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Serotonin Increases Flexion And Excitatory Junction Potential Amplitude While Octopamine Decreases Average Excitatory Junction Potential Amplitude In Crayfish.

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ABSTRACT
Serotonin and octopamine are biogenic amines that cause postural changes in crayfish. Their effectiveness is related to the social experience of the animal. In this study, the effect of serotonin and octopamine on postural changes of socially isolated crayfish was analyzed after injecting the amines into the hemolymph at the abdominal thoracic junction. Serotonin increased flexion of the tail, claws, and legs of the crayfish after 30 minutes. However, octopamine treated crayfish showed no significant postural changes. In addition, our study investigated the effects of serotonin and octopamine on excitatory junction potential amplitude (EJP) by stimulating the fast extensor muscles of the crayfish abdomen. Serotonin significantly increased and octopamine significantly decreased average EJP amplitude.

INTRODUCTION

Serotonin and octopamine are biogenic amines that are involved in the regulation of physiological processes and agonistic behaviors in crustaceans. Previous studies have suggested that serotonin serves an important role in aggression (Huber *et. al.,* 1997; Barthe *et. al.*, 1993). In addition, both serotonin and octopamine change the rate of firing of particular neurons in the stomatogastric system and their effects have a complex set of actions on the exoskeleton muscles (Kravitz *et. al.,* 1984). The injection of these amines can elicit postures typical of aggressive or submissive stances (Antonsen and Paul 1997). Specifically, serotonin induces an aggressive posture seen in dominant lobsters and octopamine induces a depressed posture typically seen in subordinate lobsters (Roeder 1999). In free-moving lobsters, injection of octopamine causes the lobster to crouch low with claws and abdomen stretched out. In contrast, the injection of serotonin causes sustained flexion of limbs and abdomen, standing on walking legs, and raised claws (Livingston *et. al.,* 1980; Roeder 1999).

Serotonin and octopamine modulate posture and aggression by regulating the response to stimuli of the lateral giant (LG) tail-flip command neuron in the crayfish (Yeh *et. al.,* 1996). Octopamine perfusion on this ganglion causes a motor pattern that leads to the contraction of extensors and the relaxation of flexors. On the other hand, perfusion with serotonin activates a motor pattern of flexion, with activation of flexors and relaxation of extensors (Roeder, 1999).

Serotonin and octopamine regulate LG's response to stimulus depending on the social status of the crayfish. In socially dominant crayfish, the injection of serotonin enhances the response of the LG to stimulus. On the

other hand, in the subordinate crayfish, response of the LG to stimulus is inhibited (Yeh, 1996).

Knowing the effects of serotonin and octopamine on crayfish behavior can provide valuable insights into aggressive behavior in humans. This is possible because serotonin has been implicated in the modulation of aggression and depression in humans. Studies have shown that a lack of serotonin causes depression. An anti-depressant, fluoxetine (Prozac), has been found to block serotonin uptake causing a build-up of this amine in the circulatory system thus treating the condition (McGrath, *et al.*, 2000). Just as the crayfish neuromuscular preparations have been used to elucidate basic synaptic transmission mechanisms in vertebrates, the study of the effects of serotonin and octopamine on the posture and neuromuscular junction of the crayfish can potentially be useful in further understanding the effects of these amines in humans.

In this study, we investigated the effects of serotonin and octopamine on posture of socially isolated crayfish. We hypothesized that serotonin would increase flexion in the tail, claws, and legs of the crayfish when injected into the hemolymph, and octopamine would increase extension. We also investigated their effects on average EJP amplitude at the crayfish neuromuscular junction. Our findings support our hypothesis that serotonin increases flexion, although we did not find evidence that octopamine increases extension. Furthermore, we determined that serotonin increases EJP amplitude whereas octopamine has the opposite effect.

MATERIALS AND METHODS

Posture Experiment

All experiments were performed on isolated crayfish in order to avoid the effects of social ranking. To isolate crayfish, we separated them into individual pots one week prior to the experiment. This isolation attempted to eliminate social dominance and subordination.

To investigate the effects of serotonin and octopamine on aggression in crayfish, we injected 0.5 mL of either serotonin (10^{-5} M) or octopamine (10^{-5} M) into the ventral hemolymph sinus at the abdominal thoracic junction. For the control crayfish, we injected 0.5 mL of saline. We observed each crayfish for approximately one hour, recording the tail position, abdominal elevation, and claw position.

