Andrews University
JN Andrews Honors Program

Proposal for Senior Honors Thesis

HONS 497 Senior Honors Thesis **Credits** 2 (2 minimum required)

Directions: Please return signed proposal to the Honors Office **at least one week prior to your scheduled meeting with the Honors Council**. This proposal must be accepted by Honors Council the semester before presentation.

Student's Name: Brent Sherwin

Primary Advisor: Dr. Gordon Atkins

Secondary Advisor: Dr. Tom Goodwin

Thesis Title: "The effect of nanoinjecting pictrotoxin into the supraesophageal ganglion of the cricket *Acheta domesticus*"

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Expected date of Graduation: May 2011

I. Provide goals and brief description of your project or research.

The main goal of my research is to document the effects of nanoinjecting the $GABA_A$ channel blocker picrotoxin (PTX) into the supraesophageal ganglion ("brain") of unselective crickets of *Acheta domesticus*. Specifically, I want to see how PTX affects phonotactic responses of the females to male calling songs (CS's). This research will help add to the larger body of knowledge about neuronal/hormonal control of cricket audition.

Generally, older female crickets (>14 days old) are unselective (Atkins et al., 2008). This means that they respond to a wider range of syllable periods, which make up the male calling song (30-90 ms). Younger females respond to only a few select syllable periods. I will take unselective female crickets and expose their supraesophageal ganglion to a small amount of the PTX. PTX blocks the chloride channel, which reduces its inhibition. PTX has been shown by Stumpner to cause selective bushcrickets to become less selective in their phonotactic response (Stumpner, 1998). However, it is not currently known whether $GABA_A$ chloride channels exist in the auditory portion of the supraesophageal ganglion of *Acheta domesticus*. If these receptors do exist, I will determine how they modulate the phonotaxis of female crickets.

II. Outline your methodology. **Please be specific**. How does this achieve your goals and how reliable is it?

Before performing the pre-test, I obtain a 20-28 day-old female cricket, which is kept under controlled temperature and daylight conditions in the lab. I use a pin and warm wax to attach a tether to the cricket's dorsal exoskeleton just below the head. This tether connects to the arm of a metal rod extending above the free-floating ball of the cricket treadmill. Following a three-minute adaptation period I will use a computer program called Optical Kugel, to play pre-recorded calling songs of male crickets for 3 minutes each. Between each test I allow the cricket to rest for 1 minute and then change the syllable period that is being played on the treadmill speaker. These syllable periods range from 30-90 milliseconds (ms) and are played in a pre-determined non-sequential order (50, 90, 60, 30, 70, 40, and 80 ms).

A positive phonotactic response of the cricket on the treadmill is determined by two factors. The first is path orientation, which is determined by the ratio of the distance the cricket goes towards the speaker vs. the distance it goes away from it. The second is angular orientation, which measures how direct the cricket's path toward the speakers is. If the path orientation has a 2:1 ratio of towards to away and the angular orientation is +/- 60° the cricket is classified as performing positive phonotaxis towards the sound source. During the pre-test if the cricket responds to 5-7 of the syllable periods it is considered to be unselective and can then be used for the experiment.

Following the pre-test, dissection of the cricket is performed. This involves placing the cricket on a wax block ventral side down. Two strips of wax, one near the head and the other over the back, are used to secure the cricket. Microscissors are then used to carefully puncture 4 holes in the exoskeleton on the top of the head. Two of the holes are in line with the antennae and the others are in line with two small white structures known as ocelli. The microscissors are then used to cut out a rectangular flap of the exoskeleton, which allows access to the supraesophageal ganglion. Once this is completed, 9.2 nL of Ringer's solution can be nanoinjected into the supraesophageal ganglion using a Drummond "Nanoject II" automatic nanoliter injector. The flap is then closed which allows the cricket hemolymph to seal up the incision lines. After approximately 10 minutes the cricket is put through the post-test, which is identical to the pre-test described above. The control experiment is extremely important to my research because it allows me to ensure that the results I find from injecting PTX are not due to any aspect of the dissection. Pre and post-test phonotaxis will be compared using a paired T-test.

