

ORIGINAL PAPER

B. L. Antonsen · D. H. Paul

Serotonin and octopamine elicit stereotypical agonistic behaviors in the squat lobster *Munida quadrispina* (Anomura, Galatheidae)

Accepted: 22 June 1997

Abstract Serotonin and octopamine have been implicated as modulators of posture and behavior in several crustaceans. Here we characterize the agonistic behaviors of normally interacting squat lobsters *Munida quadrispina* (Anomura, Galatheidae) and their responses to serotonin and octopamine injected into the ventral hemolymph sinus, in order to evaluate the potential roles of these amines in modulating agonistic behaviors. Normally interacting *M. quadrispina* do not develop lasting dominance hierarchies, although transient aggressive and submissive displays do occur. Injected serotonin elicits postures and behaviors in isolated individuals similar to those typical of aggressive, normally interacting animals. Injected octopamine can produce postures and behaviors typical of submissive animals, and elicits behaviors which imply a modulatory role for octopamine in tailflipping. The effects of both amines are reversible and dose dependent, and the dose-response curves parallel the normal progression of agonistic interactions. The social behaviors and reactions to injected serotonin and octopamine of *M. quadrispina* differ from those of lobsters and crayfish, indicating that interspecific differences in neuromodulation of behavior and motor output exist. Such differences have implications for the understanding of aminergic modulation of aggression and the evolution of aminergic modulation in crustaceans.

Key words Serotonin · Octopamine · Crustacean · Agonistic behavior · Posture

Abbreviations OA Octopamine · 5-HT Serotonin

Introduction

Serotonin (5-HT) and octopamine (OA) are involved in the modulation of many physiological processes involved in agonistic behaviors in crustaceans, as is 5-HT in mammals (Olivier et al. 1995). Although some examples of aminergic influences on neural circuits have been published, the neurological pathways by which these influences are effected are not well understood.

Injecting American lobsters or crayfish with 5-HT or OA induces postures closely resembling typical aggressive or submissive stances, respectively (Livingstone et al. 1980). In the crab *Carcinus maenas*, injected 5-HT or OA elicits, respectively, postural flexion or extension (Bevengut and Clarac 1982). The same postural motor patterns are activated in both groups, but neither study reported induction of complex behaviors typical of normal agonistic encounters by either amine, or any influence of amine dose.

A clear behavioral change is elicited in submissive crayfish perfused with 5-HT: both incidence and length of fights with dominant animals increase (Huber 1995). Glanzman and Krasne (1983) implicated 5-HT and OA in gain-setting in the lateral giant interneuron escape circuit in crayfish. Current and prior social status have been found to influence serotonergic modulation of this circuit: 5-HT increases responsiveness to sensory input in dominant animals and decreases responsiveness in submissive animals (Yeh et al. 1996). Additionally, serotonergic cells act as gain-setters specific for the flexion control circuit (Ma et al. 1992).

Injected amines also affect posture of the amphipod *Gammarus lacustris* (Helluy and Holmes 1990) and the hippid sand crab *Emerita analoga* (B.L. Antonsen and D.H. Paul, unpublished observations). In both species, injected 5-HT elicits postural flexion; in neither species, however, does injected OA alone affect posture or behavior, but it inhibits the actions of injected 5-HT. The behavioral roles and perhaps also the mechanisms of action of 5-HT and OA clearly differ among crustaceans.

B.L. Antonsen · D.H. Paul (✉)
Department of Biology, University of Victoria, PO Box 3020,
Victoria B.C., Canada V8W 3N5
Tel.: +1-250 721-8856; Fax: +1-250 721-7120
e-mail: Dhp@uvvm.uvic.ca

We have begun studying the serotonergic and octopaminergic influences on agonistic behaviors of the squat lobster *Munida quadrispina* (Anomura, Galatheidae), a decapod crustacean distantly related to the more thoroughly studied lobsters and crayfish; our goal is to use comparative analyses to elucidate further the effects of these amines on neural circuits involved in agonistic behaviors. Galatheid crustaceans usually hold their muscular abdomen loosely curled under the thorax. Their long first pair of legs are chelate, the second through fourth pairs are non-chelate walking legs, and, typical of anomurans, the fifth pair are modified as cleaning appendages. *M. quadrispina* is native to deep marine waters off the west coast of North America. They live in rocky areas, often crowded together on accessible surfaces. Most wild populations are deep, making studies difficult; no reports exist, even anecdotal, of agonistic interactions in the wild.

Several neuromuscular systems involved in agonistic encounters differ between galatheids and lobsters and crayfish. Among these are absence in galatheids of both giant escape tailflip circuits (Sillar and Heitler 1985; Wilson and Paul 1987) and differences in abdominal proprioceptors (Wallis et al. 1995), tailfan neuromusculature (Paul et al. 1985), and walking leg musculature organization (B.L. Antonsen and D.H. Paul, unpublished observations). In this paper, we describe the normal behaviors of interacting *M. quadrispina*, and examine the extent to which postures and behaviors typical of interacting animals can be induced by 5-HT and OA.

Materials and methods

M. quadrispina were collected by trawl in Saanich Inlet near Victoria, British Columbia, Canada, and maintained in recirculating natural seawater tables (temp. ca. 10 °C). Rocks and bits of netting were provided for the animals to climb. Animals were kept at densities approximating those in the wild and were fed regularly on a mixed fish and algal diet. One tank had a substrate and light regime which approximated their natural rocky habitat. They were observed for any evidence of developing dominance hierarchies or agonistic interactions. Animals of both sexes weighing between 4 and 10 g and in apparent good health were selected from this population for amine injection and isolation experiments. Some of the animals used for amine injection tests were selected directly from the main populations, while most were isolated in small groups prior to the experiments.

The small isolation aquaria had flat, textured bottoms; no attempt was made to reproduce a natural environment. We felt this was justified because prior, long-term observations showed that relative positions of animals on complex substrates had no effect on the frequency, course, or outcome of agonistic encounters. Detailed observations were made of these small groups to characterize behaviors typical of normally interacting animals for use as a baseline for the amine injection experiments. Normal stances and movements of non-interacting individuals and changes during agonistic encounters were recorded with videotape and still photographs. Leg joint angles and drawings representative of each behavior were taken from individual frames. We recorded relative positions of the antennae, claws, walking legs, and abdomen, and any movements which occurred during interactions. Frequency and course of

agonistic interactions between animals of various sizes and states of hunger, with and without food present, were noted. Signs of developing or prolonged dominance were sought in all groups. To mimic threatening situations, individual animals were challenged from outside the tank by sudden presentation of artificial visual stimuli: an artificial squat lobster and several dark shapes resembling fish. We chose to present these stimuli from outside the tank because there was no difference in responsiveness to these and stimuli approaching through the tank, and to avoid technical problems associated with pushing these sometimes quite large objects through small tanks. Additionally, squat lobsters in tanks were challenged by the introduction of an additional squat lobster to determine the reaction of animals to the appearance of non-familiar conspecifics. Results were pooled for all animals, with each group of animals serving as its own control in all comparisons of pre- and post-injection data presented in the Results. Food intake was controlled while animals were observed: some groups were kept satiated, while food was withheld from others for periods of up to 14 days.

