The effects of dopamine and octopamine on locomotor behavior in the American Horseshoe Crab, *Limulus polyphemus*

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Introduction

Locomotion of invertebrate organisms is affected by specific neurotransmitter amines such as dopamine and octopamine (Chen *et al*., 2013). For example, dopamine agonists induce locomotor activity in some species, such as *Drosophila melanogaster* (Andretic, 2000). Octopamine similarly has locomotor effects on some animals, for instance increasing the speed of locomotion of the snail *Lymnaea* as a result of injection of an octopamine agonist (Miyamae *et al*., 2010). These neurotransmitters have further effects on other organisms such as the American lobster, *Homarus americanus*. Injections of dopamine in resting lobsters have been noted to cause an increase in the organism's heart rate (Guirguis & Wilkens, 1995). In addition, dopamine injections in lobsters have induced motor activity such as extension of the claws, legs, and tail (Savage & Atema, 2003), and this neurotransmitter has also been shown to increase activity in excitatory units of extensor muscles of lobsters (Lingstone *et al.* 1980).

While a considerable amount of information is known about the American lobster, the effects of dopamine and octopamine on *Limulus polyphemus* behavior are relatively unknown. These neurotransmitters have, however, been demonstrated to have a degree of *in vitro* effects on *Limulus*, which could indicate their potential involvement in motor functions. Octopamine has been demonstrated to increase adenylate cyclase activity in *in vitro* subjects of this animal, and dopamine has been noted to reduce octopamine's effect on this activity (Atkinson *et al*.1977). These effects of octopamine and dopamine on adenylate cyclase indicate their potential to be involved in postsynaptic stimulation and transynaptic communication from the circumesophageal ring (Atkinson *et al*., 1977). Furthermore, octopamine is suggested to have an effect on the motor movement of photo receptors as well as the motor movement of pigment cells of *Limulus* (Battelle *et al*., 1982). Although studies have demonstrated some *in vitro* effects of these neurotransmitters on horseshoe crabs, little is known of the effect of dopamine and octopamine on behavioral locomotion of this species. The goal of this study was to determine if dopamine and octopamine injections have an inhibitory or contributory impact on the locomotion of *Limulus polyphemus*.

Methods

Animals and Environmental Conditions

Four juvenile horseshoe crabs (60-72 g) were collected from the Great Bay estuary and placed in a 30-gallon tank (66x81x34 cm) divided in to four quadrants (Figure 1). Specimens were not fed anything for the duration of the experimental process. Water temperature and ammonia levels were kept fairly constant (17-19 ° C) for the duration of the experiment, using a water chiller and a recirculating system with a 3x pass protein skimmer. *Limulus* were subjected to a light:dark cycle (14:10; lights on 8:00 a.m.; lights off 10:00 p.m.) which was held constant. Horseshoe crabs were allowed three days acclimation time to the holding tank and baseline locomotion was recorded using Gawker (Phil, Piwonka, Seattle, Washington). In order to better track locomotion, white contrasting tarp was laid in the bottom of the tank and red LED lights (Ikea, 60/m, 12mm wide, by 1 foot) were installed above.

Injections

Saline injections (0.10 mL) began at 8:30 am and 10:30 pm daily post acclimation period. The saline solutions consisted of RO distilled water and sea salt (0.9% NaCl, 1 L H2O) at a concentration of 32 ppt (Qadri et al., 2007). Ten minutes prior to injections Gawker was used to record pre-injection locomotion as a comparison to 10 minute post- injection locomotion. Visual observations of activity were also recorded during these times using a scale from 0-10 (Table 1). Each horseshoe crab was injected with .10 mL of saline solution through the arthrodial membrane. Saline injections were administered a total of four times (2- 8:30 a.m. and 2- 10:30 p.m.). Dopamine (Sigma, H8502-5G Milwaukee, Wisconsin) injections followed (0.10mL) with increasing concentrations from 10^-6 - 10^-1 in multiples of 10. Injections were administered daily (8:30 a.m. and 10:30 p.m.) for a period of 12 consecutive days. Octopamine, (Sigma, H8502-5G, Milwaukee, Wisconsin) injections at the same increasing concentrations followed the 12 day dopamine injection period.