After obtaining enough experimental controls I will use the same methods described above to nanoinject PTX into the supraesophageal ganglion of unselective females and compare pre and post-test phonotaxis.

III. Explain in what sense your project is original, unique, or beyond normal senior expectations. How does it relate to current knowledge in the discipline?

Although this project is unique in its objective, it builds, on prior research of cricket phonotaxis and pharmacology. Phonotaxis at its basic level is the movement of an organism towards or away from a sound stimulus. This behavioral response is regulated not only by neurons within the cricket but also by certain types of chemicals. Atkins and his colleagues discovered that inactivation of the L1 and L3 neurons in the prothoracic ganglion of *Acheta domesticus* caused angular errors in the phonotactic response (Atkins et al., 1992). This demonstrated that the prothoracic ganglion was involved in the phonotactic response.

Following this discovery more work was done by the Andrews University cricket team on the prothoracic ganglion. Nanoinjection of Junvenile Hormone III (JHIII), PTX, Benzodiazepine, and Histamine all caused changes in phonotactic selectivity, which demonstrates that these molecules influence phonotactic circuits (Atkins et al., 2008; Lee, 2010 unpublished). From this research a model has been developed to explain how neurons and hormones in the prothoracic ganglion modulate phonotaxis.

In addition to the prothoracic ganglion, the supraesophageal ganglion has also been shown to be involved in phonotaxis (Pires and Hoy, 1992; Schildberger, 1984). Thus, the Andrews University cricket team has recently begun to focus on the supraesophageal ganglion or "brain" of the cricket. Markovic nanoinjected JHIII into the supraespohageal ganglion for her senior honors project. She found that unselective females became more selective post-injection (Markovic, 2010). In addition she discovered that the response towards greater selectivity was stronger than it had been in experiments involving JHIII in the prothoracic ganglion. My project asks if PTX also modulates neuronal control of the phonotactic response in the supraesophageal ganglion as it does in the prothoracic ganglion. Thus, my project is unique in the substance and location being studied.

IV. Include a **substantive annotated bibliography** of similar or related work.

ANNOTATED BIBLIOGRAPHY

Atkins, Steven, Gordon Atkins, Mike Rhodes, and John F. Stout. "Influence of syllable period on song encoding properties of an ascending auditory interneuron in the cricket *Acheta domestica.*"*Journal of Comparative Physiology A* 165 (1989): 827-836.

Atkins et al. found that the L3 prothoracic auditory neuron excitatory response increased linearly with sound intensity. In addition, Atkins et al. discovered that the syllable periods decrements in response to longer syllable periods (50-200 ms) but does not in response to short syllable periods (>200 ms). This indicates that the L3 auditory neuron is involved in phonotactic response.

Atkins, Gordon, John Henley, Rob Handysides, and John Stout. "Evaluation of the behavioral roles of ascending auditory interneurons in calling song phonotaxis by the female cricket (*Acheta domesticus*)." *Journal of Comparative Physiology A* 170 (1992): 363-372.

Atkins et al. found that inactivation of the L1 and L3 neuron resulted in angular errors in the phonotaxic response. In addition they demonstrated the necessity of L1 for performing phonotaxis.

Atkins, Gordon, Jason Kilmer, Michael Scalfani, Benjamin Navia, and John Stout. "Modulation of syllable period-selective phonotaxis by prothoracic neurons in crickets (*Acheta domesticus)*: juvenile hormone, picrotoxin, and photoinactivation of the ON1 neurones." *Physiological Entomology* 33 (2008)**:** 322-333.

Nanoinjection of both Picrotoxin (PTX) and Juvenile Hormone III (JHIII) resulted in unselective crickets becoming more selective for specific syllable periods than in the pre-test. In addition, photoinactivation of both ON1 prothoracic auditory interneurons resulted in a more selective response of unselective crickets.

Markovic, Christianna N. and Gordon Atkins. "The Effect on Phonotaxis of Nanoinjecting Juvenile Hormone III into the Supraesophageal Ganglion of Female Crickets." *Senior Honors Thesis* (2010).

Injection of Juvenile Hormone III into the supraesophageal ganglion caused older unselective females to become more selective to male calling songs, especially within the 60 ms syllable period.