Solutions of 5-HT and OA (Sigma) were prepared at concentrations between 0.01 and 1 mg ml⁻¹ in physiological saline of the following composition (mmol·l⁻¹): NaCl 460, CaCl₂ 13.7, KCl 12.7, MgCl₂ 10, Na₂SO₄ 14, maleic acid 5, TRIS base 10, pH adjusted to 7.4. All amine solutions were stored at 4 °C and were less than 48 h old when used.

Injections were made into the ventral hemolymph sinus at the thoracic-abdominal juncture, through a 27-1/2 gauge needle. We found that this injection point gave the fastest entry into the general circulation, the fastest distribution of the injected substance (tests using dye injection indicated less than 30 s to enter the vasculature in the ventral nerve cord along its entire axis), the least chance of damaging the ventral nerve cord or muscles vital to the experiments, and the most reproducible results. Injections of each amine started at 0.001 mg g⁻¹ body weight, and were increased and decreased from this point until threshold and maximum non-toxic doses were found (the latter marked by the beginning of pathological responses). Dose responses between these points were characterized. The concentration of the injected solution was such that the volume injected was never more than 5% of the animal's weight. Tests performed at the start of the experiments indicated that varying the concentration of the injected solutions within the range used did not affect an animal's responses to a given dose, but increasing the volume of the injection beyond 7–8% of the animal's weight resulted in erratic behavior and sometimes death. At least 6 h for recovery were given between injections, with no more than two injections per day. We found that this injection rate did not result in any chronic effects on behavior. Control injections of saline were equivalent to 5% of the animal's weight. Food was offered every 2nd day, after the conclusion of that day's experiments, and any left after 30 min was removed.

Forty-four animals received 5-HT dose series, 47 received OA dose series, and 21 received dose series of both amines. Starting immediately after each injection, changes in posture and behavior of the animals, and responses to the same artificial stimuli used to challenge the untreated animals, were recorded continuously until behavior appeared to have returned to normal. Postural and behavioral responses to injections were recorded as for the untreated, normally interacting animals. Mortality of injected animals was not elevated above the low level normal in our larger seawater tables during the injection experiments, and was only slightly elevated following the experiments. Animals that recovered fully received repeat doses under a different set of conditions, such as a change in the stage of the molting or breeding cycle, or longer times in the laboratory. Following characterization of the dose-response curves of animals which had been isolated in small groups, animals with similar responsiveness to the amines were placed together, and were observed for any behavioral changes. Injections were repeated after an additional period of 1–3 weeks in the new groups, to determine if a change in social setting could induce a change in dose responsiveness.

Results

Posture and behavior during normal interactions

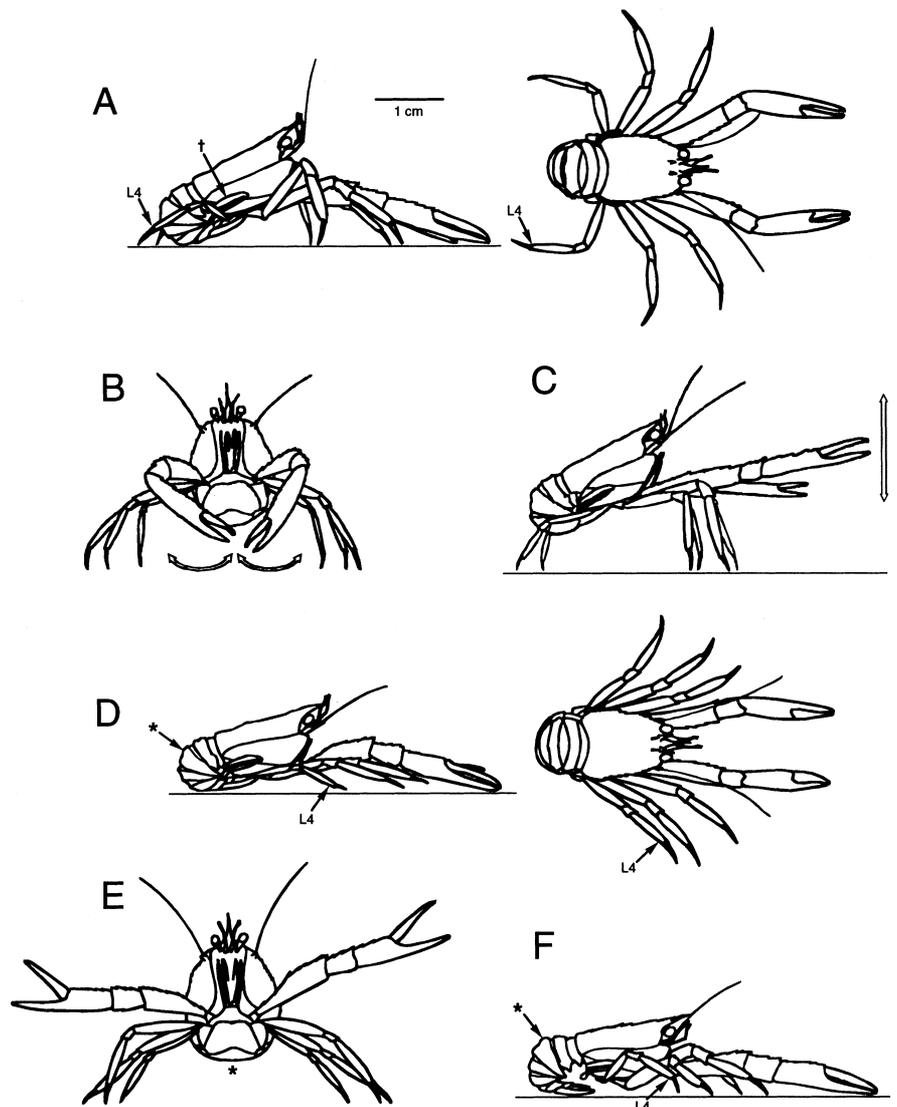
All postures and behaviors of *M. quadrispina* were consistent regardless of body orientation (i.e. sitting flat on a rock or hanging from an overhang), light levels within the limits of visual observation, or time of day. These animals normally rest in a squatting posture, with the head slightly elevated and the abdomen curled under the thorax, the dorsal surface of the tailfan resting on the substrate (Fig. 1A). The claws rest on the substrate to the front, and the three pairs of walking legs are held to the sides in the characteristic positions illustrated. *M. quadrispina* move by walking around the benthos and tailflipping short distances.

We observed no lasting dominance hierarchies among *M. quadrispina* in any of the seawater tables or observation tanks. They did not have territories of any kind,

and were rarely aggressive towards each other. Transient agonistic interactions were always resolved without lasting effects. Dominance hierarchies did not develop and the frequency of agonistic encounters did not increase during mating periods. At least in captivity, the successful male in the competition for mates was the one who got to the receptive female first, regardless of size. Frequency of aggressive displays decreased in the presence of food. Feeding was a free-for-all, success depending less on size than on speed in grabbing a piece of food and escaping to a safe spot. Larger individuals commonly used their closed claws to block competitors and shove them away; however, if many other animals were around, some quickly found ways to reach in to steal the food.