Statistical Analysis

Using a Wilcoxon test, the visually observed locomotion before and after saline injections was compared in order to determine the effects of stress on behavior. Expected results and Observed results were compared using a Chi Square with a significance value of ($p \leq 0.05$). Using Gawker, dose response curves of the visually observed locomotion as well as dose response curves of the average change in velocity before and after injections were created for both dopamine and octopamine. Each of the varying concentrations of the neurotransmitters were plotted against its effects on the horseshoe crab. Lastly, a Kruskal-Wallace test was used to determine significance of differences in the dose response curves for visual data, and an ANOVA was used to determine significant differences of change in velocity between doses for the graph of Ethovision data.

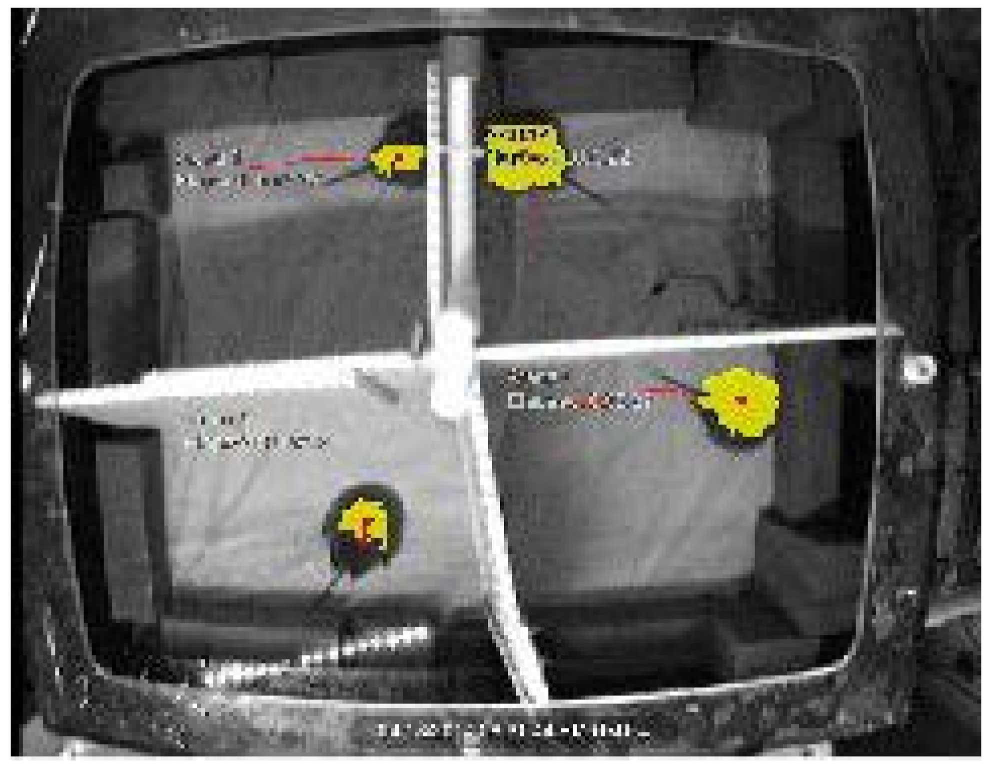

Figure 1: EthoVision image of observation tank. Note: Red lines indicate movement over 10 minutes.

0	No movement
1	Slight movement of limbs/tail
2	Slightly active, but no change in position in quadrant
3	Slightly active with change in position
4	Slight to moderate activity; change in position halfway across quadrant
5	Moderately active; movement across entire quadrant or halfway across quadrant at higher speed
6	Slightly above moderate activity; faster movement across entire quadrant
7	Active; constant movement around quadrant
8	Very active; constant movement around quadrant at constant speed
9	Extremely active; very quick movement across quadrant
10	Maximum level of activity

Table 1: Visual analysis was measured on a scale from 0 – 10.

