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The Effect of 4-aminopyridine on Phonotactic Selectivity in Female Crickets

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Honors Thesis

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Abstract

My research project was designed to explore the effects of 4-aminopyridine (a potassium channel blocker) on phonotactic selectivity in female crickets, *Acheta domesticus*. These crickets underwent pre-tests for selectivity on a non-compensating treadmill during which their response to a range of male calling songs (30-90ms syllable periods) was recorded. A solution of 9.2 nl of saline or 10^{-5}M 4-aminopyridine was nanoinjected into the supraesophageal ganglion. After a ten minute recovery period, post-tests were performed, identical to the pre-tests. There was no significant effect of 4-aminopyridine on the females' selectivity.

Keywords

Phonotaxis, 4-Aminopyridine, Plasticity, Modulation, Supraesophageal Ganglion, Nanoinjection, Selectivity

Introduction

Female crickets respond differentially to spatial and temporal features of the conspecific male calling song (Popov and Shuvalov, 1977; Stout *et al.*, 2010). Syllable period is the temporal feature of the male calling song to which female *Acheta domesticus* show the greatest selectivity (Stout *et al.*, 1983; Stout and McGhee, 1988). During a calling song, females display position phonotaxis, which is the movement towards the sound source (Atkins *et al.*, 2008). Previously, the female's response was considered a stereotyped behavior which was partially responsible for the selection of the cricket model as a model of neural function (Stout *et al.*, 1983). However, the work of Popov and Shuvalov (1977) indicated that the complexity of this behavior was greater than previously imagined. This launched an exploration of the factors which modulate behavior and contribute to plasticity (Stout *et al.*, 2010).

Auditory neurons directly synapse onto the prothoracic ganglion, which makes it an ideal location for modulation (Fig. 1). Atkins *et al.* (2008) discovered that the nanoinjection of juvenile hormone III into the prothoracic ganglion caused greater selectivity for syllable period. Following this experiment, several other modulators, including picrotoxin, glycine, serotonin, GABA and histamine, were injected to confirm the prothoracic ganglion's control and determine the mechanism by which this occurs (Atkins *et al.*, 2008; Yoon *et al.*, 2011). This data led to the proposition of a model in which the synapse between inhibitory omega neurons and L3 auditory neurons is modulated by histamine (Yoon *et al.*, 2011). The L3 auditory neurons then project towards the supraesophageal ganglion in the head of the cricket (Fig. 1).

Because of its anatomical location, the supraesophageal ganglion was suspected to play a role in phonotactic behavioral regulation. Pires and Hoy (1992) conducted differential heating experiments showing that the collective warming of the head and thorax gave rise to greater selectivity than the warming of each region separately, indicating the collaboration of the prothoracic

and supraesophageal ganglia during selectivity. Furthermore, electrophysiological experimentation performed by Schildberger (1984) showed selective firing of certain brain neurons to specific calling songs.

Thus, the modulation exercised by the supraesophageal ganglion was examined by our laboratory. First, Markovic (2010) observed increased phonotactic selectivity for mid-range syllable periods (50-70ms), similar to what was seen in the prothoracic ganglion, after the nanoinjection of juvenile hormone III into the supraesophageal ganglion. Subsequently, Sherwin (2011) nanoinjected the chloride channel blocker, picrotoxin, and found greater selectivity as expected from the results in the prothoracic ganglion (Atkins *et al.*, 2008). However, instead of a preference for mid-range (50-70ms) syllable periods as observed in the prothoracic ganglion, the females showed a preference for songs with shorter syllable periods.

Sherwin (2011) explained this selectivity for shorter syllable periods using the Schildberger model (Schildberger, 1984). Schildberger presented a model for modulation of the cricket auditory pathway involving a low-pass filter causing longer syllable periods to generate action potentials and a high-pass filter which allows shorter syllable periods to generate action potentials. The low-pass and high-pass filters combine forming a band-pass filter which synapses onto the supraesophageal ganglion (Fig. 2). This band-pass filter was used to explain the formerly stereotyped response for mid-range frequencies (50-70ms). Since the low-pass filter modulates responses to lower frequency syllable periods, and the high-pass filter does the same for high frequency syllable periods, when either filter is blocked this result in a directional shift in selectivity.