No consistent movements or actions preceded the onset of either of *M. quadrispina*'s aggressive displays. Both aggressive behaviors involved depression at the coxal-basal (second) joints and flexion at the meral-carpal (fourth) joints of the walking legs, which elevated

Fig. 1A–F Postures and behaviors of normally interacting *Munida quadrispina*: **A** side and top views of normal resting posture, with partial flexion of the walking legs at the meral-carpal joint maintaining the head-up tilt of the cephalothorax. Note leg four (*L4*) is removed and the small fifth leg (†) is held curled alongside the carapace; **B** “shaking-claws” aggressive behavior; **C** “raised-claws” aggressive behavior; **D** side and top views of the submissive, prostrate posture. Note that the abdomen is tightly curled (*) and all walking legs, including the fourth pair (*L4*), are fully promoted alongside the thorax; **E** defensive stance, with raised claws held far apart. Note the abdomen (*) is tightly curled against the underside of the thorax, well off the substrate; **F** startle response. The anterior part of the abdomen is extended (*), the walking legs, including the fourth pair (*L4*), are extended forward, although not to the same degree as in the submissive posture (compare with **D**). See Results for detailed descriptions of these behaviors



the body relative to the substrate, while the abdomen remained loosely flexed. The first behavior, which occurred while the animals were some distance apart, consisted of holding the claws in front, slightly elevated at the coxal-basal joint and slightly flexed, and, intermittently, shaking them horizontally and rapidly (approximately 2–5 Hz) at the thoracic-coxal joint in what appeared to be a warning display (Fig. 1B). During this “shaking-claws” display, the tips of the claws were kept at or below the level of the head, and the displaying individual would sometimes advance slowly towards its opponent. The second form of aggressive behavior, the “raised-claws” behavior, occurred when the animals were close or in contact. The advancing, aggressive animal extended both claws at the meral-carpal joint and elevated one or both claws high overhead to get its claws above its opponent’s to push them down and its opponent back or to the side (Fig. 1C). We observed no consistent movements of the antennae at any time during agonistic interactions.

An aggressive individual usually turned to within approximately 45° of face on to its opponent, but occasionally faced away (Fig. 2). The actions of the claws appeared to be the most important cues for the opponent, and the claws were visible regardless of the

aggressive animal’s orientation. Grasping rarely occurred, and the aggressive behaviors ended when one animal retreated or clearly showed itself to be submissive, or when both “lost interest.” Fighting was never seen in any encounter in the laboratory; the shed legs and claws occasionally found in the densely populated sea tables were probably the result of cannibalism of recently molted or unhealthy animals, which was observed in a few instances. Size was not a factor in determining whether an aggressive display would be performed: small animals displayed to large ones as often as large animals displayed to small ones. Relative size of the animals also did not influence whether the non-aggressive animal would retreat or respond in kind to the display. The only exception to this was that very large animals very rarely responded at all to aggressive displays, unless they were by equally large animals. In this case, posturing by both animals sometimes continued for several hours. Only recently molted individuals were less likely than any other to perform aggressive displays and more likely to retreat from other squat lobsters; no animal at any other time was found consistently to be particularly aggressive or submissive.

Three types of reaction to aggressive displays occurred with different frequencies (Fig. 2), and their frequencies were the same regardless of which of the two aggressive displays initiated them. In only about 10% of agonistic encounters (22 of 225 observations) did individuals respond to aggression with aggressive displays of their own. In more than 70% (164 of 225 observations) of the encounters, the aggressors were ignored or pushed away without any reciprocal displays. Pushing was done with closed claws, and was performed with sweeping claw motions or extensions directly away from the body. The claws and body were not usually raised during pushing, and motions were not repeated, as in the aggressive “shaking claws” behavior discussed above (Fig. 1B), unless the second animal continued to advance. Pushing never became violent, and transitions between pushing and any aggressive behavior were rarely seen; an individual whose space was repeatedly infringed upon usually moved away.

Behaviors which could be classified as submissive occurred in fewer than 20% (39 of 225 observations) of all agonistic encounters. Escape was the most common: animals retreated from an aggressor by walking or tailflipping away, and each behavior occurred with approximately equal frequency. Sometimes, but not always, the body was lowered while walking backwards or sideways away from the aggressor. Tailflipping was not directional; the individual often came to rest temporarily as close or closer to the aggressive animal than when it started, in which case a second attempt to retreat often followed quickly. Occasionally, submissive animals prostrated themselves before an aggressor without retreating, by lowering the body to the substrate, extending the legs, and promoting the fourth pair of legs alongside the second and third pairs (Fig. 1D). This most often occurred when no route for retreat was open.

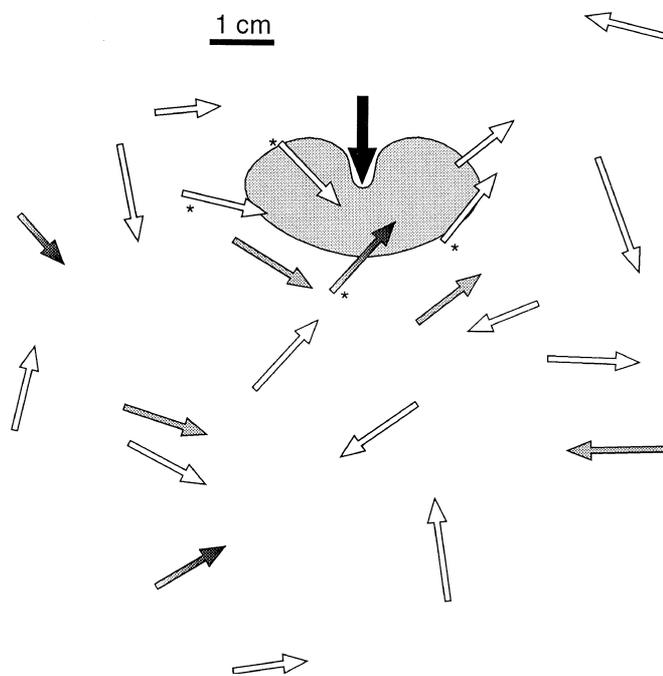


Fig. 2 Composite diagram of 23 agonistic encounters between *M. quadrispina* individuals. The large black arrow is the animal which initiated each encounter with an aggressive display; each of the other arrows represents the second animal in different encounters. Arrows indicate the direction each animal was facing, and carapace length is represented by arrow length. Reactions of the second animals are as follows: (→) no reaction, (→) an aggressive reaction, (→) a submissive reaction. The shaded area is the claw reach of the first animal. An asterisk (*) at the base of the arrow indicates short-range encounters in which the first animal was performing the “raised-claws” behavior. No asterisk indicates a “shaking-claws” display by the first animal

M. quadrispina occasionally performed a behavior in response to a threat which could be analogous to a crayfish defense response (Wiersma 1952): the body was elevated, the animal turned toward the threat, and the open claws were held wide apart (Fig. 1E). This behavior occurred only in response to handling or the sudden appearance of a potential predator (e.g. human hand, artificial fish shape), never in response to conspecifics, and was rare (approximately 15% of threatening situations not involving conspecifics). Defensive animals grasped the threatening object, sometimes violently, if it came within reach. More common were tailflipping away (50%) or startle responses with no retreat (22%). Startle responses involved a quick extension of the legs and abdomen, resulting in an almost prostrate posture resembling the first stage of an escape tailflip when the anterior abdomen has extended (Fig. 1F). The startle position (Fig. 1F) was transient, unlike the prostrate posture which was held for periods of up to 15 min. Most individuals were consistent in their defensive reactions, although no correlation existed between how an individual would react to a conspecific of any size and how it would react to potential predators.