Posture was quantified using a point system. Each of the following responses was granted 1 postural point (PP) based on the expected serotonin induced posture: raised body, raised claws, and flexed tail. Therefore, if the serotonin-injected crayfish acted as hypothesized it would receive the maximum PP of 3. On the other hand, if the octopamine injected crayfish acted as hypothesized (lowered body, lowered claws, and extended tail) then it would receive no PP. Total points were calculated and recorded every 5 minutes for 1 hour. Figure 1 illustrates a flexed tail, low body and unstretched claws, therefore it would be assigned 1 PP. Figure 2 illustrates raised body, fully extended tail, and raised claw for which we would assign 2 PP. Figure 3 illustrates raised claws and body, and a partially extended tail for which we would assign 2.5 PP.

Excitatory Junction Potential Recording

To investigate the effect of

the biogenic amines on the excitatory junction potential (EJP) of the crayfish fast extensor muscle, we dissected the crayfish tail according to procedures outlined in Teaching Physiology (Stephens 1996). Then, we placed dissected crayfish tails in bath solutions of either saline (control), octopamine (10^{-5}M in saline), or serotonin (10^{-5}M in saline). To evoke an EJP, we stimulated the nerves in the 2nd or 3rd segments of the crayfish tail using a suprathreshold stimulus from a Grass Stimulator. After recording initial EJPs for all three preparations, we treated the tails in their respective bath solutions for 1 hour and then recorded the final EJP. To record the EJP we used the MacLab/Scope program and calculated the average of 5 repetitions. Statistical significance was based on standard deviation.

RESULTS

Posture Experiment

After injecting crayfish with either serotonin (10^{-5}), octopamine (10^{-5}), or saline, we recorded the posture every five minutes. To compare the overall effect of the injections on posture, we calculated the mean PP for 0-30 minutes and for 35-60 minutes for each condition. Figure 4 shows that the serotonin-treated crayfish had a significant postural change after 30 minutes. The serotonin-treated crayfish showed an average of 0.29 PP in the first 30 minutes and increased to 0.83 PP in the second 30 minutes. This was particularly notable since this crayfish was extremely difficult to provoke to produce an aggressive posture before the injection of serotonin. The octopamine-treated crayfish showed no significant change in average PP. This may, however, represent a decrease in aggressive posture since the control crayfish showed an average of 1.21 PP in the first 30 minutes and increased to 2.75 in the second 30 minutes.

Excitatory Junction Potential Experiment

Next, we investigated the effects of serotonin and octopamine on EJP amplitude of the extensor muscles. Figure 5 shows the EJP amplitude of the serotonin, octopamine, and control preparations. The initial recordings were considered to be baseline measures because serotonin and octopamine do not take effect immediately. After 1 hour of treatment, the average EJP amplitude in the serotonin preparation increased significantly from 29 mV to 37.62 mV, a 30% increase over baseline. In contrast, the EJP amplitude in the octopamine preparation decreased from 49.38 mV to and 34.61 mV, a 30% decrease from baseline. After an hour of treatment in saline solution, no significant change in EJP amplitude was observed.

DISCUSSION

Posture Experiment

Our hypothesis that serotonin-injected crayfish would exhibit a flexed tail, flexed legs (elevated body), and raised claws (high PP), and that octopamine-injected crayfish would exhibit an extended tail, extended legs (low-lying body), and lowered extended claws (zero PP), is somewhat supported by our data. The serotonin-injected crayfish exhibited expected flexion during the last 30 minutes. However, the octopamine-injected crayfish exhibited no significant postural changes.

Our hypothesis regarding the effects of serotonin was not fully supported since the crayfish did not exhibit maximal PP changes. The lack of drastic posture changes may be explained by the effects of social status of crayfish on amine-induced postures. According to Yeh *et*

al., (1996) the efficacy of the response of two populations of serotonin receptors differs in dominant, subordinate and socially isolated crayfish. Thus, the response to sensory stimulus differs according to social status of the crayfish. In socially dominant crayfish, serotonin reversibly enhances the response to sensory stimulus in the Lateral Giant (LG) tailflip command neuron. Conversely, serotonin reversibly inhibits the response in subordinate animals and persistently enhances it in socially isolated crayfish. Therefore, social experience has a tremendous impact on the effects of serotonin on posture. In our experiment, we attempted to socially isolate the crayfish (placing them in individual pots for five days). However, we suspect that confined quarters of isolation may have affected the social experience of the animals by reducing freedom of movement, which may itself be a requirement for social isolation. Other factors, such as sex and mating cycle, may affect the response of the subject to serotonin or octopamine (Kravitz *et al*., 1984). These observations may explain why the serotonin-treated crayfish did not exhibit the maximum levels of flexion, as we expected.

Since the activities of serotonin and octopamine vary with social experience, we suspect that the behavioral characteristics of the crayfish would also play a role in mediating its response. The crayfish that was injected with serotonin showed sluggish, non-responsive, non-aggressive behavior prior to the experiment. It only responded to extreme physical aggravation. Yeh *et al*. (1996) found that injection of serotonin in a subordinate animal inhibited flexion. Because our serotonin-injected subject appeared docile, we suspect that the medium levels of flexion may be in accordance with Yeh *et al.*'s (1996) findings.