Consequently, Sherwin (2011) attributed the shift in selectivity observed after the administration of picrotoxin to the blockage of the low-pass filter allowing higher frequency calling songs through. He hypothesized that blocking the high-pass filter would result in selectivity for longer syllable periods. Because chloride and potassium independently regulate inhibition in

organisms, he also hypothesized that potassium channels may be involved in the inhibition of the high-pass filter. Thus, a potassium channel blocker would block the high-pass filter creating a preference for low frequency calling songs. To test this hypothesis, my project attempts to identify the effects of a potassium channel inhibitor (4-aminopyridine) injected into the supraesophageal ganglion on the phonotactic response of adult female *Acheta domesticus* crickets to male calling songs.

Materials and Methods

Cricket Rearing

Four week old nymphs or six week old adults of the species *Acheta domesticus* were purchased from Fluker's Cricket Farm (Baton Rouge, Louisiana). Female crickets were separated and reared in 100 L containers which were continuously replenished with water (in stopper tubes), cricket chow (Fluker's Cricket Farm) and egg cartons for shelter. They were kept at a temperature of 21˚C-23˚C and under a LD 12:12 h photoperiod with lights on at 06.00 h (Atkins *et al*., 2008).

Phonotactic Testing

A metal tether was adhered by hot wax to the pronotum of the female crickets and was left attached for the duration of the experiment. They were secured to a metal rod suspended over the free-floating Styrofoam ball of a non-compensating cricket treadmill described by Walikonis *et al.* (1991). Following a three-minute period of adaptation, synthesized calling songs (SoundStudio 3.0) were played using the computer program, Optical Kugel, from a speaker located 70 cm away from the cricket. These cricket songs, composed of three syllable chirps (chirp period of 666ms, syllable duration of 25ms) with syllable periods ranging from 30ms to 90ms, were played in a non-sequential, standard order (50, 90, 60, 30, 70, 40, and 80ms) following the stipulations of Sherwin (2011). Each calling song was played for 3 min. with 1 min. of silence before the next song. Phonotaxis was considered positive if the angular error was no greater than ±60˚ away from the speaker and if the distance walked towards the speaker was twice the distance walked away from the speaker (Atkins *et al*., 2008).

Hormonal Manipulation

Female crickets, pre-tested in the fashion described above, were mounted dorsal side up onto a wax block using narrow strips of wax (Nakiplast) to secure their legs and head. Four holes were carefully punched through the exoskeleton (two between the antennae, two between the ocelli) in a rectangular pattern as indicated by Sherwin (2011) and the flap of exoskeleton was then excised using microscissors and microforceps. This resulted in an opening through which the supraesophageal ganglion could be visualized. The nanoinjector (Drummond Nanoinject II; Drummond Scientific Co., Broomall, PA) fastened to a micromanipulator was used to nanoinject 9.2 nl of Fielden (1960) saline as a control or a 10^{-5} M 4-aminopyridine (Sigma) solution for the experimental group. After injection, the crickets recovered for ten min. before post-tests, identical to the pre-tests, were performed. The resulting phonotactic activity was recorded in a spread sheet using Microsoft Office Excel and statistically analysed using a two-tailed paired *t*-test.

Results

Individual crickets responded to a variable number of syllable periods, which occurred in different ranges, between the pre-tests and post-tests. Crickets may respond to more, fewer or the same number of syllable periods during post-tests as compared with the pre-tests. Although individual cricket's response changed between the pre-tests and post-tests (Fig. 3), there was no overall significant difference in the number of syllable periods responded to after nanoinjection of the control crickets (p= 0.256, n=21). Similarly, the comparison of the phonotactic responses during the pre-tests and the post-tests (Fig. 4) of the experimental group showed no significant change in the number of syllable periods that females responded to (p= 0.310, n=21). This lack of significant variation also extends to the distribution of the syllable periods which elicited a positive response (Fig. 5).