Effects of the injected amines on posture and behavior

In general, injected 5-HT induced aggressive postures and behaviors in *M. quadrispina*, and injected OA induced submissive postures and behaviors. Almost every animal responded with dose-dependent classes of behaviors which could be ranked in a dose-response curve. Although not all behaviors described could be elicited in every individual tested, and the dose required to produce a particular response in different animals varied considerably, the order in which the responses were elicited with increasing dose was consistent for all animals. Furthermore, individual animals' dose-response curves were consistent in order of responses and were not dependent on recent social experiences, changes in social environment, molting stage, reproductive state, or time in captivity. Control saline injections caused individuals to tailflip around the tank, assume defensive postures, and to explore the injection site with their fifth pair of legs, but these behaviors never lasted for more than 30 s; in the first 30 s following amine injections, only behaviors that were very clearly different from these control responses were recorded as part of the aminergic effect. The characteristic amine-induced behaviors usually began 30–60 s following the injection.

Clear, dose-dependent responses to injected 5-HT occurred in every animal tested, with four distinct classes of response elicited by the range of doses used in these experiments (Fig. 3A). These classes were quite discrete, although intensity of a particular induced response often increased within the dose range capable of eliciting that response. Transitional stages between classes of behaviors occurred only rarely, and could not be repeatably induced. On two occasions, animals injected with large

doses of 5-HT proceeded, within 1 min, through all four of the response classes in order, and the transitions between classes were remarkably abrupt.

The first observable effect of injected 5-HT was an increased likelihood and sometimes intensity of aggressive reactions to real or artificial squat lobsters, but without a sustained change in posture. If left alone and undisturbed after the injection, their behavior under this lowest effective dose of 5-HT was indistinguishable from that of untreated squat lobsters. However, these animals responded aggressively to untreated squat lobsters immediately after the latter had been placed in the observation tank in more than 90% (40 of 43 observations) of all tests, as compared to untreated animals responding aggressively in fewer than 10% of similar encounters (3 of 35 observations). Aggression by untreated animals towards treated animals was always responded to in kind. On two occasions (in 43 tests), animals injected with low doses of 5-HT actively pursued other squat lobsters, initiating combat when successful in catching their opponent, a behavior never seen in untreated animals. Potentially threatening stimuli usually elicited aggressive responses in animals injected with their lowest effective dose, as opposed to the defensive or escape responses elicited in untreated animals. The effects of this dose wore off 20–30 min after injections.

The next two classes of behavior induced by larger doses of injected 5-HT (Fig. 3 A–C) closely resembled the two classes of aggressive display seen in normally interacting animals (see Fig. 1B, C). Several points distinguish the induced responses from natural behaviors, however. Most importantly, the aggressive displays induced by injected 5-HT were performed in the absence of any external stimulation, and were performed with greater frequency and for longer periods than the intermittent displays of untreated animals. Furthermore, as was the case under lowest effective doses, aggressive acts towards conspecifics increased in frequency and intensity, and threats were usually responded to aggressively. The degree of leg flexion and depression was greater and the cephalothorax, therefore, more tilted than during aggressive displays by untreated animals (Table 1; cf. Fig. 3B, C with Fig. 1B, C), and the differences between elicited and normal aggressive displays increased with increasing 5-HT dose. 5-HT doses which produced the “raised-claws” behavior also caused the folded fifth leg to rotate up from its usual horizontal position alongside the body until it stuck straight up above the thorax (Fig. 3C). The induced “shaking-claws” and “raised-claws” behaviors lasted for 30–90 min depending on dose, and were followed by a period of increased aggressiveness similar to that induced by the lowest effective 5-HT doses.

Even higher doses of 5-HT caused extreme rigidity, with the legs and claws depressed and tightly flexed under the thorax (Fig. 3A, D). Animals in this state would often tip over and remain on their side or back for up to several hours depending on dose. If sufficiently disturbed by handling, however, these animals could

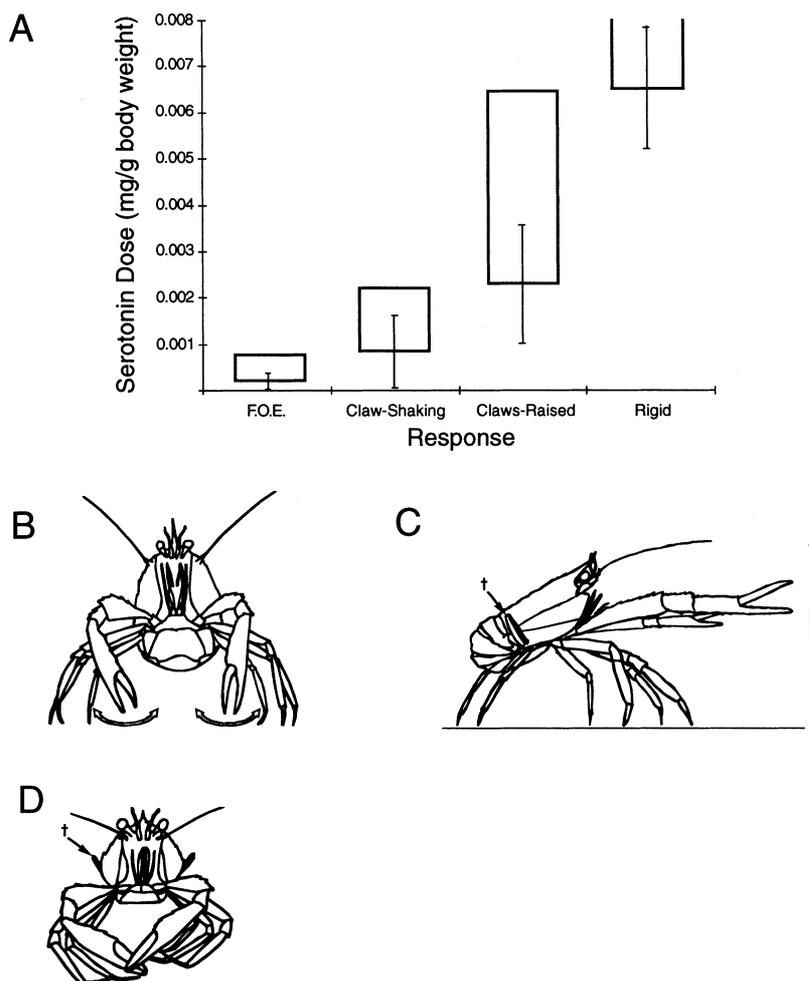
Fig. 3A–D *M. quadrispina*'s responses to injected 5-HT:

A dose-response graph for injected 5-HT. The responses correspond to the four classes of behavior described in the Results. The boxes are the dose ranges which elicit each class of behavior. Thresholds and maxima (respectively, bottom and top of boxes) for each behavior class are means of all tests.