Our observations of the octopamine-treated crayfish were contrary to our expectations. This divergence could potentially be due to the behavioral characteristics of the crayfish. The octopamine-injected crayfish responded with an erect, defensive stance when objects moved near it prior to the experiment. This suggested a dominant behavioral characteristic. We speculate, on the basis of our results, that the dominance of the crayfish may have had an inhibitory effect on its response to octopamine.

The control crayfish also exhibited aggressive characteristics before the study, but to a lesser degree. Our results show that the control crayfish exhibited a predominately flexed posture. This was consistent with its aggressive behavior, since aggression is associated with flexion in crayfish. Since the control crayfish exhibited a significant increase in flexion while the octopamine treated did not, this suggests that Octopamine did in fact have an effect on normal posture.

Excitatory Junction Potential Experiment

In this experiment, the average EJP amplitude after 1 hour in serotonin was significantly greater than the baseline EJP amplitude. On the other hand, the average EJP amplitude after one hour in octopamine was significantly lower than the baseline EJP amplitude.

Serotonin has been shown to increase the amount of neurotransmitter release up to 5 fold (Kravitz *et. al*., 1984). Kravitz *et al*. (1984) studied the mechanism through which serotonin increases neurotransmitter release and identified it to be presynaptic in origin. This research found that serotonin has a dual effect acting on both the excitatory and inhibitory nerve terminal. They suggest two

mechanisms through which serotonin increases neurotransmitter release. First, serotonin may cause an alteration in either the buffering or storage of calcium ion levels. Second, serotonin could increase the sensitivity of the transmitter release machinery in the synaptic cleft to existing calcium ion levels. This increase in calcium sensitivity causes an increased amount of neurotransmitter release. We speculate that the average EJP amplitude in our serotonin-treated preparations increased after 1 hour of treatment because of presynaptic changes that increased the amount of neurotransmitter released. However, we cannot make definitive conclusions because the serotonin-enhancing machinery is currently unknown.

According to Breen and Atwood (1997), octopamine enhances the average EJP amplitude by mediating presynaptic mechanisms leading to an increase in quantal release of neurotransmitter. However, our data do not support this finding. We propose that octopamine decreases neurotransmitter release in the crayfish extensor muscle.

Future research

Future studies could strive to analyze the effects of external spatial environment on the social development of crayfish. By placing some in pots and others in larger containers it would be possible to analyze the effects of confinement on the development of dominant and subordinate characteristics. Another possible area of research could be to analyze the long-term effects of octopamine and serotonin on neurotransmitter release and the mechanism through which it occurs. Lastly, future research can investigate the effect of amine activation time on crayfish postural changes.

In summary, our results for the postural experiment were somewhat consistent with our expectations. We found that serotonin causes significant increases in flexion whereas octopamine has no significant effect on posture. Moreover, we observed that serotonin increases and octopamine decreases average EJP amplitude at the crayfish neuromuscular junction.

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Fig. 1: Crayfish illustrating flexed tail, stretched claws, and lowered body. 1 postural point.

Fig. 2: Crayfish illustrating fully extended tail, raised claws, and raised body. 2 postural points.

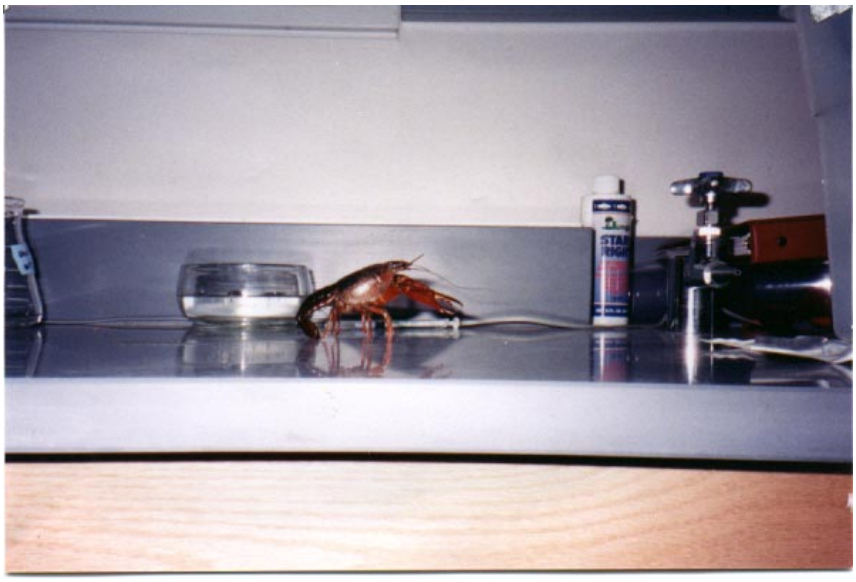

Fig. 3: Crayfish illustrating partially flexed tail, raised claws, and raised body. 2.5 postural points.

