MODULATORY EFFECTS OF BIOGENIC MONOAMINES IN AGONISTIC BEHAVIOR OF THE RED SWAMP CRAYFISH *PROCAMBARUS CLARKII.*

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Running head: MONOAMINES AFFECT AGONISTIC BEHAVIOR IN CRAYFISH.

Abstract

Three biogenic monoamines, serotonin, octopamine, and dopamine, were injected ventrally into the haemolymph of *P. clarkii*. At high doses (1.0 mg per 10 g of body weight), each chemical induced unique postures in the animals. Exogenous serotonin and octopamine at lower doses (0.1 µg, 1.0 µg, and 10 µg per 10 g of body weight) were unable to induce consistent aggression and submission during agonistic encounters. Alteration of endogenous serotonin levels by differential illumination suggested a possible correlation between abundance of serotonin in the central nerve cord and aggression in the red crayfish. Results also indicated that exogenous dopamine produced subordinate behavior, typically through elicitation of rapid withdrawals by tailflip. These findings demonstrated that octopamine, dopamine, and serotonin have distinctive effects on biasing behavioral output during highly stereotyped social interactions in *P. clarkii*. Possibly, octopamine primes the crayfish to retreat from aggressive encounters, and dopamine modulates withdrawals by enhancing escape behavior, which may be terminated by actions of serotonin.

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Based on our personal experiences, it is easy to understand the concept that human behaviors are subject to the influence of many chemicals. Caffeine keeps people alert, while Valium cools off hyper heads. A little alcohol may boost people's mood, while too much may wear them down. Addictive drugs not only alter people's perceptions of reality, but can come to dominate their existence and abolish dignity. The human body also constantly produces chemicals to modify behavior. Familiar ones include adrenaline (epinephrine) which regulate the classic "fight or flight" response, androgens and estrogens which modulate sexual drives, and endorphins which temper pain and pleasure. It is important for us to unravel the interactions between the chemicals and the body, so that desired behaviors can be induced by chemical treatment.

One class of endogenous compounds that have attracted much attention in recent research are monoamines. These amines are subdivided into two categories: 1) catecholamines, including norepinephrine (or more commonly known as noradrenaline, NE), epinephrine (EP), octopamine (OA), and dopamine (DA); and 2) indolamines, represented by serotonin (5-HT). Figure 1 depicts the molecular structures of most of these amines. Also pictured is L-DOPA, the amino acid precursor of DA, which is the subsequent precursor of NE.

Insert Figure 1 about here

These biogenic monoamines, together with their amino acid precursors and metabolites, play major roles in numerous contemporary studies of several intriguing topics. For example, EP and NE are the modulating factors in stress, emotional responses, and immunological activities (Pinel, 1993, pp. 580-590). According to the monoamine theory of depression, underactivity of noradrenergic and serotonergic systems is the major cause of depression (Pinel, 1993, p. 612). The catecholamine hypothesis of affective disorder speculates that, in general, behavioral depression maybe associated with deficiency, usually in NE, at central adrenergic systems, while mania may be precipitated by an abundance of the catecholamines (Copper, Bloom, and Roth, 1991, p. 278).

Degeneration of dopaminergic cells within substantia nigra and striatum and depletion of DA are consistent observations in patients with Parkinson's disease, and injection of L-DOPA seems to alleviate behavioral deficits (Pinel, 1993, p. 173). On the other hand, the dopamine theory of schizophrenia suggests that schizophrenia is due to hyperactivity at central dopaminergic synapses (Pinel, 1993, pp. 607-610). In addition, the mesotelencephalic dopamine system is believed to be involved in the rewarding effects of intracranial self-stimulation that resembles addictive behavior (Pinel, 1993, p. 450).

Serotonin seems to participate in the PAG-raphe-dorsal-column analgesia circuit relevant to migraine headaches (Pinel, 1993, p. 252). Also, impaired serotonergic function is related to suicidal behavior (Cooper, Bloom, and Roth, 1991, p. 357).

Although the monoamines have extraordinary significance in human beings, elucidation of their detailed actions within mammalian nervous systems has been difficult, primarily due to the incredible complexity of such neural organizations. Rigorous cellular and molecular studies face ethical limitations, and appropriate non-invasive methods are yet to be developed. Alternatively, researchers have been looking into invertebrate systems, hoping that the parallelism between vertebrates and invertebrates allows them to make accurate extensions from the neural organizations underlining invertebrate behavior to the behavior of vertebrates. Fortunately, most of the monoamines exist throughout animal phylogeny. The only exceptions are that while OA, the phenolic analogue of NE, is present exclusively in invertebrates, EP is only detected in vertebrate systems.

Extensive studies on invertebrates have used crustaceans as subjects. Crustaceans are remarkably suitable material for the examination of fundamental processes in the nervous system. Aside from being cost-effective, they offer neuronal simplicity in combination with stereotyped motor output patterning (Sandeman, Sandeman, and Aitken, 1988; Sandeman and Sandeman, 1990; Schneider, Trimmer, Rapus, Eckert, Valentine, and Kravitz, 1993). The

crustaceans also have accessible and well-understood sense organs for studies of primary processes in sensory transduction and control, and a well-defined motor system with readily identifiable motorneurons. Not only is their behavioral repertoire limited, the presence of a rigid exoskeleton makes their movements more reproducible and measurable. All these properties link up to provide a very special opportunity to combine, in a rigorous way, observations at the cellular, or even the molecular, level with behavior of the whole organism.