Standard error bars are shown for the threshold doses for each behavior. (*F.O.E.* first observable effect); **B** induced "shaking-claws" behavior typical of early aggressive displays; **C** induced "raised-claws" display performed late in aggressive performances. Note that in the induced stances the walking legs are more depressed and flexed, and the body tilt is greater than in the natural behaviors (cf. Fig. 1B, C).

Also note the fifth legs which are elevated and remotated, stick straight up above the animal, but remain flexed at the meral-carpal joint (†); **D** the rigid posture elicited by very high doses of injected 5-HT.

The animal is illustrated upright, but often tips over to lie on its side or back. The walking legs and chelae are very strongly depressed and flexed; the flexed fifth leg sticks straight up (†)



overcome their rigidity and tailflip away, with apparently normal progression (movements), but their rigidity returned immediately after they settled down. Aggressive responses to any external stimulus could not be elicited from animals in this rigid state, or for several hours after they regained mobility.

M. quadrispina's responses to injected OA also fell into four classes (Fig. 4A), although the divisions be-

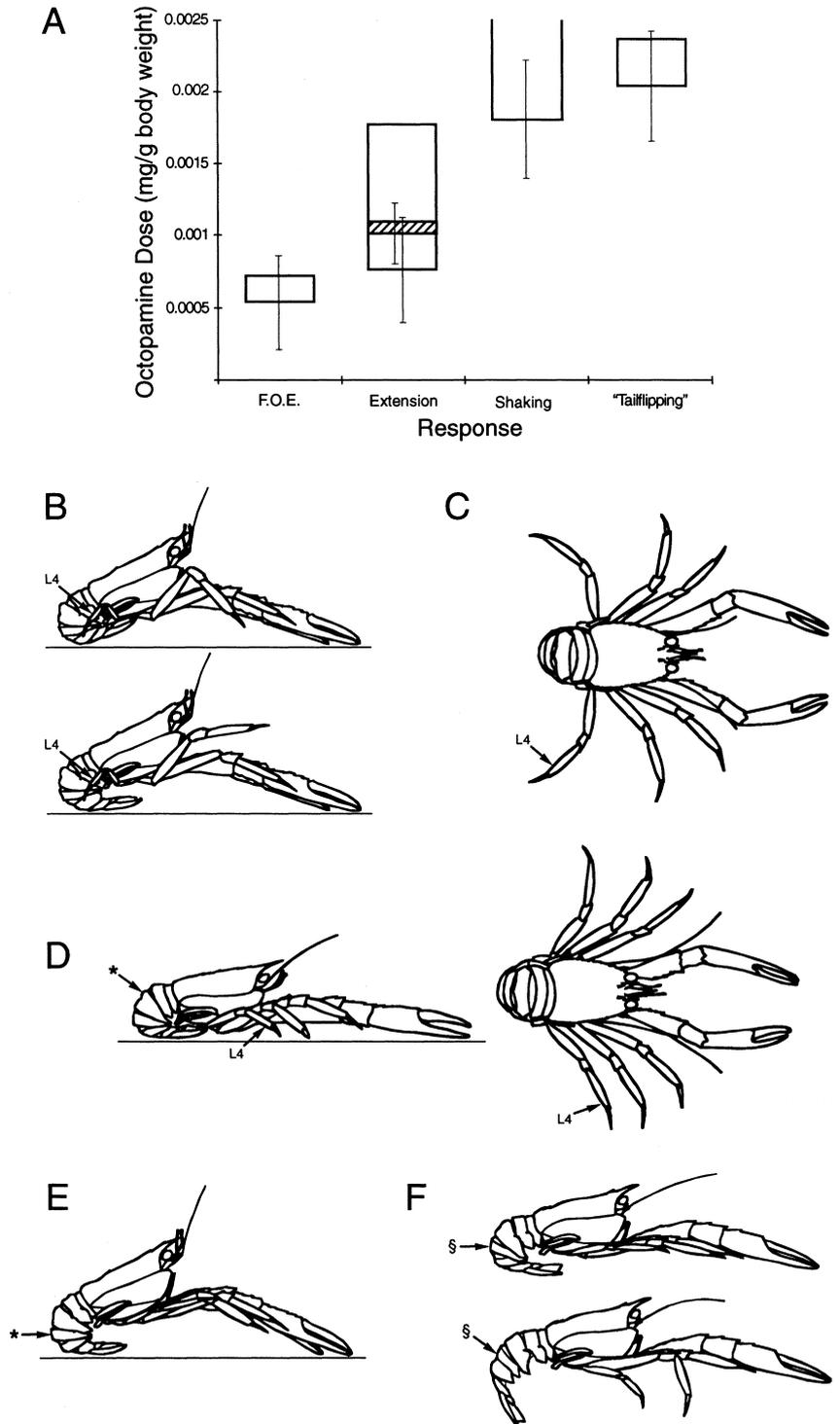
tween them were not as distinct as between the response classes in the 5-HT dose-response curve. The lowest doses of OA to have an observable effect caused no sustained change in posture or behavior of undisturbed animals, but increased the likelihood of escape responses when another, untreated squat lobster was put in the tank from 33% (15 of 45 observations) to more than 90% (23 of 25 observations) compared to untreated animals. Likewise, escape or startle responses when presented with an artificial threatening stimulus increased from 55% (110 of 200 observations) in untreated animals to very nearly 100% (123 of 125 observations) in OA-treated animals. Escape responses consisted of two behaviors which occurred with approximately equal frequencies: tailflipping, which was indistinguishable from tailflipping in untreated animals, and rapid scuttling away from the perceived threat, a behavior rarely seen in untreated animals.

