Dopamine, Histamine, and Octopamine Modulation of Efferent Nerve Action in the Pedal Nerves and Ventral Nerve Cord of *Limulus polyphemus*

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NH-INBRE

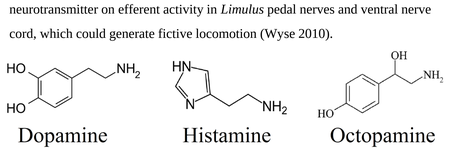
Introduction

Invertebrates are excellent organisms to study neurobiological structure and function, in particular, the American Horseshoe crab *Limulus polyphemus*. *Limulus'* primitive brain structure is anatomically and functionally segregated into response ganglia (Corning et al, 1965), which Chamberlain and Wyse (1986) compiled into a detailed atlas. This atlas helped localize neurotransmitters (NT's) histamine (HA), and octopamine (OA) (Batelle, 1999), while dopamine (DA) was localized by O'Connor *et al.*(1981).

Octopamine and dopamine have been shown to produce a dose dependent increase in both contractile amplitude of muscle fibers and frequency of impulses in motor ganglia (Watson et al. 1985, Groome & Watson 1990, Watson & Augustine 1982, Rane et al. 1983). Similarly, Groome and Lent (1992) found octopamine to have an excitatory effect on visceral muscle while dopamine was found to act as an antagonist, decreasing these effects. Not only has octopamine been shown to have a greater effect than dopamine on frequency and contractility, it has been shown to modulate behavior as well (Augustine, 1981, Lee & Wyse, 1991, Wyse, 2010).

Lastly, histamine is an afferent neurotransmitter in the peripheral visual system (Battelle & Hart 2002, Battelle et al. 1991), and has been localized outside the visual system in areas such as the medulla and lamina. However its role in these areas is not known (Batelle,1999). Histaminergic cells within the medulla and the lamina, could potentially affect the ventral nerve cord or motor nerves in *Limulus*.

If the pedal nerve and ventral nerve cord are affected by these neurotransmitters, *In Vitro*, it can be suggested that they may contribute to fictive motor activity. This research will investigate the potential roles of these neurotransmitter on efferent activity in *Limulus* pedal nerves and ventral nerve cord, which could generate fictive locomotion (Wyse 2010).



Methods

Materials and Environmental Conditions:

Male *Limulus* (N=12) were collected from Adams Point in Great Bay, Durham, NH, kept in a circulating pool, and staged in a 12:12LD cycle. Salinity levels were maintained at 32±1 ppt, in accordance with their natural environment. Artificial sea water was made using Instant Ocean© aquarium sea salt and distilled water, and continuously run through a filtration system. Super distilled water was purified using a Barnstead NANOpure Diamond water purifier.

To record from the brain, the walls of the containment chamber were cooled to 4±1°C to preserve the brain, and maintained at a salinity of 32±1 ppt. A reservoir chamber was set up to establish a flow-through system which enabled the brain to live for approximately three days. Suction electrodes were fitted to the diameter of pedal nerve and ventral nerve cord to ensure tight seals. These electrodes were attached to Amplifiers and LabChart® (ADINSTRUMENTS, LabChart V7 2008) software to analyze electrical output of the pedal nerve and ventral nerve cord. The neurotransmitters used: dopamine hydrochloride (H8502-5G), histamine dihydrochloride (H7250-5G), and octopamine hydrochloride (O0250-1G) were all obtained from Sigma-Aldrich, Lifesciences Corporation, St. Louis, MO.

Procedure:

Limulus brains were harvested by removing the shell from the main body, cutting surrounding nerves, and removing connective tissue and fascia, and then submerged in the containment chamber. Two suction electrodes were attached to the nerves of interest and electrical difference between the charge of the outer saline solution and charge emitted from the nerve were measured using LabChart© to monitor efferent electrical activity. The pedal nerve and the ventral nerve cord were mounted to each electrode respectively. Baselines of efferent output were recorded from each nerve before administering neurotransmitters; they were then compared the efferent activity of the nerves after administration of dopamine (DA), histamine (HA), and octopamine (OA) at concentrations of 10^{-6} through 10^{-1}M . The neurotransmitters were prepared via serial dilutions with super distilled water.