A literature search indicated that the monoamines affect a variety of behaviors in invertebrates, ranging from swimming in leeches to learning in molluscans (Baker, Kushner, and Hooper, 1979; Barthe, Bevengut, and Clarac, 1993; Laxmyr, 1984). Within these paradigms, intraspecific agonistic behaviors in lobsters and crayfish seem to be of some interest. Using the American lobster (*Homarus americanus*), Kravitz (1988) postulated that 5-HT and OA function as neuromodulators at the level of interneurons in thoracic and abdominal ganglia, biasing the animals towards aggression and subordination, respectively.

Supporting evidence included the first demonstration by Livingstone, Schaeffer, and Kravitz (1980) and Kravitz, Glusman, Livingstone, and Harris-Warrick (1984) that both 5-HT and OA freely circulate at low concentrations in the haemolymph of the lobsters. Ventral injections of 5-HT and OA brought out drastically different behavioral displays in the lobsters which were interpreted as

postures of dominance and subordination, respectively. Both amines induced contractions when perfused at neuromuscular junctions (Bishop, Krouse, and Wine, 1990; Breen and Atwood, 1983). Their application on the abdominal ganglia was ineffective on phasic networks, but caused differential modifications in tonic circuits. Overall, 5-HT facilitated flexion and inhibited extension of the abdomen, while OA activated the opposite patterns (Livingstone, et al., 1980; Kravitz, 1986; Kravitz, Beltz, Glusman, Goy, Harriz-Warrick, Johnson, Livinstone, Schwartz, and King-Siwicki, 1981; Kravitz, et al., 1984; Ma and Kravitz, 1990).

The pericardial organs and the associated plexuses along each of the second roots were identified as two peripheral neurosecretory sites releasing the amines into the open circulartory system (Kravitz, 1988). Kravitz (1986) also found a serotonergic system in lobsters and crayfish mediating a state in which fighting and defense predominated over flight behavior. More impressively, Robert Hubber at the University of Graz in Austria (1994) recently claimed that exogenous injection of the two amines at certain general concentrations could generate significant correlation with behavior. Animals injected with 5-HT became dominant and aggressive, while those injected with OA became subordinate and submissive. His team is working on the 5-HT/OA interpretation of dominance relationships within pairs, as well as the linear hierarchy within groups (Copp, 1986).

On the other hand, objections to the serotonin-aggression and octopamine-submission relationships were raised from laboratory observations as well as arousal studies in other species of crustaceans. Stanley Cobb at the University of Rhode Island (1994) argued that the postures induced with amine injections as reported by Kravitz's group did not closely resemble the dominant and subordinate postures observed in intact animal pairs. In fact, the doses used for postural induction were more than eight (8) orders of magnitude higher than the concentrations of the amines in the general circulation (Livingstone, et al., 1980; Kravitz, et al., 1981), suggesting that the postural effects of the amines were more likely to be pharmacological than physiological.

Also, Cobb (1994) believed that behaviors, rather than postures, were more appropriate and reliable indices in defining aggression and submission in the lobsters. Quantitative analysis of the amines' levels in lobster haemolymph showed that neither 5-HT nor OA concentrations displayed smooth transitions over the molt cycle (Fadool, Brown, Cobb, and Kass-Simon, 1989) as aggressive and submissive behaviors did (Tamm and Cobb, 1978). Since the lobsters are primarily nocturnal, postural displays probably exert fewer critical effects than behaviors do on the establishment and maintenance of intraspecific hierarchies.

In virtually all species engaging in social interactions, aggressors are usually more active (or aroused) than subordinates, initiate more attacks and display more spontaneous locomotions. So it

is reasonable to assume that aggression is positively correlated with behavioral arousal. Arnesen and Olivo (1988) showed that 5-HT and OA respectively abolished and provoked spontaneous walking and compensatory optokinetic movements in the crayfish *P. clarkii*, locomotive elements elicited by behavioral arousal (Olivo and Thompson, 1982). Such results seemed to discredit the proposal that 5-HT promotes aggression and OA enhances submission.

However, the amines induced similar postures in *P. clarkii* as in the case of the lobster (Livingstone et al., 1980; Kravitz, 1986; Kravitz, et al., 1981; Ma and Kravitz, 1990), and both amines at much lower concentrations amplified the optokinetic response in crabs in a dose-dependent pattern (Erber and Sandeman, 1990). Again, the extremely high doses of injection (about 5 to 7 orders of magnitude higher than endogenous concentrations in the haemolymph) used for the crayfish complicated the situation. As Arnesen and Olivo (1988) acknowledged, there was an "unlikely possibility that the amine[s were] creating contractions so severe that the animal [was] immobilized for hours." Apparently, additional studies are required to clarify the effects of 5-HT and OA on agonistic behavior in lobsters and crayfish.

Another amine mentioned in the literature with relevance to crustacean agonistic behavior is dopamine (DA). In comparison to the extent of research and understanding on 5-HT and OA systems, DA in crustaceans is very under-explored. Neural networks subjected to investigation with dopamine application include cardiac ganglia of

crabs (Miller and Benson, 1984), the pyloric circuit within the stomatogastric system in lobsters (Anderson and Barker, 1981; Flamm and Harris-Warrick, 1986a, Flamm and Harris-Warrick, 1986b; Harris-Warrick and Flamm, 1986; Kushner and Barker, 1983), the feeding motor program in *Limax maximus* (Weiland and Gelperin, 1983), and the swimmeret system in lobsters (Barthe, Mons, Cattaert, Geffard, and Clarac, 1989; Cattaert and Clarac, 1987), all of which are simple rhythmic activities originated within the central nervous system by limited ensembles of neurons called central pattern generators. The only effect on posture was reported in the lobster, *Homarus gammarus*, by Barthe et al. (1989), who interpreted the gross action with short latency and duration as being similar to agonistic behavior observed in lobsters.