Extension of the walking legs and abdomen was elicited by a range of OA doses beginning at a point slightly higher than that required to induce the increased escape tendencies (Fig. 4A, B). Within this range, increases in OA dose produced increasing extension of the walking legs and abdomen. Extension and elevation of

Table 1 Thoracic tilt and leg 2 joint angles (means \pm standard errors, $n = 15$) of *M. quadrispina* in normal resting posture, normal aggressive postures, and 5-HT-induced aggressive postures. Data for the two types of aggressive behavior, "shaking-claws" and "raised-claws," were pooled, as the angles were similar. Thoracic tilt is the angle of the dorsal surface of the carapace from the horizontal; the coxal-basal joint angle with respect to the sternal plate corresponds to leg depression (negative indicates the leg is elevated); the meral-carpal joint angle, measured from straight, corresponds to leg flexion

Posture	Thoracic tilt	Coxal-basal angle	Meral-carpal angle
Normal resting	22.0 \pm 3.4	-9.0 \pm 2.5	55.2 \pm 7.5
Normal aggressive	22.5 \pm 2.6	17.2 \pm 3.1	40.2 \pm 2.3
Induced aggressive	26.1 \pm 4.0	23.5 \pm 5.2	62.0 \pm 4.3

Fig. 4A–F *M. quadrispina*'s responses to injected OA: **A** dose-response graph for injected OA. The responses correspond to the classes of behavior described in the text. The prostrate response occurs in the *hatched area* within the range of doses which elicit extension of the walking legs and abdomen. The *boxes* are dose ranges which elicit each behavior class. The threshold and maximum dose of each class (bottom and top of boxes, respectively) are means of all tests, and standard error bars are for each threshold dose. (*F.O.E.* first observable effect); **B, C** postures of one individual induced by OA doses within the range which causes extension of the legs and abdomen, but which are not typical submissive postures: **B** (*top*) low dose within this range and (*bottom*) high dose; **C** top view of animal injected with a high dose to show clearly the position of the fourth pair of legs (*L4*), which are only slightly promoted from their normal position. The claws are also kept close to their normal resting position, resulting in the walking legs being elevated off the substrate (evident in **B**); **D** side and top view of prostrate stance induced by injected OA. The fourth pair of legs (*L4*) are promoted to lie alongside the second and third pairs, but the walking legs are not held as tightly to the thorax, and the abdomen is more extended (*), than in the natural submissive stance (Fig. 1D); **E** the most extended position during a typical example of the slow "tailflipping" behavior induced by injected OA. The animal is balanced on the tips of its claws and dorsal surface of the tailfan; **F** positions of the legs and abdomen at the end of the extension phase during normal slow (*top*) and fast (escape) tailflipping (*bottom*) (Wilson and Paul 1987; B. L. Antonsen and D. H. Paul, unpublished observations). The abdominal extension (*) during the induced "tailflipping" is within the range of maximal abdominal extension (§) during normal tailflipping



the walking legs could become so extreme that they stuck straight out and up, leaving the animal balanced on the tips of its claws and dorsal surface of the tailfan (Fig. 4B). Leg extension and elevation as extreme as pictured in Fig. 4B (bottom) could not be elicited in every individual, but all showed increasing extension with increasing dose. The fourth pair of legs was not promoted alongside the second and third pairs in these

elicited postures (Fig. 4C). With increasing OA dose within this range, frequency of escape reactions increased to 100%, and intensity and/or duration of escape or startle responses increased. At the highest doses within this range, the animals were very jumpy in the absence of any additional stimulation, and scuttled and tailflipped around the tank seemingly without provocation.

A posture resembling normal submissive postures (see Fig. 1D) was elicited by a narrow dose range within the dose range which produced the increasing extensions of the legs and abdomen just described, in 14 of the 47 animals tested. In this elicited prostrate stance, in contrast to the other OA-elicited extended stances, the claws were elevated, resulting in the body being lowered to the substrate, and the fourth pair of legs promoted alongside the second and third pairs (Fig. 4D). This induced prostrate posture differed from submissive postures in untreated animals in the greater extension of the rostral abdomen and flattening of the caudal abdomen against the substrate and more elevated and lateral position of the walking legs (Fig. 4D; cf. Fig. 1D). Additionally, the animals would scuttle about, usually backwards, and periodically assume the "legs-extended" postures described above. The narrowness of the dose range over which prostrate stances could be induced ($< 0.0002 \text{ mg g}^{-1}$ body weight) could explain why this behavior was not induced in most of the test animals, but for those animals in which this stance could be induced the result was repeatable.

Rapid shaking involving the walking legs, claws, and abdomen was elicited by OA doses at the high end of the dose range which induced increasing postural extension. The degree of elevation of the walking legs and extension of the walking legs and abdomen decreased at the threshold dose for shaking, but always remained greater than in the normal resting posture. Further increases in OA dose resulted in shaking of increasing severity. Shaking could be quite violent, and could be either periodic or continuous for up to several minutes. Tailflipping and scuttling without any apparent provocation were frequent during these periods. Animals in this condition did not often respond to visual stimuli, but tailflipped immediately when touched.

At a dose slightly higher than the threshold required to induce shaking, the animals performed an unusual behavior which resembled very low repetition tailflipping. This behavior consisted of abnormally slow extensions punctuated by sudden, normal-looking, fast flexions. Starting with the legs and abdomen somewhat extended and the body balanced on the tips of the claws and the dorsal surface of the tailfan (as in Fig. 4B, top), the animal slowly extended, elevated, and promoted its walking legs alongside the thorax and extended its abdomen (Fig. 4E). The extension phase took from 2 to 90 s, and was followed by a quick abdominal flexion, which brought the animal to a posture similar to the resting posture of untreated animals. Within 10 s, the legs again became extended, stiff, and shaky, and the cycle began again, to be repeated for periods of up to several hours depending on the dose. The extension of the abdomen and the positions of the legs alongside the thorax very closely resembled those assumed during the return stroke of a normal tailflip; in particular, the degree of extension of the abdomen during this OA-induced behavior was well within the normal range for tailflipping in untreated animals (Fig. 4F; see also Wilson and

Paul 1987). Much higher OA doses resulted in very severe shaking and completely immobilized the animals.

Discussion

The social structure of *M. quadrispina* populations differs substantially from that of crayfish: aggressive acts are rare and dominance hierarchies do not exist, whereas in populations of crayfish and American lobster, aggression and fights are common and used to establish dominance hierarchies (Scrivener 1970; Bruski and Dunham 1987; Huber and Kravitz 1995). Aggression is used solely for transient space claims in *M. quadrispina* populations, and most aggressive acts appear to be bluff, for threats which are ignored are almost never followed by aggressive acts. *M. quadrispina*, like most benthic galatheids, are omnivorous scavengers and filter feeders (Nicol 1932; B.L. Antonsen and D.H. Paul, unpublished observations). In high population densities, "scramble competition", similar to what we regularly observe in our laboratory populations, is likely the most efficient method of scavenging food items of unpredictable availability. The frequency of aggressive acts does not increase during the mating season, in keeping with the absence of territoriality.

Several crustacean species which normally live in low population densities become less aggressive towards conspecifics if kept at high densities (Hazlett 1968; Courchesne and Barlow 1971; Ulmer and Grant 1971; Dunham 1972; Vannini 1981). However, *M. quadrispina* (this study) and the hermit crab *Pagurus pubescens* (Ulmer and Grant 1971), both of which live at high population densities, do not become more aggressive when kept at low density. This indicates that although aggressive species can be conditioned to become less aggressive, the lack of aggression in gregarious species is genetically ingrained.