Discussion

As one from a small number of models for neuronal modulation of phonotaxis currently derived, the Schildberger model offered a logical explanation for the shift in selectivity observed from the experiment involving the nanoinjection of picrotoxin (Sherwin, 2011). Nevertheless, this model was originally used to explain the stereotypical responses to mid-range frequencies of 50-70ms and thus contains several disparities. Stout *et al.* (2010) mentioned that the Schildberger model does not account for the presence of skipping which is the response to calling songs in various syllable period ranges. Additionally, this model does not include selective processing at the prothoracic level. Stout *et al.* (2010) rightly reveals that this model does not explain the amounts of plasticity demonstrated by female crickets.

Our results indicate that 4-aminopyridine had no effect on phonotactic selectivity which may be due to several factors. Although 4-aminopyridine was suggested as a relatively unselective potassium channel blocker, it is still not comprehensive. This chemical only blocks a family of potassium channels (Tseng, 1999). Subsequently, some potassium channels may remain active in the inhibition of the high-pass filter causing no change in phonotactic selectivity. Without molecular probes, the variety of potassium channels present in the cricket, as well as the impact of 4-aminopyridine on these channels, remains unknown.

An alternative explanation could be the concentration of 4-aminopyridine administered. When tested at higher concentrations, the injection of other neural modulators, such as juvenile hormone and picrotoxin resulted in seizures and loss of function in crickets which led to the selection of the concentration chosen (Atkins *et al.*, 2008). To enable direct comparison between the work of Sherwin and my experiment, the drug concentration of 10^{-5} M was kept the same. Yet, the variability in the number of channels and the effectiveness of this blocker may render the concentration negligible. On the other hand, the picrotoxin of Sherwin's experiment (2011) presents

similar questions. Although picrotoxin has a clear and significant effect in both the prothoracic and supraesophageal ganglia, the difference in selectivity in the brain may be due to the concentration of picrotoxin present, the number of chloride channels present or the selectivity of chloride for certain channels (Atkins *et al.*, 2008, Sherwin and Atkins 2011). Again, without molecular probes, the nature of the chemical binding cannot be determined.

Finally, it is possible to conclude that potassium channels may not be involved in phonotactic behavioral inhibition. Potassium channels were proposed as a mechanism for control due to the role they play parallel to chloride channels in creating inhibitory postsynaptic potentials which reduce the likely of a certain action taking place. Because this relatively unselective potassium channel blocker shows no significant effect of phonotactic selectivity, it can be reasonably inferred that potassium channels may not be involved.