Both DA and its metabolic precursor L-DOPA (see Figure 1) were found to be present in the nervous systems of crayfish (Elofsson, Laxmyr, Rosengren, and Hansson, 1982) and spiny lobsters (Sullivan, Friend and McCaman, 1976) at concentrations comparable with that of 5-HT and OA. Obviously, possible effects of DA and L-DOPA in modulating particular behavioral outputs should not be underestimated. Besides, Elofsson et al. (1982) discovered that the L-DOPA level in the ventral nerve cord of crayfish increased dramatically (by up to a magnitude) upon prolonged handling, a form of high level stress for the animals. This was another piece of evidence that strongly suggested potential functions of L-DOPA other than merely being the precursor of DA, and possible modulation of

this precursor/metabolite pair of compounds in agonistic behavior of the crustaceans.

The present study was to 1) demonstrate the distinctive postural effects of exogenous 5-HT, OA, and DA at pharmacological concentrations, 2) elucidate possible correlations between the elevation of amines, either by differential illumination or by injections into the haemolymph, and the outcome of dominance relationships in sex and size matched pairs, and 3) propose the collaboration between the amines in modulating agonistic behavior in the red swamp crayfish, *Procambarus clarkii.*

Methods

Materials and Protocols

Form I intermolt male and non-gravid female *P. clarkii* with body length of 3-4 inches were obtained from Carolina Biological Co. They were held in individual pans, each filled with 2 liters of aged and well-aerated tap water and a layer of gravel as substrate. The animals were visually isolated from each other, and were marked on their carapace with white fingernail polish for identification. The crayfish were maintained on a 14:10 L:D cycle at 18-20°C, and fed with fish chunks twice a week. Experiments took place from January to April, 1994. Observations were made under red illumination in an otherwise dark aquarium lab between 1900 and 0200 hrs, when the animals were most active (Page and Larimer, 1975). Only intrasexual agonistic behavior was studied so as to avoid intersexual courtship

that would reduce aggression dramatically in the red crayfish (Ameyaw-Akumfi, 1981).

Exogenous amines were injected through the ventral sinus into the open circulatory system via a 100 μL Hamilton syringe. Serotonin HCl, DL-octopamine, and dopamine HCl were obtained from Sigma Chemical Co., and were dissolved in cold crayfish saline just before use (saline composition in mM: NaCl 195, KCl 5.5, $CaCl_2$ 13.5, $MgCl_2$ 2.5). Typically, 10 mg of an amine was dissolved in 5.0 mL of saline, with a resulting concentration of 2 mg/mL, and subsequent dilutions were made so that for each injection of desired doses the medium was always about 50 μL. In the study of postural effects, 20 mg of each amine was dissolved in 1.0 mL of saline for 1.0 mg doses. All solutions were stored in brown bottles under refrigeration and applied within 24 hrs after preparation, due to rapid degradation rates of the amines (Fadool, et al., 1989). The one-day-old solutions were discarded, and new solutions were prepared when needed.

For evalution of agonistic encounters, size-matched pairs (differences of body length being less than 2 mm) of the same sexes were transferred to a 28 x 22 x 23 cm styrofoam box filled with 5 gallons of aged and well-aerated tap water. A winner/dominator was determined if it initiated the last 10 consecutive attacks towards its opponent and elicited nothing but withdrawals, and a loser/subordinate was assigned if it responded to the opponent's last 10 successive aggressive attacks all with retreats. All encounters between a pair from the time they were transferred into the

styrofoam box to the 10th consecutive winning/losing confrontation were videotaped under red illumination. The following three measurements were extracted from the recordings for statistical analysis.

The first measurement was the duration of fighting in the first agonistic encounter between the pair, a period from the time both individuals of the pair started striking and pushing at each other until the time that one of the subjects stopped these movements and adapted a submissive posture, with chelipeds closed and pointed medially, followed by escaping from the confrontation. This time index was measured with a stopwatch, and results were rounded off to the nearest second. The second measure was the ratio of strikes between the loser and winner (loser : winner) during their first fight. The third was the ratio of two types of retreats, one by backward tailflips and the other by backward walking, within the total amount of withdrawals displayed by the subordinate before a stable dominance status was established. For example, 15 encounters were observed in a pair after the initial combat and before a dominance relationship was established according to the criteria above. The subordinator retreated in all 15 occasions, using multiple tailflips in 5 encounters, backward walking in 7 encounters, forward walking in 2 enounters, and motion less in one encounter. Then the ratio of tailflip to backwalking was 5 : 7, and recorded in decimal form as 0.71.

One animal of each pair was designated as the target and described according to the precedures below. Since there was no gender related difference in intrasexual aggression according to a preliminary study, the behavioral data of males and female were pooled together for analysis. Three experiments were conducted as follows:

I. Postural Elicitation.

On day 1, a male and a female crayfish were both injected ventrally with 5-HT once and put back into their own individual holding pans for observation. A second male and a second female were injected in the same in the same way but with OA. A third male and a third female were injected with DA. The injection dose used for all three amines was 0.01 mg per 10 g of body weight. The durations of injection induced posture displays and the appearance of the postures were recorded in the form of notes and videotape clips. All 6 subjects were given a two-day resting period to recover. On day 4, the same 6 animals were injected with the same amines individually, but this time with a dose an order of magnitude higher (0.1 mg per 10 g of body weight). The postures and their durations were recorded. Another two days of recovery followed. On day 7, the same animals were once more injected with the three amines, with a dose of 1.0 mg per 10 g of body weight).

