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The Effects of Pyrilamine on the Selectivity of Phonotaxis

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ABSTRACT

Hormones modulate phonotactic behaviors of female crickets (*Acheta domesticus)*. This project seeks to observe the changes in phonotactic behaviour when anti-histamine is injected in the prothoracic ganglion. The anti-histamine used, Pyrilamine, resulted in older, unselective females becoming more selective to the male calling songs. Females injected with saline (controls) remained unselective. The experiments with Pyrilamine (anti-histamine) validates histamine's proposed role on phonotactic selectivity by enhancing inhibition of syllable period selective neural circuits in the prothoracic ganglion.

INTRODUCTION

The auditory system of the cricket has been used as a model to understand how circuits of neurones and their modulators control behaviour. Female crickets recognize and phonotactically behave to various conspecific male cricket-calling songs (Stout *et al.* 2002). The most important parameter used for selective phonotaxis by *Acheta domesticus* is syllable period (SP) (McGee & Stout, 1988). Phonotactic responses are influenced by levels of naturally produced Juvenile Hormone III (JHIII) in female *A. domesticus* (Walikonis *et al.* 1991). Atkins *et al.* (2008) demonstrated that older crickets became less selective upon addition of JHIII; this correlates to the tendency of decreasing levels of JHIII as crickets age (Walikonis *et al.* 1991). Atkins *et al.* (2008) showed that nanoinjecting Picrotoxin (PTX) into the prothoracic ganglion caused crickets to become more selective indicating a role for chloride channels. This demonstrates that neurones in the prothoracic ganglion take part in making crickets selective towards various SPs, and that hormones modulate these inputs. There are four possible neurotransmitter candidates that mediate chloride channels: Histamine, GABA, glycine, and seratonin. Yoon *et al.* (2011) demonstrated that Histamine caused phonotactic behaviour to become unselective. None of the other three inhibitory neurotransmitters (GABA, Glycine, and Serotonin), which mediate chloride currents, did so. The present study tests the effect of an antihistamine, Pyrilamine. Since histamine significantly changes phonotactic selectivity; an anti-histamine should also change phonotactic selectivity opposite of histamine.

METHODS

Four to five week-old nymph crickets were purchased from Fluker's Cricket Farm in Baton Rouge, Louisiana, and raised in 100-L containers. Cricket food, from Fluker's Farm, was provided along with water and egg cartons (for shelter). Environmental temperatures were maintained within a range of 21-23°C under a 12:12 h LD photoperiod (Atkins *et al.* 2008). Females were removed as they molted into adults.

Four to five-week old adult crickets were randomly selected and placed in a circular sand arena (Atkins *et al.* 2000, Fig. 1) with a centrally located, omni-directional speaker. After 5 min of silence for crickets to adjust to the arena, the crickets were exposed to seven computer-generated male chirps in a non-sequential order (50, 90, 60, 30, 70, 40, and 80 ms) for five minutes each, or until the cricket displayed positive phonotaxis. Positive phonotaxis was defined as movement from the edge of the arena towards the central auditory source of the arena (Atkins *et al.* 2008). In between each syllable period test, the crickets were given three minutes of silence. We pre-selected older crickets that would display less selectivity in the pre-test.

Following the pre-test, crickets were placed ventral side up on a wax block, with wax strips and dots to fasten and prevent movement (Fig. 2). A small incision was made between the two abdominal plates, right below the crickets' front legs, until the prothoracic ganglion was viewed. Twenty experimental crickets were nanoinjected with 9.3 nl of 10^{-5} M Pyrilamine, and 20 control crickets were nanoinjected with 9.3 nl of saline. Nanoinjection was directly on the ventral side of the prothoracic ganglion. Afterwards, the flap of exoskeleton was closed, and the cricket was given 5 min of recuperation before release and post-tested. Crickets in post-testing were exposed again to the same syllable periods as the pre-test, played in the same order.

Differences and similarities in phonotaxis between the pre- and post-test were tallied and analyzed statistically with a one-tailed paired *t*-test.

RESULTS

Twenty crickets were injected with the control solution (9.2 nl saline). Slight variation was seen between individual crickets of the pre- and post-tests; however, average number of syllable periods responded to was not significantly different (paired *t*-test, $p = 0.110$, Table 1). For the experimental group, twenty crickets were injected with 9.3 nl of 10^{-5} pyrilamine (Table 2). The number of syllable periods with positive phonotaxis decreased significantly (paired *t*-test, $p = 3.12 \times 10^{-11}$). On average, individual crickets responded to more SPs in the pre-test than the post-test. The pyrilamine group showed a higher attraction towards SPs 50-80 ms in the pre-test, and 50-60 ms in the post-test (Figure 3).