Acknowledgements

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Figures

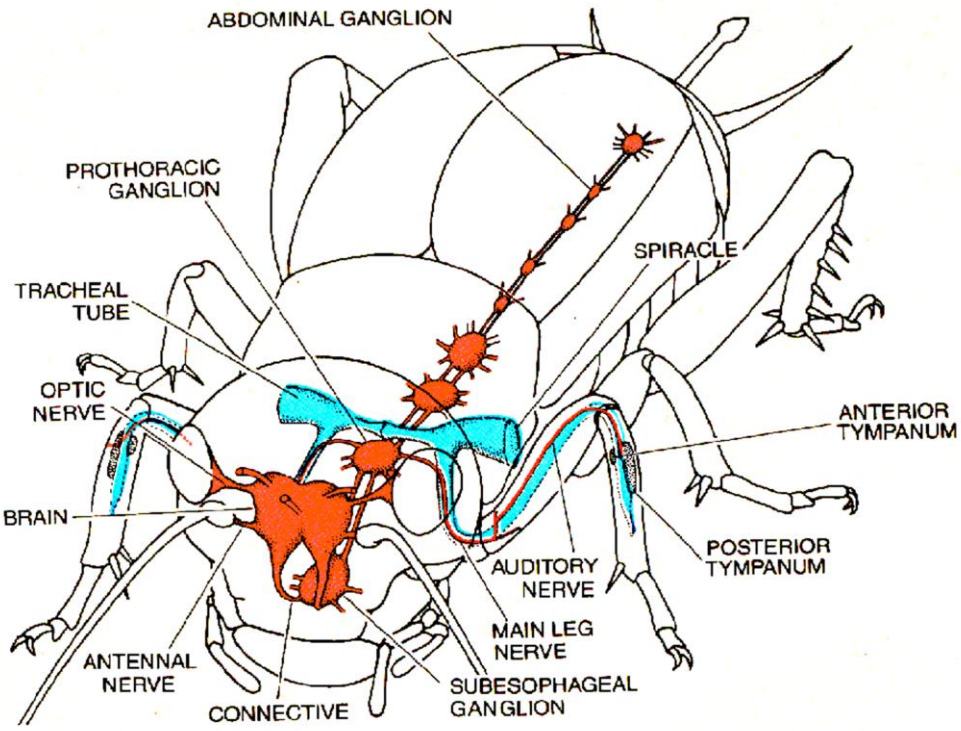

Figure 1. Diagram of cricket neural anatomy showing auditory nerves projecting to the prothoracic ganglion then synapsing on the supraesophageal ganglion in the head as referred to as the brain (Schildberger, 1984).

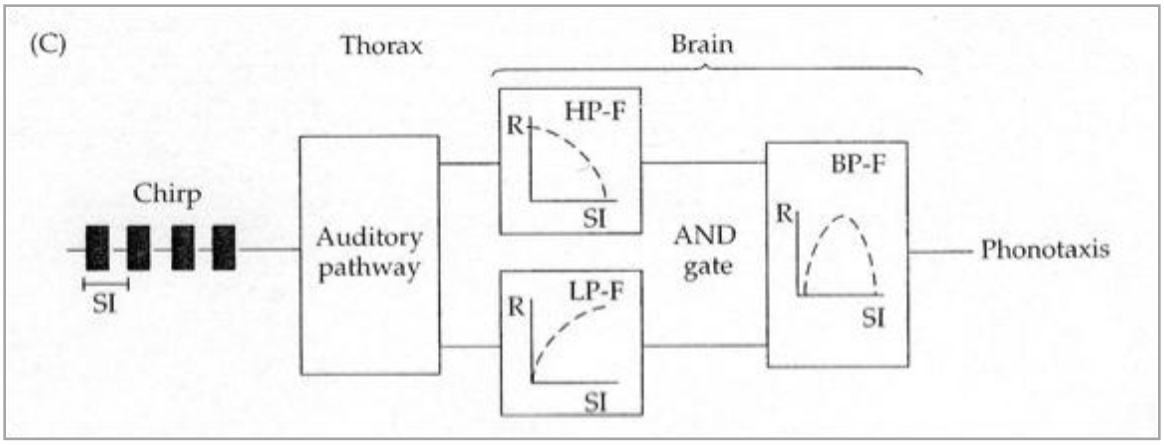

Figure 2. Diagram of the Schildberger model showing the high-pass filter (HP-F) and the low-pass filter (LP-F) combining to form a band-pass filter (BP-F), used to explain the optimal phonotactic response to mid-range syllable periods (modified from Carew, 2000).