Pires, Anthony, and Ronald R. Hoy. "Temperature coupling in cricket acoustic communication." *Journal of Comparative Physiology A* 171 (1992): 79-92.

Pires and Hoy found that warming of both the head and thorax resulted in a preference of females for a faster male calling song. In addition, they showed that the male calling song is strongly influenced by thoracic temperature. This showed that both the prothoracic and supraesophageal ganglion are involved in female phonotaxis.

Scalfani, Michael, and Gordon Atkins. "Effects of pictrotoxin (PTX) on phonotactic selectivity in cricket *Acheta domesticus*." *Senior Honors Thesis* (2006).

Injection of picrotoxin (PTX) into the prothoracic ganglia caused unselective female crickets to become more selective in the 50-70 ms syllable period.

Schildberger, Klaus. "Temporal selectivity of identified auditory neurons in the cricket brain." *Journal of Comparative Physiology A* 155 (1984): 171-185.

Schildberger showed that the relationships between the auditory neurons AN1, AN2, BNC 1, and BNC 2 provide a specific framework for how the male calling songs are recognized by females.

Stout, John F., Gordon Atkins, Michael Bronsert, Jing Hao, and Randall Walikonis. "Influence of Juvenile Hormone III on the Development and Plasticity of the Responsiveness of Female Crickets to Calling Males through Control of the Response Properties of Identified Auditory Neurons." *Hormones, Brain, and Behavior* Volume III (2002): 167-193.

Addition of Juvenile Hormone III (JHIII) to the prothoracic ganglia resulted in unresponsive females becoming maximally responsive faster than those with lower levels of JHIII.

Stout, J., Benjamin Navia, Jason Jeffery, Leslie Samuel, Laura Hartwig, Ashley Butlin, Mary Chung, Jessica Wilson, Erica Dashner, and Gordon Atkins. "Plasticity of the phonotactic selectiveness of four species of chirping crickets (*Gryllidae)*: Implications for call recognition." *Physiological Entomology* 35 (2010): 99-116.

Stout et al. showed that the plasticity of phonotaxis in four species of female crickets does not cause them to adjust their calling song phonotactic response to the calling song of males.

Stumpner, Andreas, Gordon Atkins, and John F. Stout. "Processing of unilateral and bilateral auditory inputs by the ON1 and L1 interneurons of the cricket *Acheta domesticus* and comparison to other cricket species." *Journal of Comparative Physiology A* 177 (1995): 379-388.

The ON1 and L1 neurons of *Acheta domesticus* mainly receive excitation and inhibition from male calling songs on opposite sides. The two-tone experiments performed by Stumpner et al. suggest that the model described above is valid.

Stumpner, Andreas. "Picrotoxin eliminates frequency selectivity of an auditory interneuron in a bushcricket." *Journal of Neurophysiology* 79 (1998): 2408-2415.

When picrotoxin is applied to the interneuron of the bushcricket *Ancistrura nigrovittata* it prevents subthreshold inhibitory postsynaptic potentials (IPSP's) from occurring. This causes a specifically tuned cricket neuron to respond to a wider range of stimulation.

V. Provide a statement of progress to date.

I have spent the past school year developing my skills and collecting some data. First I have helped develop and have subsequently practiced the dissection techniques used in nanoinjection of substances into the supraesophageal ganglion of the cricket. Second, I have become familiar with and practiced the techniques needed for nanoinjection. Third, I have learned how to use the computer software for collecting my phonotactic data and I have helped build switch boxes for the computers to make data collection more convenient for the project team. Fourth, I have participated in cricket research team meetings which have helped me to get a better sense of how my projects relates to the broader research program of the team. Finally, I have done preliminary dissections and phonotactic experiments with the crickets in order to improve my skills for further data collection during this fall, winter, and spring.

This student's performance in his/her major field is acceptable, and I understand that he/she plans to graduate with honors.

_____
Department Chair (signature)

I have read and support this proposal: _____
Primary Advisor (signature)

I have read and support this proposal: _____
Secondary Advisor (signature)

If human subjects or if live vertebrate animals are involved, evidence of approval from the Institutional Review Board or an Animal Use Committee is needed through the campus scholarly research offices (Ext. 6360).