DISCUSSION

From the data, it is evident that our anti-histamine, pyrilamine, affected phonotactic behaviour significantly, by observing an increase in the crickets' post-test selectivity (Table 2). Previous researches show histamine to be the neurotransmitter modulating selectivity of phonotactic behaviour; histamine displayed a decrease in cricket selectivity (Yoon *et al.* 2011). As a result, data from the anti-histamine validates the identification of histamine as the neurotransmitter controlling phonotactic selectivity. Data from pyrilamine (Fig 3) also resembled previous data from Atkins *et al.* 2008 using JHIII (Fig 4); both pyrilamine and JHIII showed post-test trends with lower counts of number of crickets responding to various syllable periods, allowing us to suggest that our anti-histamine works on the neuronal circuit like JHIII. In other words, JHIII in crickets changes its selectivity by up regulating histamine release.

Future research can explore the biochemical steps involved between JHIII and histamine by nanoinjecting JHIII and a phosophokinase C blocker (PKC) into the prothoracic ganglion. In doing so, the chemical pathway JHIII works in order to release histamine can be determined. Modulation of phonotaxis takes place by neurones in the prothoracic ganglion with its axons travelling into the supraesophageal ganglion (Atkins *et al.* 2008); as a result, we can also test whether nanoinjection of pyrilamine into the supraesophageal ganglion displays the same modulating effects as the prothoracic ganglion. Testing the effects of injected histamine and antihistamine on the L3 neurone, which is thought to be the key neural basis of the plasticity of phonotaxis. If the model is correct, L3 should be sensitive to histamine and antihistamines as it is to JHIII and PTX (Stout *et al.* 2002) and would demonstrate the role of prothoracic neurones in controlling phonotactic selectivity.

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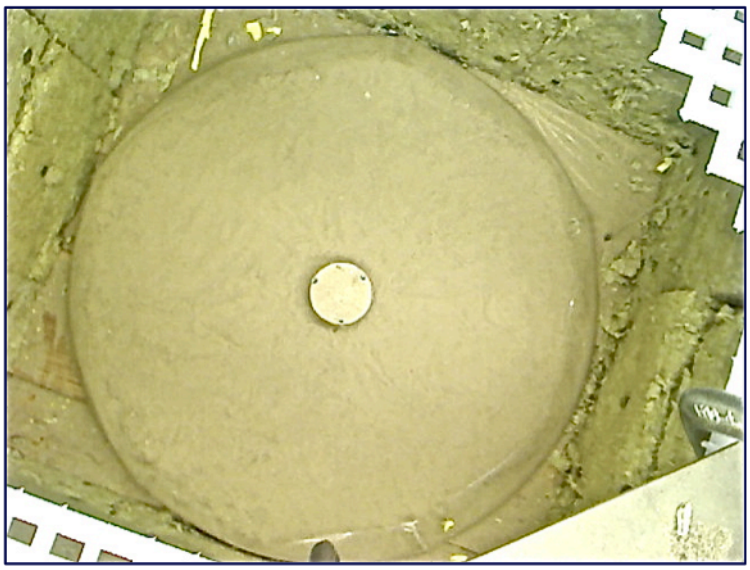
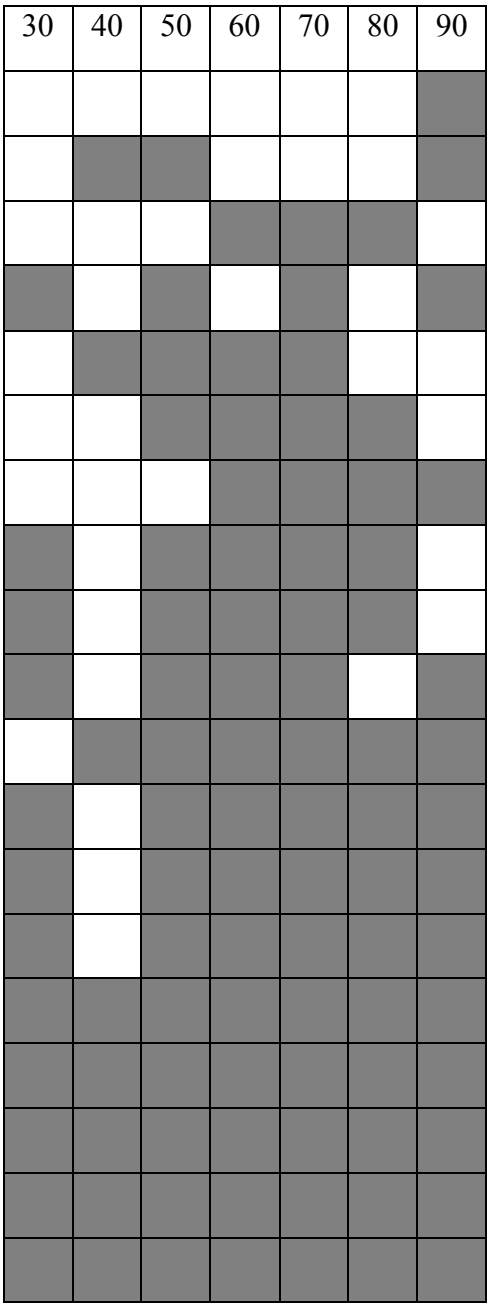