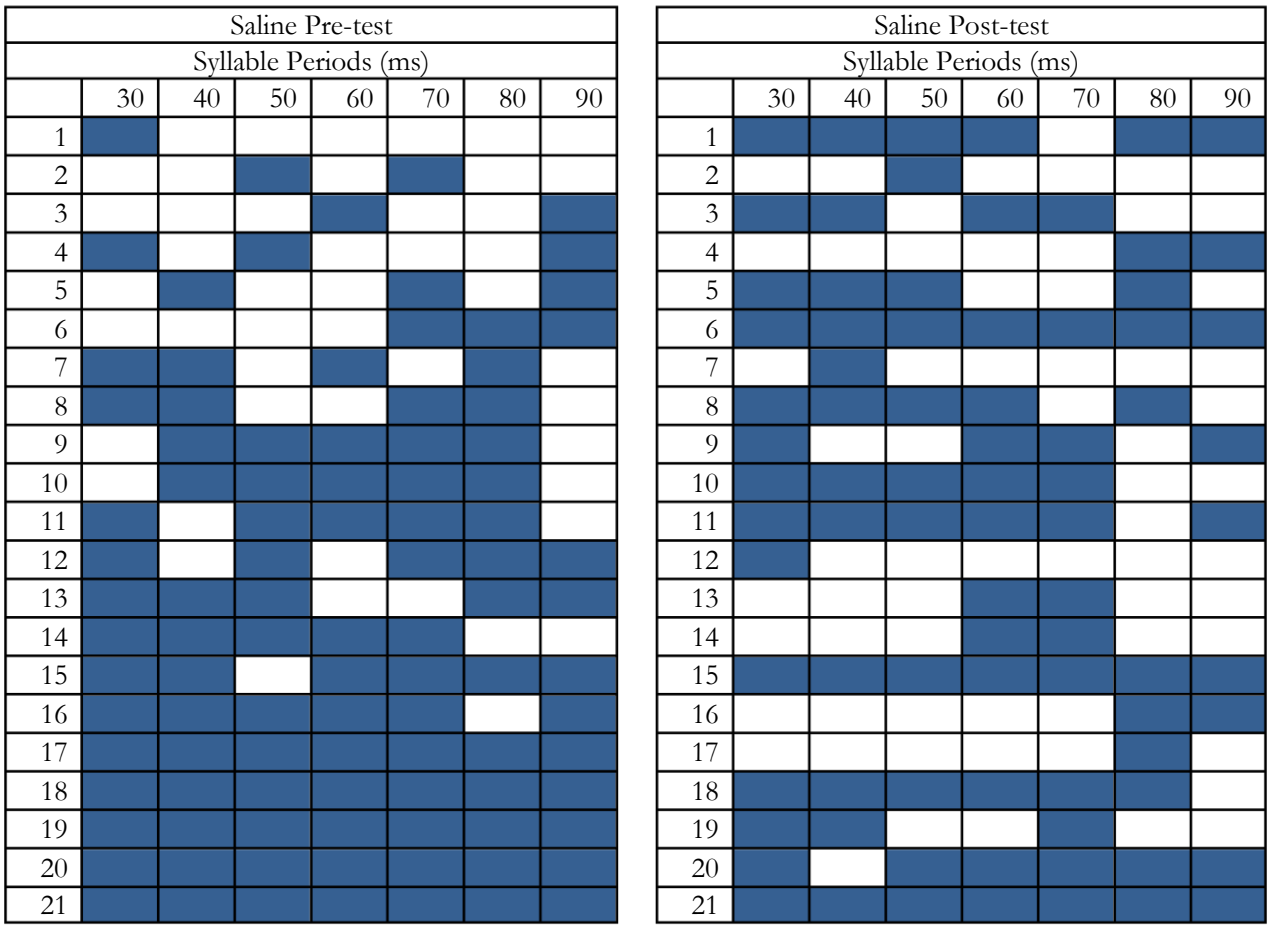

Figure 3. Table showing phonotactic selectivity before and after nanoinjection of 9.2 nl of Fielden's (1960) solution into the supraesophageal ganglion. Each row represents an individual cricket's responses (n=21) to the syllable periods listed above (30-90ms). Shaded boxes indicate positive phonotaxis, while unshaded boxes indicate no response or negative phonotaxis.

