The Effects of Picrotoxin and Ethanol on Crayfish Escape and Central Motor Pattern Neural Circuits

Nathan Hardcastle, Colin Istvan, and Rebecca Bierman
Dr. Black

Introduction

Two behavioral circuits that have received intensive study in crayfish are coordinated walking movements and the lateral giant interneuron (LG) mediated tail-flip response. Appropriate sensory stimuli applied to the tail of a crayfish will stimulate the LG, which innervates phasic flexor motorneurons to contract abdominal muscles. This reflex circuit exists to propel crayfish away from predators (3). Coordinated walking is mediated by central motor pattern generators (CPGs) located in the ventral nerve cord (1). GABA is a neurotransmitter that acts on GABA1a receptors in both the tail-flip circuit and motor pattern walking circuit (5). When activated, GABA1a receptors allow chloride ions to enter into the cell preventing excitation. It is important to understand how various pharmacological agents modulate the GABA1a receptors in each circuit. We designed an experiment to study the effects of two drugs, ethanol (EtOH) and picrotoxin (Ptx), on these circuits. EtOH is a GABA agonist while Ptx blocks the actions of the chloride channels.

Methods

We propose a mixed (between- and within-subjects) factorial design to study the isolated and combined effects of EtOH and Ptx. For behavioral tests, subjects will be given either vehicle or 50μM Ptx injections after soaking in 1500mL of water or 100mM EtOH solutions. To measure tail flip responses, subjects will first acclimate for 20 minutes, then have an appropriate stimulus applied to their tail. To measure spontaneous walking behavior, crayfish will be acclimated as before and then filmed for five minutes. The video will be scored with the Jwatcher application, noting the initiation of coordinated walking movement. Electrophysiological data will be recorded using pin electrodes on a dissected crayfish ventral nerve cord (VNC). Each nerve cord will be bathed in saline to establish baseline data and then in EtOH and/or Ptx solution. Data from each application will be recorded in Dataview to quantify and compare firing frequency.

Conclusions/Discussion

Results from this study will lead to a better understanding of the effects and interactions of EtOH and Ptx on crayfish neural circuitry.

References

1. Cattaert, Daniel Araque, Alfonso Buno, Washington. Short Communication Motor Neurons of the Crayfish Walking System Posses TEA +- Revealed Regenerative Electrical Properties J. exp. Biol. 188, 339-345. 1994.
2. Chrachri, Abdessiam and Claraca, Francois. Fictive Locomotion in the Fourth Thoracic *Procambarus clarkii* Ganglion of the Crayfish. The Journal of Neuroscience 10(3): 707-719. March 1990

3. Herbenholz, J., Antonsen, B., & Edwards, D. (2002). A lateral excitatory network in the escape circuit of crayfish. The Journal of Neuroscience, 22, 9078-9085. Retrieved from http://www-ncbi-nlm-nih-gov.ezproxy.gsu.edu/pubmed/1238861
4. Nagayama, T. Namba, H. and Aonuma, H. Distribution of GABAergic Premotor Nonspiking Local Interneurons in the Terminal Abdominal Ganglion of the Crayfish. The Journal of Comparative Neurology 389:139-148, 1997.
5. Vu, Eric T. and Krasne, Franklin B. Crayfish Tonic Inhibition: Prolonged Modulation of Behavioral Excitability by Classical GABAergic Inhibition. The Journal of Neuroscience, October 1993, 13(10): 43944402.