Short-term Action of Neuromodulator on Dopamine and Octopamine

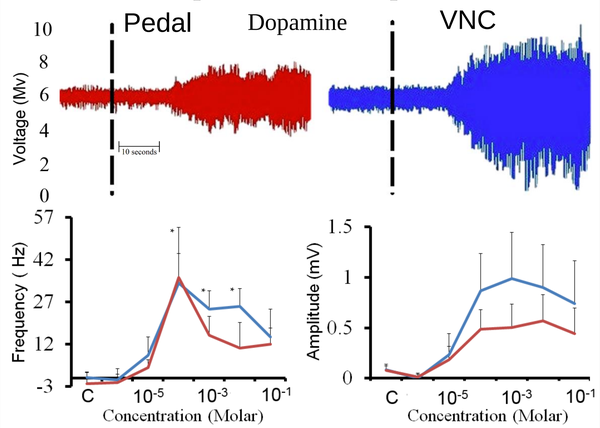


Figure 1: The effects of dopamine on pedal nerve and ventral nerve cord activity (top panels). Raw electrical output data from pedal nerve and VNC in response to 10^{-3}M DA administration, indicated by vertical dashed line. Bottom panel data represent average response for each concentration ± S.E.M.
• ANOVA tests showed no significant differences across the concentration gradient, post hoc Bonforonni tests were performed and indicated no significant differences ($p>0.05$).

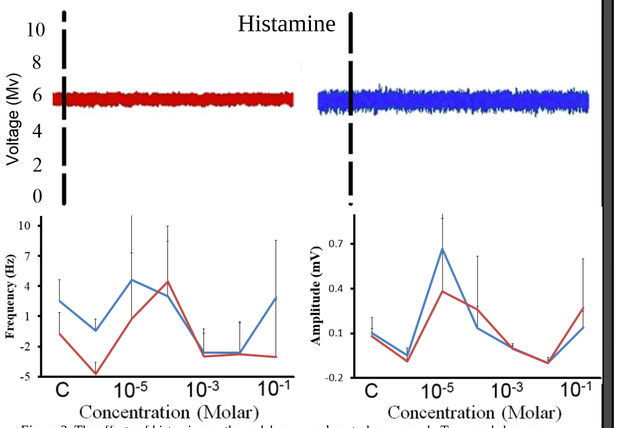


Figure 2: The effects of histamine on the pedal nerve and ventral nerve cord. Top panel shows raw electrical output data after administration of 10^{-3}M.
• ANOVA tests showed no significant differences across the concentration gradient, post hoc Bonforonni tests were performed and indicated no significant differences ($p>0.05$).

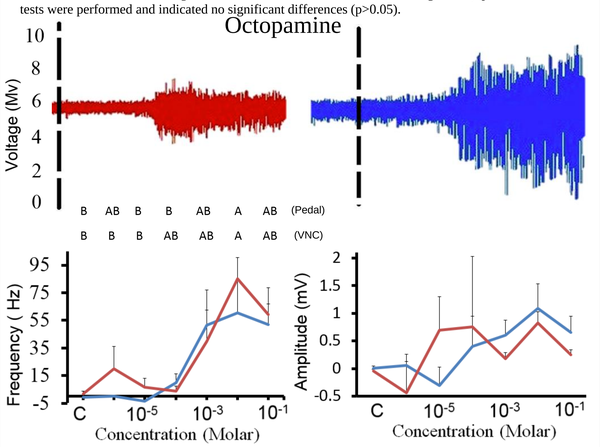
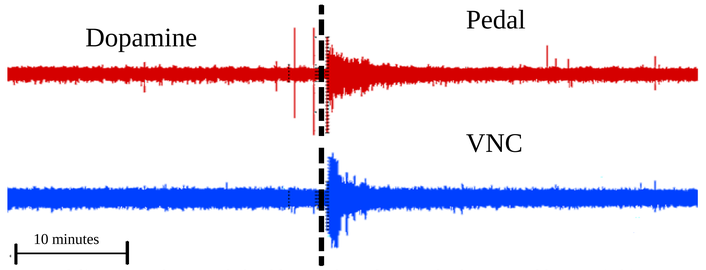
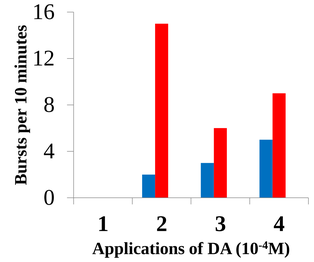


Figure 3: The effects of octopamine on the pedal nerve and ventral nerve cord (top panels). Raw electrical output of pedal nerve and ventral nerve cord was recorded in response to 10^{-3}, as indicated by vertical dash line. Bottom panels represent average response for each concentration ± S.E.M.
• ANOVA tests showed significant differences across the concentration gradient ($p<0.05$. Post hoc Bonforonni tests were performed and indicated significant difference ($p<0.05$) between 10^{-2}M concentration and control.