Figure 1: Circular sand-bottomed arena (152 cm in diameter) located in an insulated box (183 cm x 183 cm x 124 cm), surrounded by a 10 cm transparent plastic order.

Figure 2: Wax block for cricket mounting. Crickets fastened with wax dots and strips to restrict appendage movement, as well as a U-shaped wire to maintain a propped head position.

Table 1: Saline (Control) data with syllable periods (ms) at the top and each horizontal line indicating an individual cricket. White spaces indicate no phonotaxis and shaded in blocks signify positive phonotaxis towards the speaker ($p = 0.110$).

Pre-Test



Post-Test

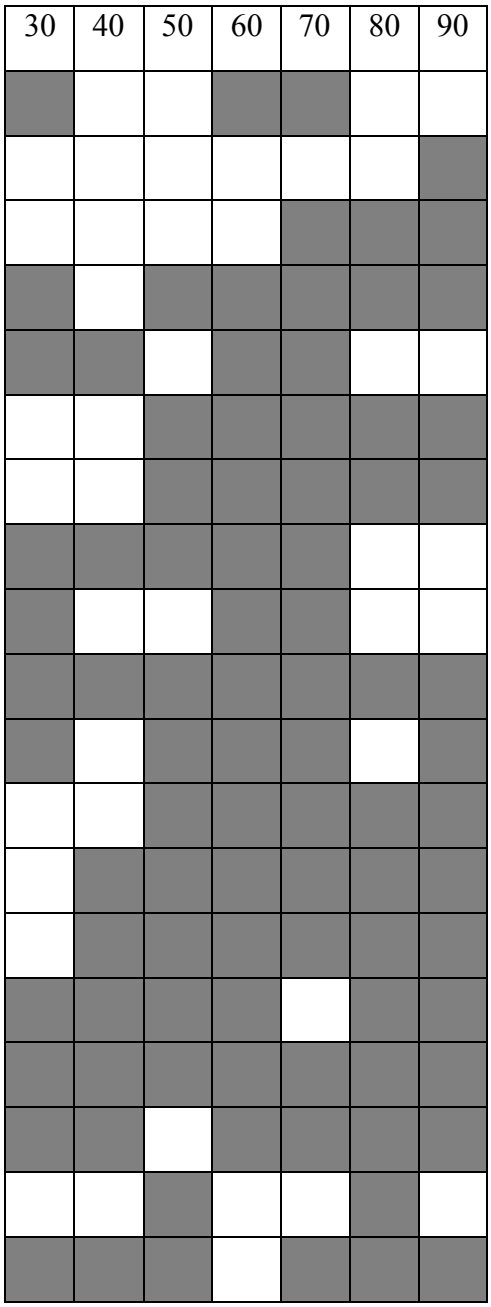
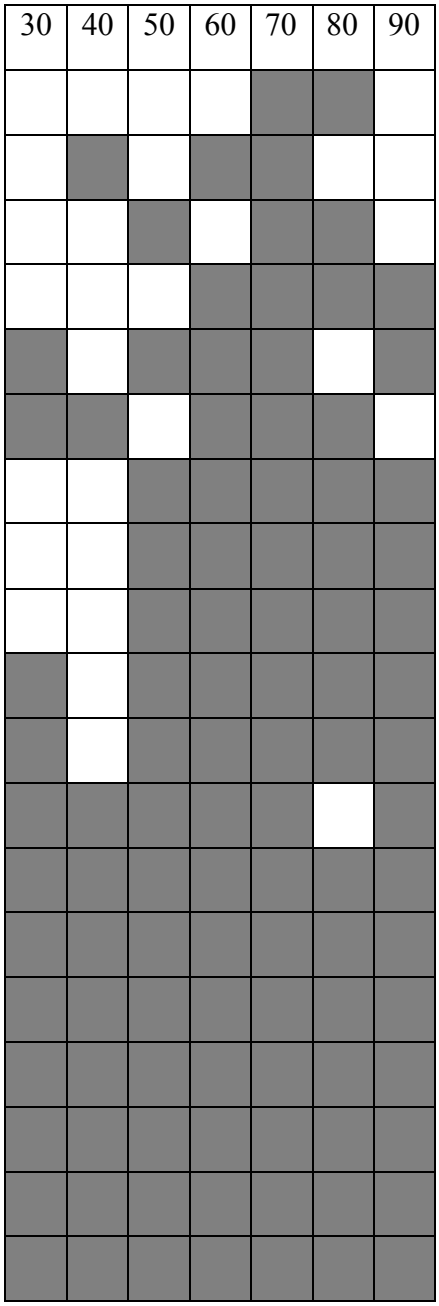