II. Differential Illumination.

Preliminary tests on exogenous injections of serotonin and octopamine at 0.01 µg, 0.1 µg, and 1.0 µg per 10 g of body weight did not seem to magnify aggression and subordination, respectively. Alteration of endogenous central storage of 5-HT was attempted using the differential illumination method described by Kulkarni and Fingerman (1992). Seven crayfish (4 males and 3 females, the L group) were held under fluorescent light (Triton F40CW Cool White, 40W) and another group of seven crayfish (4 males and 3 females, the D group) were held in complete darkness for 84 hrs. Both groups started and ended their treatments simultaneously. Crayfish in the D group (the targets) were paired with size and sex matched animals from the L group and observed for their agonistic behavior. In a control group, a group of 10 crayfish (5 males and 5 females, the target group) and another group of 10 crayfish (also 5 males and 5 females) both were held under 14:10 L:D photocycle for three days, after which they were were paired between the groups and studied for their interactions.

III. DA Injection.

One group of 10 crayfish (5 males and 5 females) were each injected with dopamine at a dose of 0.1 mg per 10 g of body weight, and another group of 10 crayfish (5 males and 5 females) were each injected with 50 µL of pure saline. A 10 min recovery period was given to eliminate possible postural rigidity, after which size and sex

matched between-group pairs were formed and transferred to the styrofoam box for observation. After the dominance relationships were identified, the pairs were separated into their original holding pans and left undisturbed for two days. On the third night, the same pairs were formed and each given a rematch. In another control group, one group of 9 crayfish (5 males and 4 females, the target group) and another group of 9 crayfish (5 males and 4 females) were all injected with 50 μL of pure saline. Between-group pairs were formed and their confrontations recorded.

Results

1. Postural Elicitation.

Injections of serotonin, octopamine, and dopamine each had dramatic, distinctive, and highly reproducible behavioral effects in the red crayfish, *P. clarkii*. Within seconds of the injections, vigorous tailflip responses ensued, after which the animals were "frozen" into amine-specific stances. Serotonin typically caused hyperextension of chelipeds and walking legs, complete closure of all fingers, severe abdominal flexion, and a general abolishment of locomotions. When placed back into the holding pan, the animals lay down with an extremely rigid stance, claws outstretching anteriorly and medially, walking legs spreading laterally, antenna pointing anteriorly, thorax touching the substrate, abdomen elevating off from the substrate and tugging in tightly. The duration and intensity of the stance were dose-dependent. An injection of 0.01 mg induced the posture for

about 10 min, with 0.1 mg, over an hour, and with 1.0 mg of 5-HT the posture lasted approximately four hours, after which a recovery period, indicated by gradually increasing leg and claw movements, took place.

Octopamine, on the other hand, produced quite opposite effects. Upon injections, subjects showed a distinctive increment in levels of arousal-like activities, such as jittery leg movements and irregular eyestalk movements. Other characteristic postural elements included hyperopening of the chelipeds and dactyls, anterior projection of the antenna, abdominal hyperextension, laying flat on the substrate, slight uplifting of the thorax, occasional spontaneous cheliped raising, and instant cheliped raising upon predatory threats. A dose dependency of the postural effects of similar magnitude to that of 5-HT was also observed.

Last but not least, postural effects of dopamine were even more interesting. The whole body was highly elevated from the substrate, with supporting walking legs and chelipeds mildly flexed and fingers open moderately to maximally. The rostrum end was much higher than the uropod end, forming an angle of about 20° - 30° between the longitudinal axis of the body and the substrate. The uropod was loosely dangling down vertically, perpendicular to the substrate, and expanded laterally. The chelipeds pointed medially and were much lower than the body position. Highly frequent but irregular swimmeret beating was also observed. In addition, there was a severe lateral expansion of both exopodites, with the antennae

pointing to the sides. Although this posture was dose-dependent as well, the magnitudes were smaller in comparison to that of 5-HT and OA. With 0.01 mg of DA, the posture lasted under a minute, about 5 min with 0.1 mg, and 30 min with 1.0 mg. During such periods, spontaneous tailflips were occasionally noticed.

II. Differential Illumination.

Out of the 7 subjects in the L group (with continuous illumination), 6 (3 males and 3 females) became the winner/dominator. That was an 86% winning probability, considerably higher than the chance level (50%). The last male in the L group lost its battle to the one from the D group,which were held in complete darkness. In the control group, however, only 6 (2 males and 4 female) out the 10 targets declared their victory, a 60% winning probability which was at chance level. When the 6 L group winner pairs were compared with the 10 pairs in the control group, no significant difference were found in the indices chosen for this study (see Tables 1, 2, and 3, Figures 2, 3, and 4).

Insert Tables 1, 2, and 3 and Figures 2 , 3 and 4 about here

III. DA Injection.

In the experimental group, the first round match ended with all 10 subjects injected with DA losing their battles against their

saline treated opponents. That was a perfect 100% probability of being defeated. In contrast, only 3 (1 male and 2 females) out of the 9 targets in the control group were defeated, a probability close to chance level. Statistical analysis indicated that relative to the losers in the control group, DA treated subordinates fought for significantly shorter periods during the first encounter ($t(17) = 2.48$, $p = 0.023$), striked less in reference to the winners ($t(17) = 3.06$, $p = 0.007$), and used tailflips more frequently to escape when attacked by their aggressive opponents ($t(17) = 3.11$, $p = 0.006$) in the first matches, while the injections of saline medium were obviously unrelated to these remarkable differences in a causal way (see Tables 1, 2, and 3, Figures 5, 6, and 7).

During the rematches, 6 pairs (2 male couples and 4 female couples) of the experimental group, in which one of each pair was injected with DA in the first round, displayed dominance reversal. The previously subordinate crayfish, which were treated with DA two days earlier, defeated their conquerors of the first matches and forced the latter to retreat in all enounters during the rematches. In contrast, none of the pairs in the control group, which were injected with saline only, reversed their hierarchies during the rematches. Further observations indicated that these 6 reversal pairs in the experimental group preserved their relative status during later enounters. Behavioral data reported in Tables 1, 2, and 3 were of the first matches only.