Saline Injections – Induce Locomotion in Dark

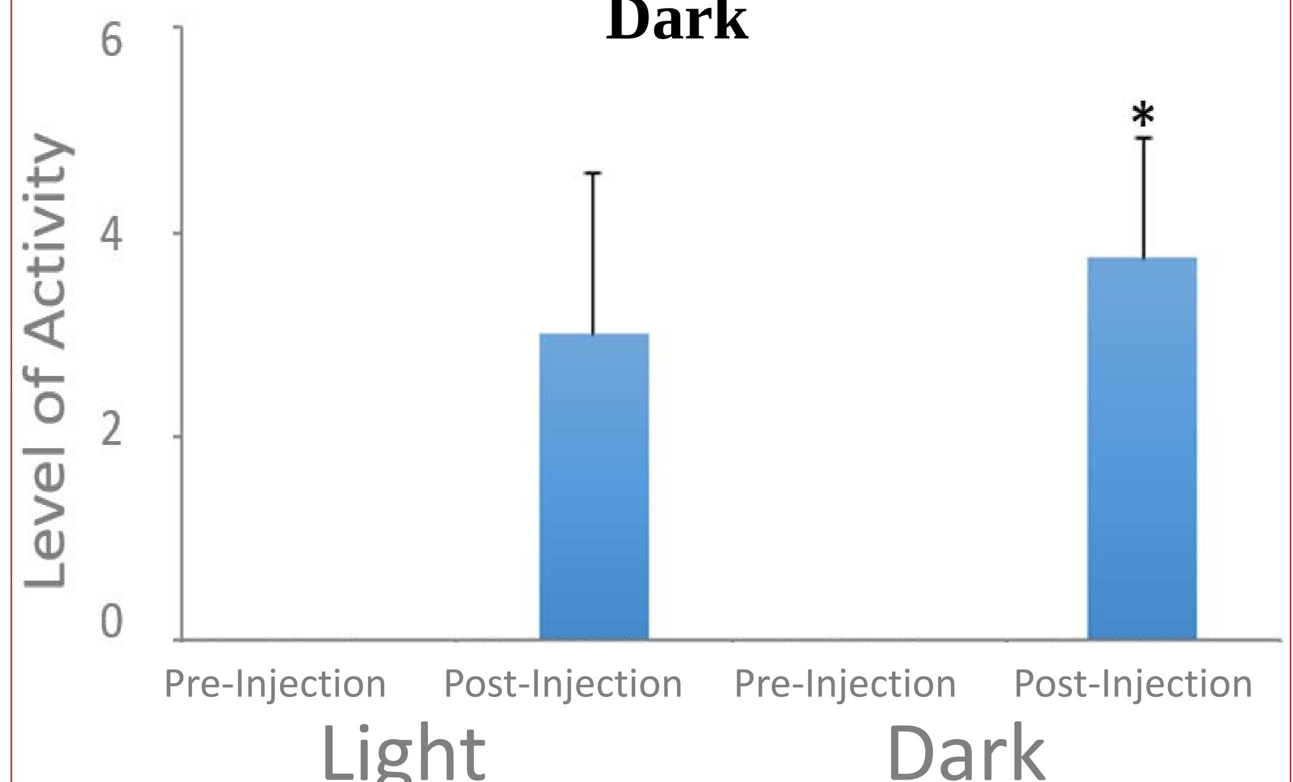

Figure 2: Average level of activity before and after injections of saline, during periods of light (H(1)=4.5, p=.053) and dark (H(1)=2.4, p=.01). Values = $\bar{x} \pm$ SEM

- Increased activity in dark
  - ➢ Suggests time of day effects of injection on locomotion