Long Term Action of Neuromodulator

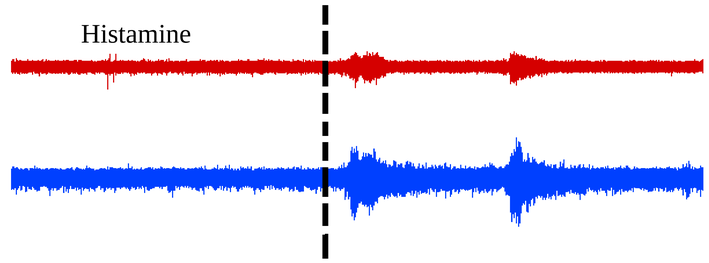




Red: 0-10 minutes after administration

Blue: 10-20 minutes after administration

Figure 4:(left) raw electrical activity in both pedal nerve and ventral nerve cord in the presence of dopamine. (right) Bursts were quantified in 10 minute intervals post administration and post-post administration of dopamine. Significant effects on the pedal nerve and ventral nerve cord were not observed when compared with frequency of bursts prior to administration of DA.



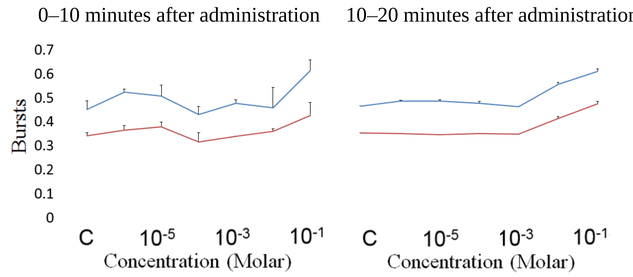
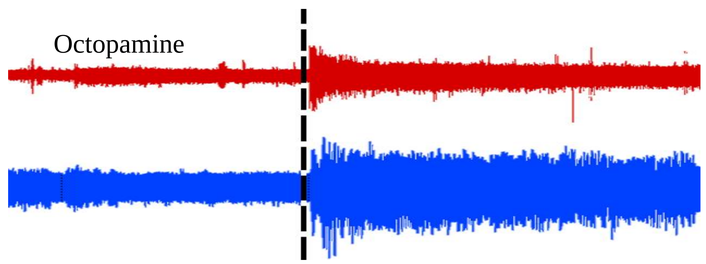


Figure 5: (left) raw histamine electrical output showing spontaneous bursts in both the pedal nerve and ventral nerve cord. (right) Bursts were quantified in 10 minute intervals post administration and post-post administration of histamine. No significant effects were noted when comparing baseline frequency of burst to frequency of bursts after administration.



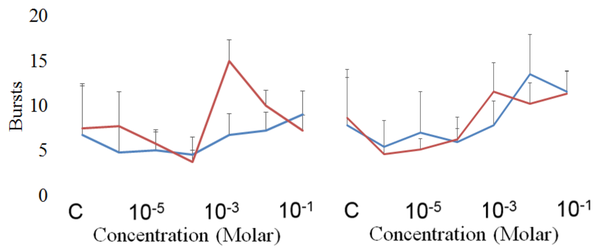
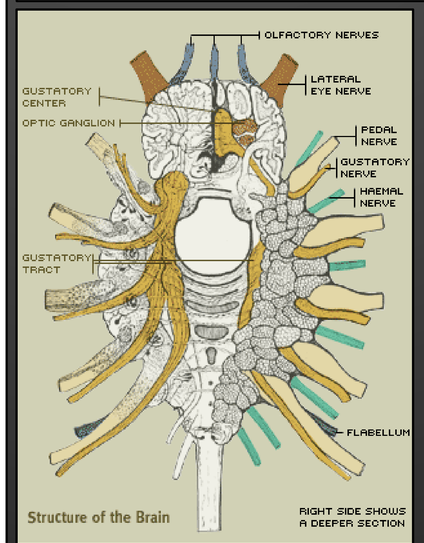
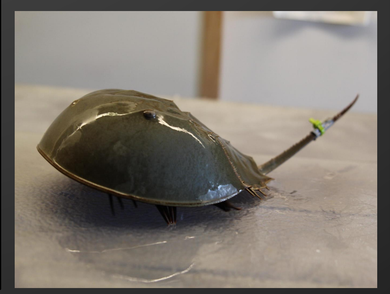


Figure 6: (left) raw octopamine data showing increase burst amplitude and frequency after administration. (right) Quantified bursts in 10 minute intervals post administration and post-post administration of octopamine.





Conclusions

- Dopamine and Octopamine appear to show short-term increases in both amplitude and frequency of peaks in the pedal nerve and VNC at higher concentrations, suggesting they normally modulate efferent nerve activity.
- Since the pedal nerves control the legs the, innervation of these nerves by neurotransmitters may be involved in fictive locomotion (Wyse, 2010).
- Histamine showed no effect on efferent nerve activity, unlike what Stuart *et al*, (1997) saw on eccentric cells in the limulus retina, indicating it is not involved in motor regulation.
- No long term effect was noted in regard to frequency of bursts in any neurotransmitter, however octopamine did appear to elicit long term (several hours) increase amplitude.

Acknowledgements

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