Insert Figure 5 , 6, and 7 about here

Discussion

Distinctive and dramatic postural effects of 5-HT, OA, and DA in *P. clarkii* were clearly demonstrated in this study. However, the serotonin and octopamine induced postures did not seem to resemble dominant and subordinate postures, respectively. Serotonin-elicited posture was characterized by tightly closed chelipeds and fingers of the walking legs, as well as tightly tugged uropods and hyperflexed abdomen. These elements were observed more often during submissive displays of this species, rather than being dominant signals as interpreted by Livingstone et al. (1980). On the other hand, octopamine-induced posture, with fully opened chelipeds and fingers of walking legs, hyperextended abdomen and uropods, and enhanced responsiveness to threats, resembled aggressive behaviors more than submissive ones.

An obvious artifact of these amine-induced postures was that the injection doses applied were much greater (about 4 to 6 orders of magnitude higher) than the entire endogenous stock of the amines in the animals. The exogenous amines basically overloaded all amine receptors in the body and caused tetanic contractions in the muscles, either directly or via actions of interneurons and motor neurons, "freezing" the animal into the observed postures. In fact, during the

posture presenting period, all simple reflexes such as eyestalk withdrawal, claw closing, and claw opening were abolished, and the animals frequently fell down on the substrate and were not able to re-adjust their positions.

Since both 5-HT and OA facilitated muscle contractions when perfused directly (Bishop, et al., 1990; Breen and Atwood, 1983; Livingstone, et al., 1980; Kravitz, 1986; Kravitz, et al., 1981; Kravitz, et al., 1984), their opposite effects on the chelipeds, walking legs, and abdomen should not be the result of the amines' direct actions on the muscles. Instead, 5-HT and OA selectively and specificly modulate various regions of the nervous system, which could express the amines' effects by producing unique neural activity patterns that operate on distinctive sets of muscles and result in very specific behavioral output. Such modulatory actions of the amines were confirmed at least in the abdominal positioning circuits (Barthe, et al., 1993; Glanzman and Krasne, 1983; Livingstone, et al., 1980; Kravitz, 1986; Kravitz, 1988; Ma and Kravitz, 1990; MacMillan and Pasztor, 1988; Murchison and Larimer, 1992) and the lateral giant escape reaction pathway (Bellman and Krasne, 1983; Bustamante and Krasne, 1991; Glanzman and Krasne, 1983; Kramer, Krasne, and Wine, 1981; Krasne and Lee, 1988; Kasne, Vu, and Lee, 1990; Wine and Krasne, 1982) in the lobsers and crayfish.

If 5-HT and OA do function, at least partially, as neuromodulators in crustaceans, how are they released and transported to their taget sites? Based on their detection of freely

circulating 5-HT and OA in the lobster haemolymph, Livingstone et al. (1980) suggested that the amines exerted their modulatory effects through peripheral circulation. However, Elofsson et al. (1982) reported that the amines were much more abundant in crayfish's brain and eyestalk than in their haemolymph (about 2 to 3 orders of magnitudes difference), with much smaller variations among the subjects, in comparison to the large variations in the haemolymph concentrations of the lobsters (Livingstone et al., 1980; Kravitz, et al., 1981), implying that the monoamines have more important functions at central neural sites than at peripheral sites. Kulkarni and Fingerman's (1992) quantitative analysis further evidenced significant variation of 5-HT even within parts of the central nerve cord of *P. clarkii*, suggesting possible functional differentiation along the system. Undoubtedly, complex behavior such as those appearing in agonistic combat are always under direct or indirect control of the central nervous system (CNS). By manipulating the amines' levels in the CNS and analyzing subsequent changes in behavioral outcome, possible roles of the amines in connection with aggression may be deduced.

In this study, selective alteration of endogenous 5-HT was achieved by differential illumination (Kukarni and Fingerman, 1992), a non-invasive method that could generate significant changes in serotonin levels while concomitantly avoiding irreversible confounding effects. As the results indicated, after being illuminated continuously for 84 hrs, which presumably had elevated the

endogenous abundance of 5-HT, 6 out of 7 subjects overpowered their opponents which had been subjected to complete darkness for the same amount of time and were supposedly depleted of 5-HT in their CNS (Kulkarni and Fingerman, 1992). Although the behavioral elements were insignificant, the high probability of winning after elevation of CNS 5-HT level suggested that studies with a larger sample might determine a possible correlation between 5-HT and aggression.

On the other hand, the same differential illumination procedure modified norepinephrin (NE) levels within the CNS in the same direction and to a similar extent as it did to the serotonin levels (Fingerman and Kulkarni, 1993). Even though NE has rarely been addressed in connection with behavior in crustaceans, future studies should nontheless examine possible behavioral effects of NE in the crayfish so as to clarify the relative importance of 5-HT and NE in modulation of agonistic behavior. In addition, neurochemical experiments are required to demonstrate whether the illumination effects on 5-HT level is through reduction of the amine's biosynthetic process or an increase in the releasing of the amine into haemolymph, and whether OA levels are alterred by the illumination. Such information is critical for the interpretation of the nocturnal nature of the crayfish and the fact that they are more active in the dark.

