pair of deutocerebral giant serotonergic neurons in the supra-esophageal ganglion is essential for the development and growth of central processing centers [Benton et al., 1997]. Further explorations of these neurons and their target synaptic regions will be required to assess whether they may be the source of higher order decisions, such as when to give up in an agonistic encounter.

Results presented above, coupled with those of other investigators, argue for a highly dynamic association between serotonin and aggressive behavior in decapods [Doernberg et al., 2001], although our understanding is far from complete. Consistent with recent reports of compensatory mechanisms in murine genetic knock-out models of behavior [Hen, 2000], we suspect the actions of similar, yet-unidentified mechanisms for the behavioral results reported here. The previously mentioned caveat in working with the crustacean species of our research focus thus reflects a caution common to all aspiring to understand amine systems in general. Changes in behavior that are regulated by biogenic amines, whether experimentally or naturally induced, do not

seem to be controlled solely by the molecule itself, but rather through a dynamic orchestration of the system in which they are imbedded [Kravitz, 1988]. Such a 'systems view' of behavior requires that causal explanations be framed within the molecule's own unique physiological and behavioral context. Moreover, exploration of a neuromodulators functional role in healthy and diseased nervous systems requires that we arrive at an integrated understanding of their effects for complex behaviors [Lederhendler and Shulkin, 2000], such as aggression. Future studies linking molecular and biochemical changes to pharmacological manipulations of serotonin and behavioral plasticity will help bridge the gap between the conclusion of flexibility and specific aspects of the serotonin system in decapod crustaceans.

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