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

Logsdon, J. D.*, Glazner, R. M.*, Collins, K. J.*, and Chabot, C. C. Department of Biological Sciences, Plymouth State University, Plymouth, NH

**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 (Guzinski & Wilkens, 1995). In addition, dopamine injections in lobsters have induced motor activity such as extension of the claws, legs, and tail (Savage & Atima, 2005), and this neurotransmitter has also been shown to increase activity in excitation 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 effect on movements in *Limulus*, which could indicate their potential involvement in motor functions. Octopamine has been demonstrated to increase adenylyl cyclase activity 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 dopamine and octopamine on adenylyl 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 vivo 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 into 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 through the holding tank and baseline locomotion was recorded using Gawker (Phil, Piwonka, Seattle, Washington). In order to better track locomotion, white wide, by 1 foot) were installed above. Animals and Environmental Conditions

**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 (39.9% NaCl, 1.4 H2O) at a concentration of 3.3 ppt (Qadri et al., 2007) and 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, while contrasting tape was laid in the bottom of the tank and red LED lights ( instrumentation, using a water chiller and a recirculating system with a 3x pass through the holding tank and baseline locomotion was recorded using Gawker (Phil, Piwonka, Seattle, Washington). In order to better track locomotion, while contrasting tape was laid in the bottom of the tank and red LED lights (installer) were installed above.

**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 differences of change in velocity between doses for the graph of Ethovision data.

**Results**

**Dopamine Injections - No Significant Effects**

**Octopamine Injections - No Significant Effects**

**Conclusions**

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

- **Contrasts with studies conducted on *H. americanus* (Savage & Atema, 2003)**
- **Dopamine and octopamine based on *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.

**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.

*All authors contributed equally.*