As the picture for serotonin and octopamine becomes even more complex, dopamine seems to stand out as clear cut in its effects

on agonistic behavior of the crayfish. Posture wise, the overall display in *P. clarkii* was in excellent agreement with reported observations in *H. gammarus* (Barthe, et al., 1989), and also resembled avoidance behavior during social interactions of the red crayfish, which usually precedes combat, submission, or escape. During agonistic encounters, all DA treated subjects (10 out of 10) became subordinate. These animals fought briefly at first, then quickly assumed submissive postures with closed chelipeds flexed and pointed medially, abdomen flexed and uropods tugged, and withdrawal by backward walking. Upon further aggressive threats, they frequently used their characteristic tailflip responses to get away. Behavioral analysis showed that the the initial combat in DA was significantly shorter than the control, with less strikes and more tailflip retreats (see Figures 5, 6, and 7).

More interesting, though, was the fact that more than half (6 out of 10) of the DA treated animals became dominant in the rematch, reversing the hierarchies formed only two days earlier in the first round. This was not observed in the control group, which showed perfect consistancy in hierarchies during both matches. In the experimental group, dominance status after the rematch seemed to be stable in the absence of further amine manipulations. Such a "dominance status reversal" phenomenon in the DA treated subjects has been shown to occur naturally in the lobster *H. americanus* (Atema and Cobb, 1980). If the dominant lobster of a pair molted before the subordinate one, two hours after ecdysis, the previously

dominant animal assumed submissive postures and exhibited avoidance behavior while the former subordinate displayed dominant characteristics. After a period of 10 days the original dominator regained its status. It seems appropriate to assume that DA plays a critical role in bringing out submission in the dominator.

In *P. clarkii*, molt-related neurosecretory cells have been identified in all thoracic and abdominal ganglia, with the most abundant site being the last (6th) abdominal ganglion (Roth, 1978). These cells contain two populations of granules that fluctuated systematically and inversely shortly before ecdysis until 4-6 weeks after molting. Discharge of one type of granules has been observed with a concomitant transition from aggressive to escape behavior in the animals. Apparently, these secretory cells may have some connections with the three aminergic systems that modulate agonistic behavior in the crayfish. The products of the granules in the cytoplasm are not likely to be related to the 5-HT/OA pair, because these two amines have been found essentially within the second thoracic roots (Livingstone, et al., 1980; Kravitz, et al., 1981). Thus a plausible alternative speculation is that the contents of those two classes of granules are a pair of precursor/product, possibly related to the synthetic machinery of DA (see Figure 1).

Although the grand scheme of neurological entities underlying agonistic behavior in crustaceans is far from complete, it is clear that several aminergic systems, namely 5-HT, OA, and DA play pivotal roles in modulating the final behavioral output. Collaborative

modulation of these amines has been studied in other invertebrates such as moths (Claassen and Kammer, 1986), and it is clear that similar cooperation among the monoamines takes places in crustacean systems.

Based on the present study and previous findings on lobsters and crayfish, the following postulation is given for crustaceans: Octopamine is the primer for submissive behavior by increasing the arousal level of different escaping circuits (Bellman and Krasne, 1983; Bustamante and Krasne, 1991; Glanzman and Krasne, 1983; Kovac, 1984a; Kovac, 1984b; Kramer, Krasne, and Wine, 1981; Krasne and Lee, 1988; Kasne, Vu, and Lee, 1990). In the presence of dopamine, these excited circuits generate neuromuscular output patterns that are identified as backward walking and tailflips and other escape behavior (Wine and Krasne, 1982). The selective release of serotonin inhibits activities modulated by octopamine and dopamine, thereby terminating the output of escape behavior.

A similar collaborative monoamine system is proposed for the flight responses in moths (Claassen and Kammer, 1986). But the crustacean systems may include participation of norepinephrine, L-DOPA, and other precursors and metabolites of the monamines as well. Hopefully, this crude proposition can set a narrowly defined goal and attract a more coherent organization of research pursuing the details, which will accelerate the process of generating exciting results.

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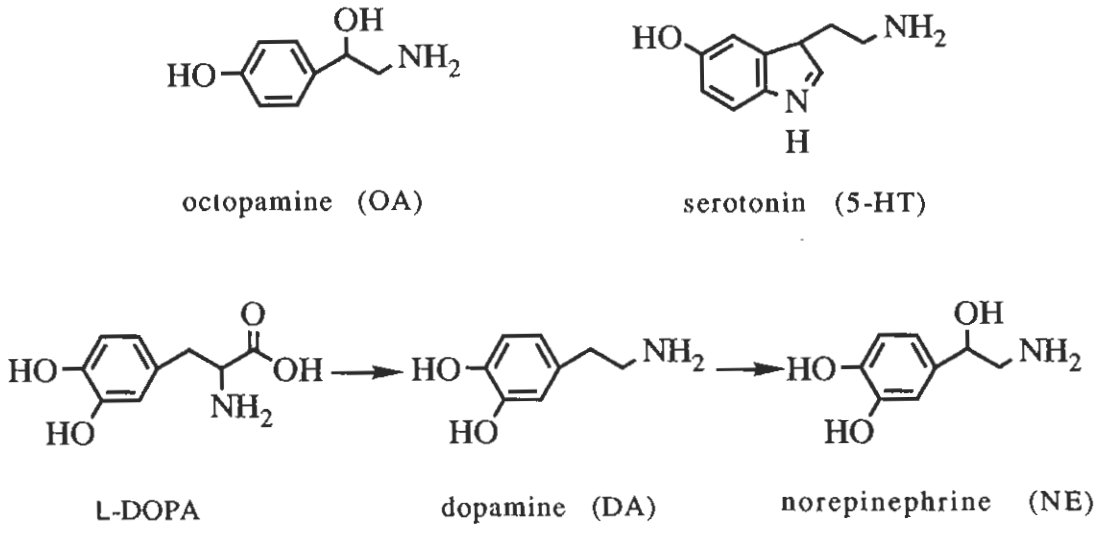

Figure 1. Biogenic monoamines identified in crustacean nervous systems and haemolymph. Also shown here is the biosynthetic scheme of DA and NE from L-DOPA.