Several lines of evidence strongly implicate 5-HT and OA in the induction of aggressive and submissive behaviors in *M. quadrispina*. First, complex behaviors exhibited by untreated, normally interacting animals can be mimicked in isolated individuals, in the absence of any additional stimulation, by injection of one of the amines. Second, injected amines prime the animals to react so that frequency and intensity of responses to external stimuli are increased. Third, injection of an amine can, in at least one situation, reverse an untreated animal's typical response. That is, animals which are under the influence of injected 5-HT react aggressively to an artificial potential predator, as opposed to displaying their normal escape or defense reactions. These data indicate that the amines act on one or more higher centers responsible for the induction and orchestration of the behaviors (Fig. 5; a). Given the influence 5-HT and OA have on these behaviors in *M. quadrispina*, it seems reasonable to anticipate that future studies on crayfish will uncover additional roles beyond the role in fighting readiness which has been reported (Huber 1995).

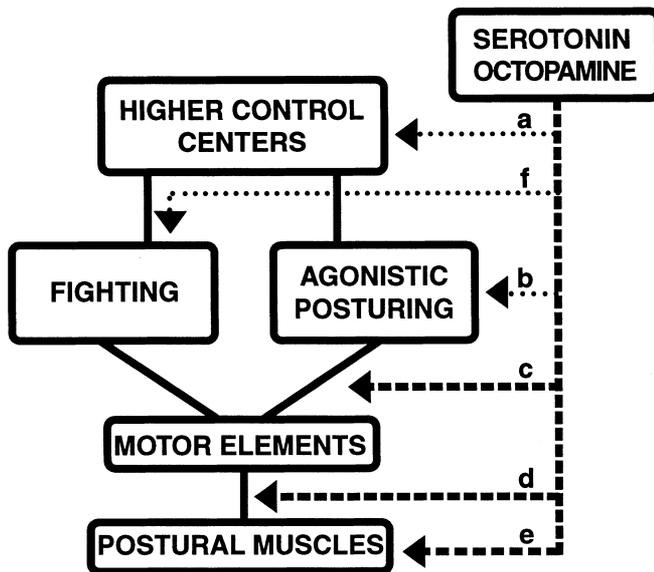


Fig. 5 Possible sites of aminergic modulation on agonistic control circuits. The *boxes* represent centers for the production or control of aggressive behaviors. The *dashed lines* indicate serotonergic modulatory sites confirmed physiologically in lobsters and crayfish. *Dotted lines* indicate additional possible sites of influence suggested by comparisons of behavioral studies on lobsters and crayfish and this study on *M. quadrispina*. *Arrows pointing to boxes* indicate serotonergic effects directly on the hypothetical centers; *arrows pointing to lines* indicate modulatory influences on the circuits involved in communication between two levels of control

The results of this study and others suggest that 5-HT and OA also influence pathways for postural control directly, independently of behavioral control pathways. In *M. quadrispina* certain doses of OA cause extension of the walking legs and abdomen without concurrent submissive postures or behaviors. Likewise, large doses of 5-HT, above those that produce aggressive behaviors, result in leg flexion far more severe than during normal behaviors. Additionally, within the dose range for each class of behavior induced by the amines, the degree of postural modulation by each of the amines increases with increasing dose. Large doses of 5-HT or OA induce, respectively, flexion or extension of the legs and abdomen in lobsters and crayfish without inducing any distinct natural behaviors (Livingstone et al. 1980). These results suggest an influence in the areas controlling postural coordination (Fig. 5; b) and postural extension and flexion (Fig. 5; c). Additionally, some sites of action of the amines within postural control circuits have been identified in lobsters and crayfish: at the level of command neuron interaction with motor elements (Fig. 5; c) (Harris-Warrick 1985; Ma et al. 1992), at the neuromuscular junction (Fig. 5; d) (Florey and Rathmayer 1978; Glusman and Kravitz 1982; Breen and Atwood 1983; Fischer and Florey 1983; Dixon and Atwood 1985), and on the muscles themselves (Fig. 5; e) (Kravitz et al. 1980). We are currently working to characterize analogous sites in *M. quadrispina*.

Perfusion of 5-HT into crayfish promotes fighting readiness in an animal which would normally be

submissive, but does not influence any other aspects of the aggressive encounter (Huber 1995), suggesting serotonergic influences, direct or indirect, on "fighting centers" in crayfish (Fig. 5; f). Injection of 5-HT can also induce fighting in *M. quadrispina*. Considering that fighting is not a part of *M. quadrispina*'s normal behavioral repertoire, its induction by injected serotonin suggests that intact behavioral pathways for fighting exist but are never activated in untreated animals. This implies the loss of fighting behavior in this species, and may explain how bluffs which are never backed up by action could have evolved. Alternatively, 5-HT's influence could be on a "higher center" which is separate from the "aggression pathways" per se. A candidate could be within the defense reaction pathway, as defensive reactions in *M. quadrispina* can involve violent grasping, which could appear as fighting among conspecifics.

This study implicates OA in control of tailflipping circuitry in *M. quadrispina*. High doses of OA induce motions which are very much like individual tailflips performed by untreated animals, except that the extension phases are very much slower and, therefore, the frequency is much lower. OA facilitates giant interneuron-mediated escape tailflip reactions in crayfish (Glanzman and Krasne 1983), but high doses of OA have not been reported to induce escape tailflips in lobsters or crayfish, or to influence any aspect of non-giant interneuron-mediated tailflipping.

Although doses of either amine required to produce any single response varied between individuals, dose-response curves for individual animals remained consistent over time and regardless of the animals physiological state or social experience. Interestingly, dose-response curves for individual animals remained consistent even under circumstances which alter behavioral repertoires of untreated animals. For example, animals became more likely to retreat from conspecifics when recently molted, but responses to injected amines did not change over the same period. This leaves unanswered the question of the causes of the observed individual variation in responsiveness. Environmental conditions during the egg or larval stages may influence development of the modulatory circuits, or the variability in the circuits may be genetically determined.

Clearly, both amines act at two levels, postural and behavioral, in influencing agonistic encounters, although these actions probably occur at many sites. Although these two levels are interconnected, our results reveal that they are subject to (at least partially) independent, aminergic influences. The "higher control centers" that are responsible for behavior are influenced by lower doses of the amines than are the postural circuits, as evidenced by behavioral facilitation at low doses in the absence of sustained postural change. This influence may be on central neurons, or on sensory afferents with inputs to these "higher centers." Aminergic influences on several sensory pathways with potential influences on agonistic behaviors or posture have been described (El Manira et al. 1991; Pasztor and MacMillan 1990;

Pasztor and Golas 1993; Rossi-Durand 1993; Yeh et al. 1996). Increasing doses of the amines have a punctuated effect on behavior, with changes between classes occurring over a very narrow dose range. This suggests that changing concentrations of the amines could change recruitment in sets of higher-level neurons responsible for effecting behavioral choice. At the level of postural control, the aminergic influences start at a higher dose, and are graded, increasing in intensity with increasing dose. We are currently working to identify sites within the postural control circuits influenced by the amines (Fig. 5; b, c, d, or e).