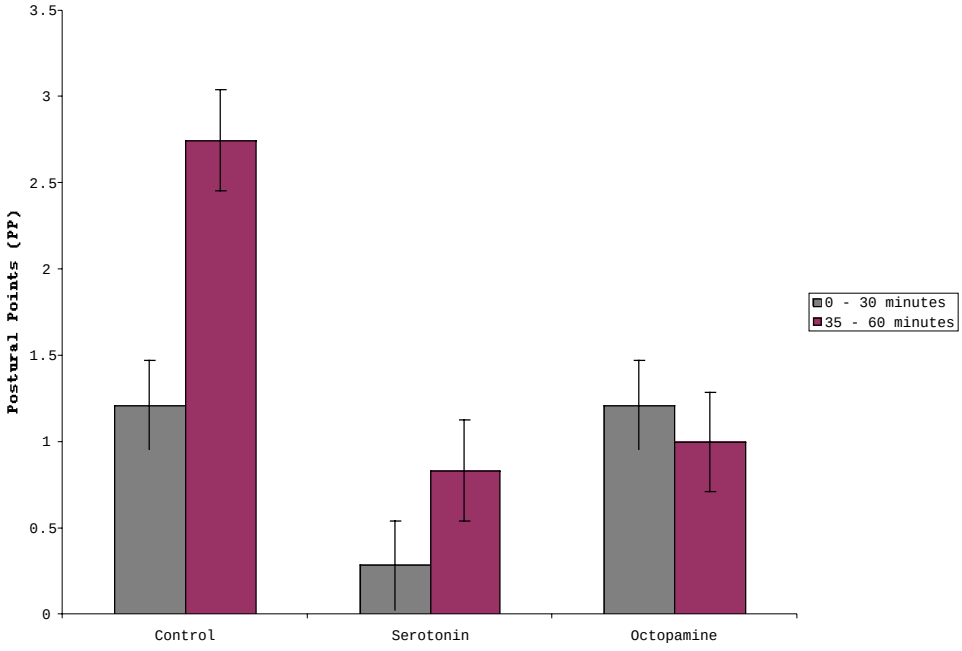

Fig. 4: Effects of octopamine and serotonin on posture in crayfish. Error bars are based on standard deviation. The control and serotonin treated crayfish showed a significant increase in PP after 30 minutes. The octopamine treated did not show any significant change in PP.

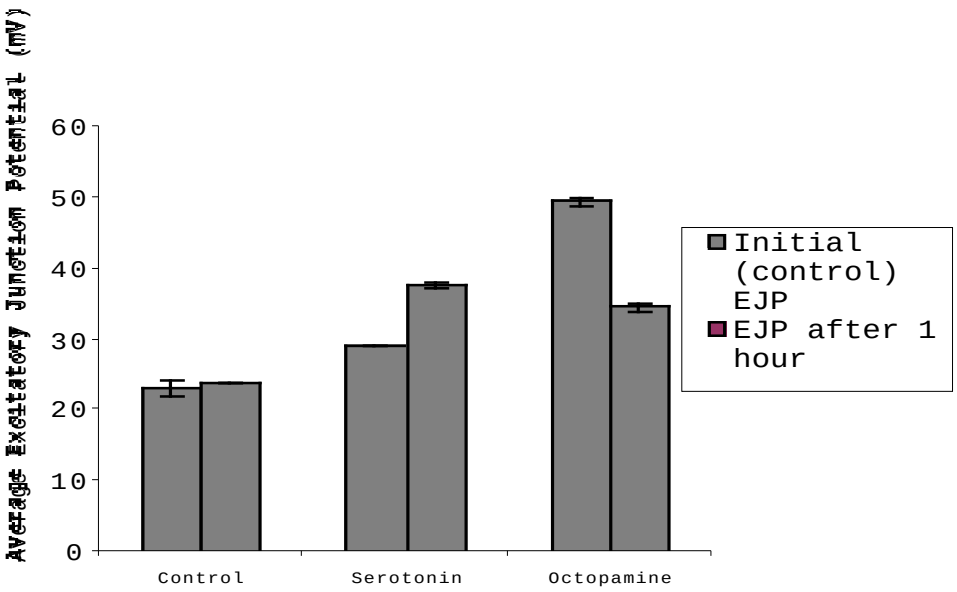

Fig. 5: Effects of serotonin and octopamine on average EJP amplitude after 1 hour. Serotonin significantly increases serotonin amplitude and octopamine significantly decreases EJP amplitude. Error bars based on standard deviation.