Octopamine Injections -No Significant Effects

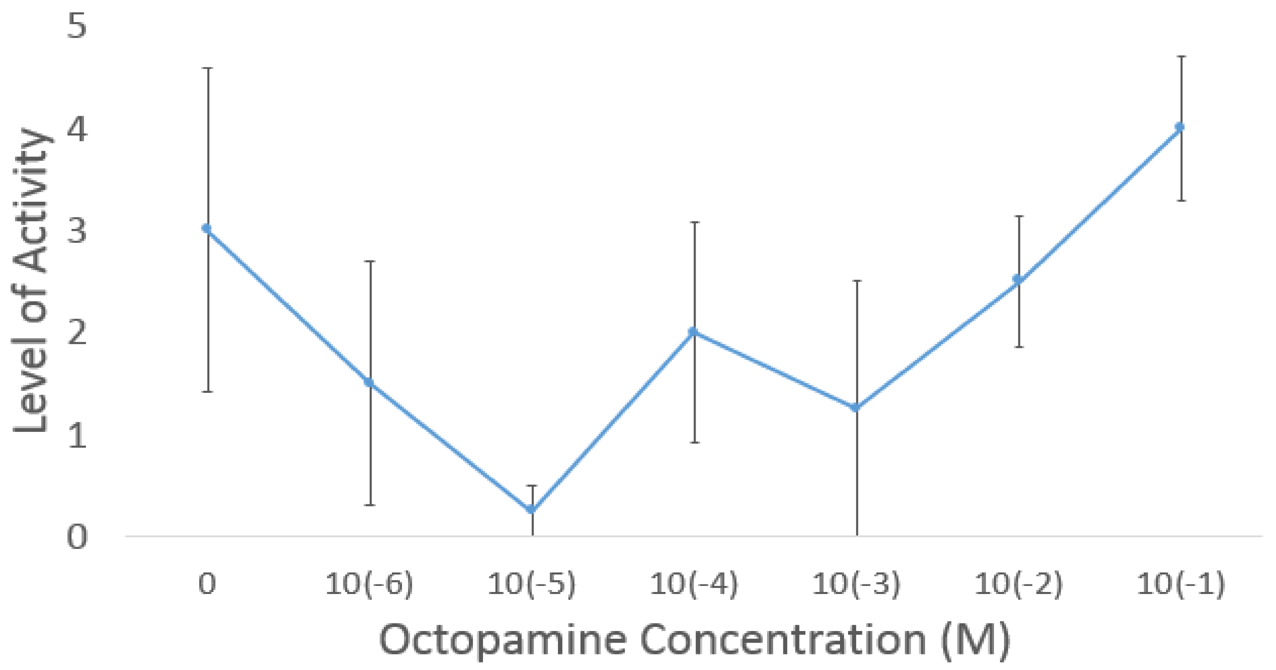

Figure 3: Level of activity 10 minutes post-injection for several concentrations of octopamine (H(6) = 8.895, p=0.18).

- ➢ Suggests that octopamine is broken down very quickly or there are no octopaminergic synapses

Dopamine Injections - No Significant Effects

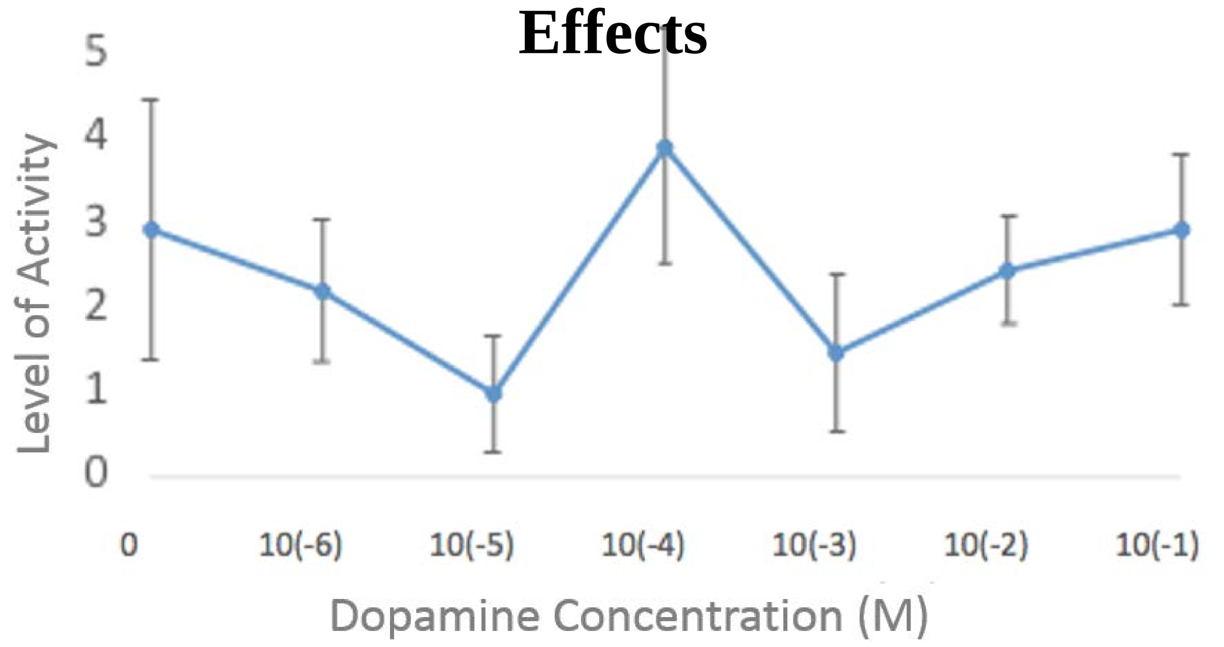

Figure 4: Level of activity 10 minutes post-injection for several concentrations of dopamine (H(6) = 5.362, p=.503).