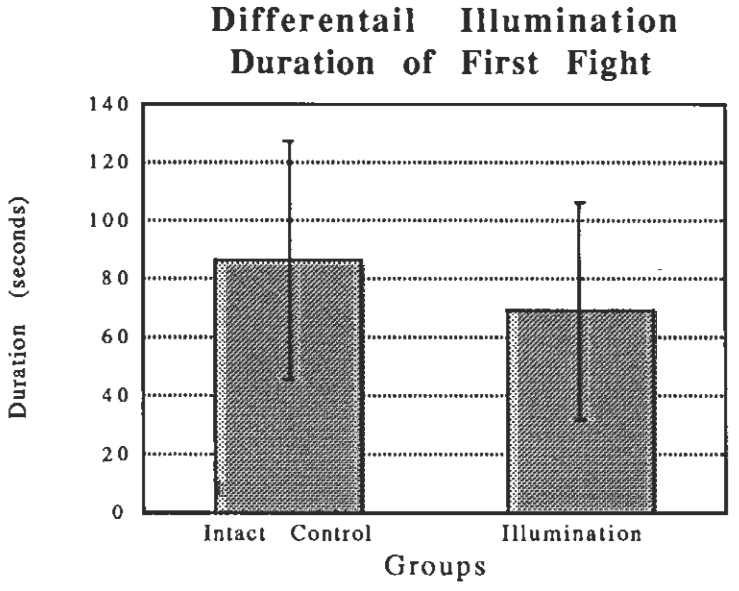

Figure 2. Duration of first fights in the differential illumination experiment. Standard deviations were used as the error bars. For data see Table 1.

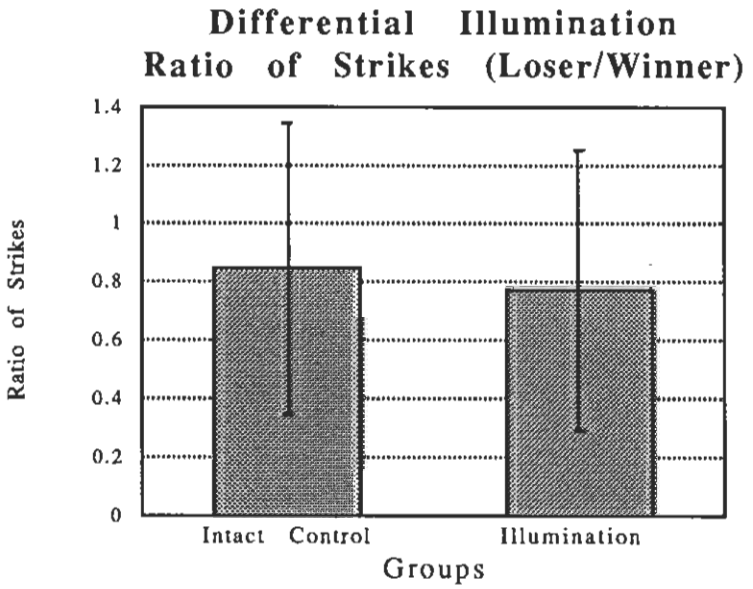

Figure 3. Ratio of strikes (loser/winner) in the differential illumination experiment. Standard deviations were used as the error bars. For data see Table 2.

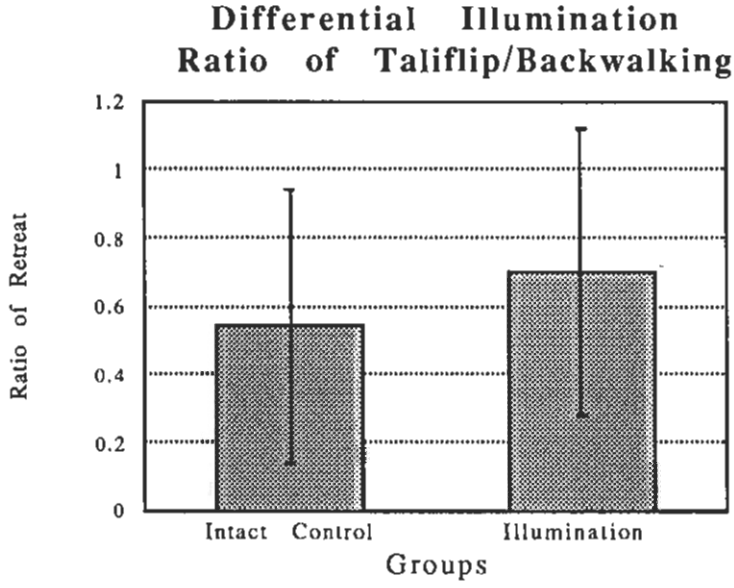

Figure 4. Ratio of withdrawals (tailflip/back walking) in the differential illumination experiment. Standard deviations were used as the error bars. For data see Table 3.