These results place aminergic neurons at the center of behavioral and postural induction. Activity levels of individual neurons could influence behavioral and motor output by changing local amine concentrations. The number of serotonergic neurons is greatly reduced in *M. quadrispina* as compared to lobsters (Antonsen and Paul 1994; B.L. Antonsen and D.H. Paul, unpublished observations); therefore, identifying individual neurons involved in modulating aggressive responses may be easier than in lobsters.

Acknowledgements This work was supported by the Natural Science and Engineering Council of Canada (Research Grant no. OGP08183 to DHP). B.L.A. is a recipient of an NSERC PGS-B Scholarship.

References

- Antonsen BL, Paul DH (1994) The effects of the amines serotonin and octopamine on the posture of the squat lobster *Munida quadrispina*. Soc Neurosci Abstr 20: 379
- Bevengut M, Clarac F (1982) Contrôle de la posture du crabe *Carcinus maenas* par des amines biogènes. C R Acad Sci Paris 295: 23–28
- Breen CA, Atwood HL (1983) Octopamine – a neurohormone with presynaptic activity-dependent effects at crayfish neuromuscular junctions. Nature 303: 716–718
- Bruski CA, Dunham DW (1987) The importance of vision in agonistic communication of the crayfish *Orconectes rusticus*. I. An analysis of bout dynamics. Behaviour 63: 83–107
- Courchesne E, Barlow GW (1971) Effect of isolation on components of aggressive and other behavior in the hermit crab *Pagurus samuelis*. Z Vergl Physiol 75: 32–48
- Dixon D, Atwood HL (1985) Crayfish motor nerve terminals' response to serotonin examined by intracellular microelectrode. J Neurobiol 16: 409–424
- Dunham PJ (1972) Some effects of group housing upon the aggressive behavior of the lobster *Homarus americanus*. J Fish Res Board Can 29: 598–601
- El Manira A, Rossi-Durand C, Clarac F (1991) Serotonin and proctolin modulate the response of a stretch receptor in crayfish. Brain Res 541: 157–162
- Fischer L, Florey E (1983) Modulation of synaptic transmission and excitation-contraction coupling in the opener muscle of the crayfish *Astacus leptodactylus*, by 5-hydroxytryptamine and octopamine. J Exp Biol 102: 187–198
- Florey E, Rathmayer M (1978) The effects of octopamine and other amines on the heart and on neuromuscular transmission in decapod crustaceans: further evidence for role as a neurohormone. Comp Biochem Physiol 61C: 229–237
- Glanzman DL, Krasne FB (1983) Serotonin and octopamine have opposite modulatory effects on the crayfish lateral giant escape reaction. J Neurosci 3: 2263–2269
- Glusman S, Kravitz EA (1982) The action of serotonin on excitatory nerve terminals in lobster nerve-muscle preparations. J Physiol (Lond) 325: 223–241
- Harris-Warrick RM (1985) Amine modulation of extension command element-evoked motor activity in the lobster abdomen. J Comp Physiol A 156: 875–884
- Hazlett BA (1968) Effects of crowding on the agonistic behavior of the hermit crab *Pagurus bernhardus*. Ecology 49: 573–575
- Helluy S, Holmes JC (1990) Serotonin, octopamine and the clinging behaviour induced by the parasite *Polymorphus paradoxus* (Acanthocephala) in *Gammarus lacustris* (Crustacea). Can J Zool 68: 1214–1222
- Huber R (1995) Serotonin controls decision making aspects of fighting in crayfish. In: Burrows M, Matheson T, Newland PL, Schuppe H (eds) Nervous systems and behaviour. Thieme, Stuttgart, p 168
- Huber R, Kravitz EA (1995) Quantitative analysis of agonistic behavior in juvenile American lobsters (*Homarus americanus* L.). Brain Behav Evol 46: 72–83
- Kravitz EA, Glusman S, Harris-Warrick RM, Livingstone MS, Schwarz T, Goy MF (1980) Amines and a peptide as neurohormones in lobsters: actions of neuromuscular preparations and preliminary behavioral studies. J Exp Biol 89: 159–175
- Livingstone MS, Harris-Warrick RM, Kravitz EA (1980) Serotonin and octopamine produce opposite postures in lobsters. Science 208: 76–79
- Ma PM, Beltz BS, Kravitz EA (1992) Serotonin-containing neurons in lobsters: their role as gain-setters in postural control mechanisms. J Neurophysiol 68: 36–54
- Nicol EAT (1932) The feeding habits of the Galatheidæ. J Mar Biol Assoc UK 18: 78–106
- Olivier B, Mos J, Oorschot R van, Hen R (1995) Serotonin receptors and animal models of aggressive behavior. Pharmacopsychiatry 28 [Suppl]: 80–90
- Pasztor VM, Golas LB (1993) The modulatory effects of serotonin, neuropeptide F1 and proctolin on the receptor muscles of the lobster abdominal stretch receptor and their exoskeletal muscle homologues. J Exp Biol 174: 363–374
- Pasztor VM, MacMillan DL (1990) The actions of proctolin, octopamine and serotonin on crustacean proprioceptors show species and neurone specificity. J Exp Biol 152: 485–504
- Paul DH, Then MA, Magnuson DS (1985) Evolution of the telson neuromusculature in decapod crustacea. Biol Bull 168: 106–124
- Rossi-Durand C (1993) Peripheral proprioceptive modulation in crayfish walking leg by serotonin. Brain Res 632: 1–15
- Scrivener JCE (1970) Agonistic behaviour of the American lobster *Homarus americanus* (Milne-Edwards). MSc. thesis, University of Victoria, Victoria, BC
- Sillar KT, Heitler WJ (1985) The neural basis of escape swimming behaviour in the squat lobster *Galathea strigosa* I. Absence of cord giant axons and anatomy of motor neurones involved in swimming. J Exp Biol 117: 251–269
- Ulmer KM, Grant WC Jr (1971) Influence of shell size and isolation on con-specific aggressive behavior in hermit crabs. Bull Mt Desert Isl Biol Lab 11: 96–97
- Vannini M (1981) Notes on some factors affecting aggressive behaviour in *Carcinus mediterraneus*. Mar Biol 61: 235–241
- Wallis EJ, Paul DH, Antonsen BL, Hollenburg R (1995) Variations on a segmental theme: muscle receptor organs and extensor neuromusculature in the squat lobster *Munida quadrispina* (Anomura, Galatheidæ). J Exp Biol 198: 2453–2463
- Wiersma CAG (1952) The neuron soma: neurons of arthropods. Cold Spring Harbor Symp Quant Biol 17: 155–163
- Wilson LJ, Paul DH (1987) Tailflipping of *Munida quadrispina* (Galatheidæ): conservation of behavior and underlying musculature with loss of anterior contralateral flexor motorneurons and motor giant. J Comp Physiol A 161: 881–890
- Yeh S-H, Fricke RA, Edwards DH (1996) The effect of social experience on serotonergic modulation of the escape circuit of crayfish. Science 271: 366–369