- ➢ Suggests that dopamine is broken down very quickly or there are no dopaminergic synapses

No Significant Change in Velocity

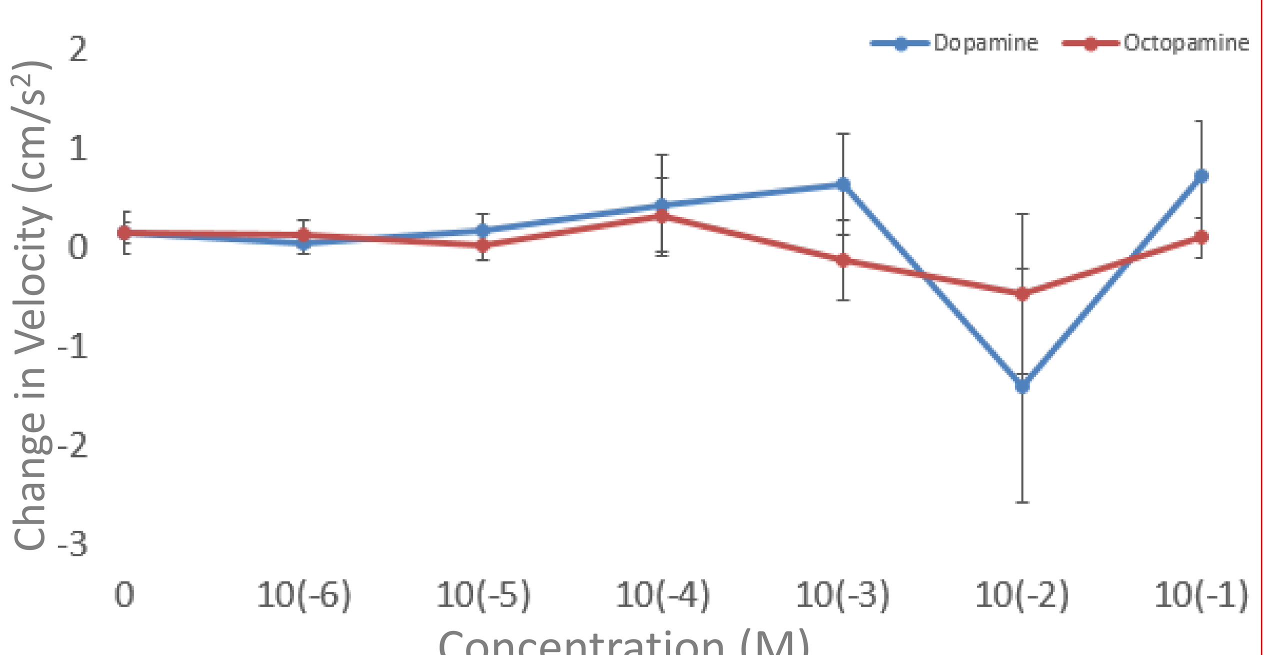

Figure 5: Change in velocity after injections of dopamine and octopamine based on EthoVision analysis. There was no significant change in velocity after either dopamine (F(6,26)=1.5 , p=0.23) or octopamine (F(6,26)=1.6, p=0.18).

Conclusions

No effect of dopamine or octopamine on locomotion of *Limulus polyphemus*

- Contrasts with studies conducted on *H. americanus* (Savage & Atema, 2003), *Drosophila melanogaster* (Andretic, 2000), and *Lymnaea* (Miyamae *et al*., 2010)
- Interestingly, Pham *et al.* (2014) found fictive motor effects after *in vitro* administration of dopamine and octopamine in *Limulus*

Overall Implications:

- Octopamine and dopamine may not regulate motor synapses
- These neurotransmitters could be enzymatically degraded before they can cause an effect

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http://www-tc.pbs.org/wnet/nature/files/2008/06/590_crash_anatomy.jpg

National Science Foundation WHERE DISCOVERIES BEGIN

Acknowledgements: This work was supported by Plymouth State University, NSF IOS 0517229 and NIH INBRE to CCC and Plymouth State University Biology Department. A special thanks to John Rieder and Christopher Wilk.

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