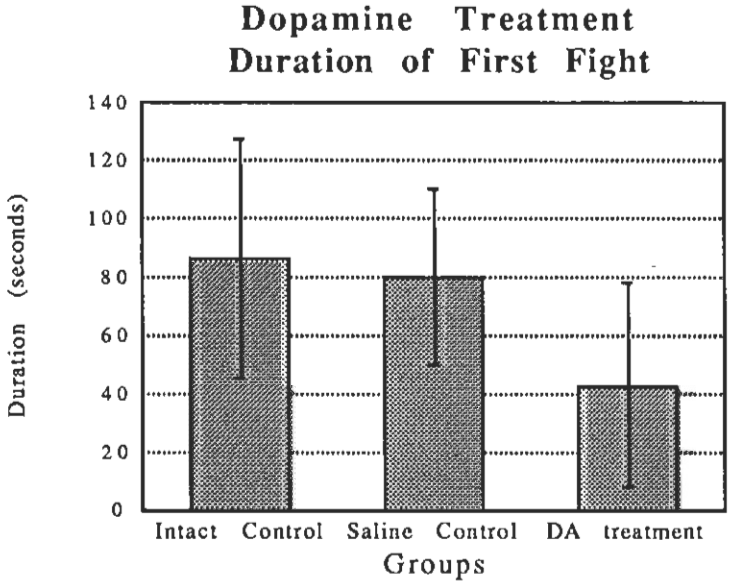

Figure 5. Duration of first fights in dopamine treatment experiment. Two control groups, one with saline injection and one without, are included for comparison of injection effects. Standard deviations are used as error bars. For data see Table 1.

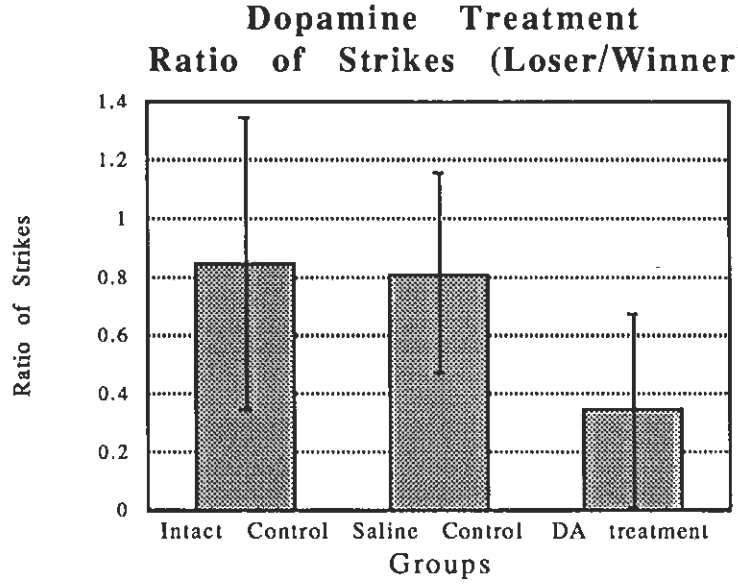

Figure 6. Ratio of strike between loser and winner in dopamine treatment experiment. Two control groups, one with saline injection and one without, are included for comparison of injection effects. Standard deviations are used as error bars. For data see Table 2.

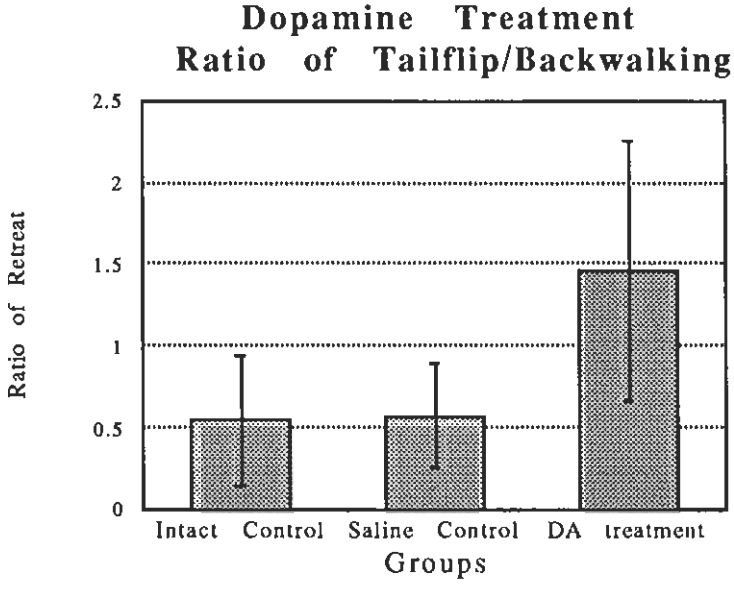

Figure 7. Ratio of withdrawals (tailflip/back walking) in dopamine treatment experiment. Two control groups, one with saline injection and one without, are included for comparison of injection effects. Standard deviations are used as error bars. For data see Table 3.

Intact Control	Illumination	Saline Control	DA Treatment
95	93	124	78
22	44	83	34
67	38	42	69
95	26	79	31
100	92	96	38
88	119	66	116
62		89	19
54		111	16
102		31	27
177			10

Table 1. Duration of first fights of all control and experimental groups. All measurements are in seconds.

Intact Control	Illumination	Saline Control	DA Treatment
0.92	0.11	1.00	0.58
0.06	1.04	0.87	0.23
1.22	0.33	0.63	0.07
1.09	1.09	1.25	0.24
1.48	0.98	0.75	0.78
1.26	1.16	0.30	0.58
0.60		0.67	0.12
0.16		1.33	0.86
0.50		0.50	0.25
1.12			0.18

Table 2. Ratio of strikes (loser/winner) of all control and experimental groups. All measurements are unitless.

Intact Control	Illumination	Saline Control	DA Treatment
0.83	0.98	0.10	1.25
0.92	0.60	0.75	1.25
0.14	0.10	0.67	2.67
0.25	1.16	1.00	0.29
0.50	0.33	0.28	2.00
0.20	1.00	0.07	1.00
0.40		0.87	1.67
0.67		0.62	1.22
1.33		0.70	2.68
0.14			0.59

Table 3. Ratio of withdrawals (tailflip/back walking) in losers of all control and experimental groups. All measurements are unitless.

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