4-Aminopyridine Pre-test							
Syllable Periods (ms)							
	30	40	50	60	70	80	90
1							■
2			■				■
3			■	■	■	■	
4		■	■	■	■		
5	■	■		■		■	
6	■				■	■	■
7	■				■	■	■
8	■				■	■	■
9	■		■	■	■	■	
10	■	■		■	■	■	
11		■	■	■		■	■
12	■		■	■		■	■
13	■	■	■			■	■
14	■		■	■	■	■	■
15	■	■		■	■	■	■
16	■	■	■	■		■	■
17	■	■	■	■	■	■	
18	■	■	■	■	■	■	
19	■	■	■	■	■	■	■
20	■	■	■	■	■	■	■
21	■	■	■	■	■	■	■

4-Aminopyridine Post-test							
Syllable Periods (ms)							
	30	40	50	60	70	80	90
1	■	■		■	■	■	
2		■					■
3	■	■	■	■	■	■	■
4			■	■	■		
5	■				■		■
6			■	■			■
7	■	■					■
8	■						■
9	■	■	■	■	■	■	
10	■	■	■	■	■	■	■
11	■	■				■	■
12	■		■	■			■
13	■	■	■		■	■	■
14		■	■	■			■
15	■	■	■	■	■	■	
16	■			■			■
17	■						■
18		■	■	■	■	■	
19	■	■	■	■	■	■	■
20	■	■	■	■	■		■
21	■	■		■	■		■

Figure 4. Table showing phonotactic selectivity before and after nanoinjection of 9.2 nl of 10^{-5}M 4-aminopyridine solution into the supraesophageal ganglion. Each row represents an individual cricket's responses (n=21) to the syllable periods listed above (30-90ms). Shaded boxes indicate positive phonotaxis, while unshaded boxes indicate no response or negative phonotaxis.

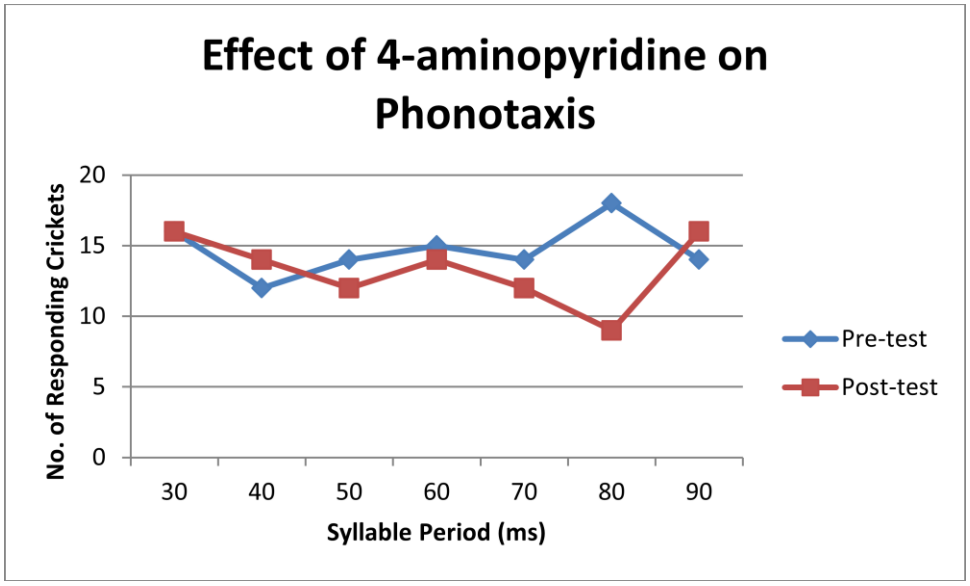

Figure 5. Graph illustrating the number of crickets (n=21) responding to each of the syllable periods (30-90ms) before and after the nanoinjection of 9.2nl of 10^{-5}M 4-aminopyridine solution into the supraesophageal ganglion.