Table 2: Pyrilamine (Experimental) data with syllable periods (ms) at the top and each horizontal line indicating an individual cricket. White spaces indicate no phonotaxis and shaded in blocks signify positive phonotaxis towards the speaker ($p = 3.12 \times 10^{-11}$).

Pre-Test



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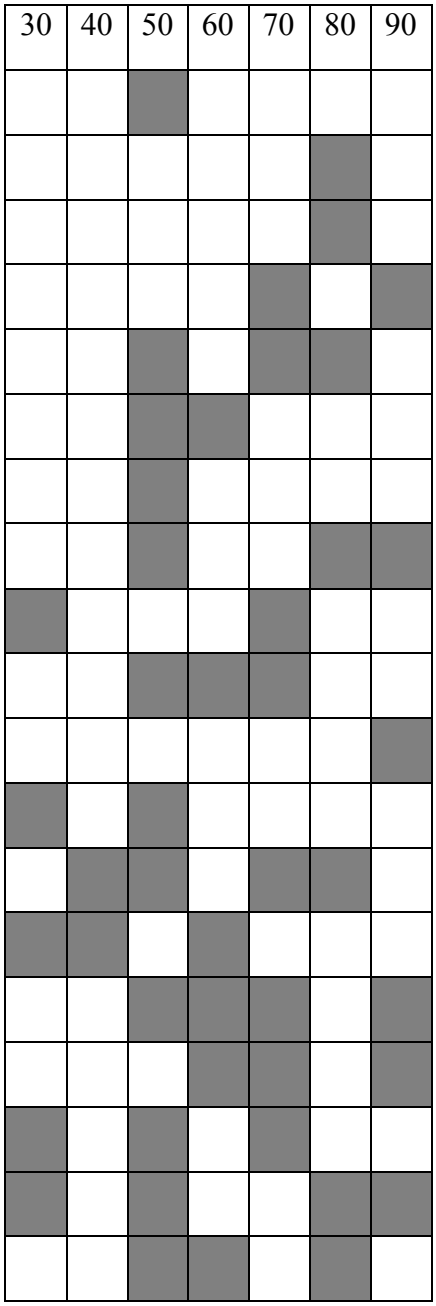

Table 3: Histamine data with a p-value of 0.0000454 (Yoon *et al.* 2011).

Pre-Test

30	40	50	60	70	80	90
				■		
				■		
	■			■		
		■		■		
		■		■		
			■	■		
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		■	■	■	■	■

Post-Test

30	40	50	60	70	80	90
				■		
■		■		■	■	■
■		■		■	■	
■	■	■	■	■		
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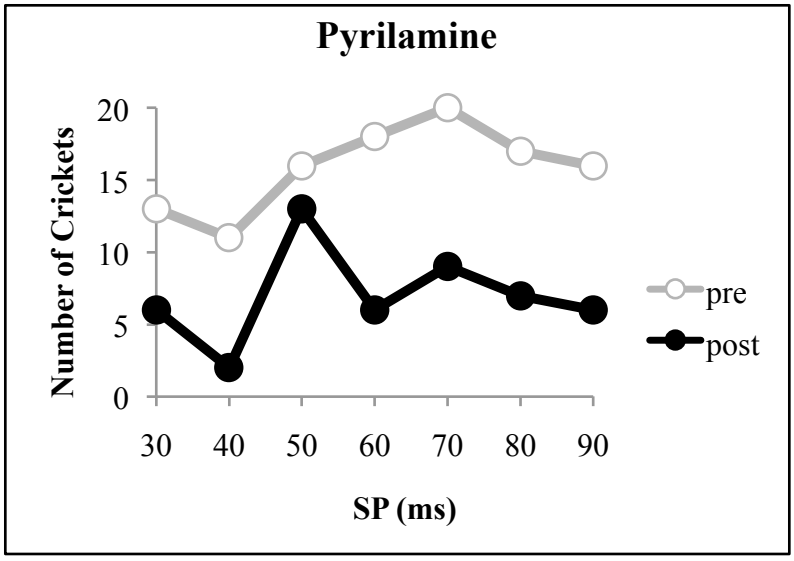

Figure 3: Frequency of 50 ms syllable period was most preferred in Pyrilamine in both the pre- and post-test from the lower number of responses to the 40 ms syllable period.

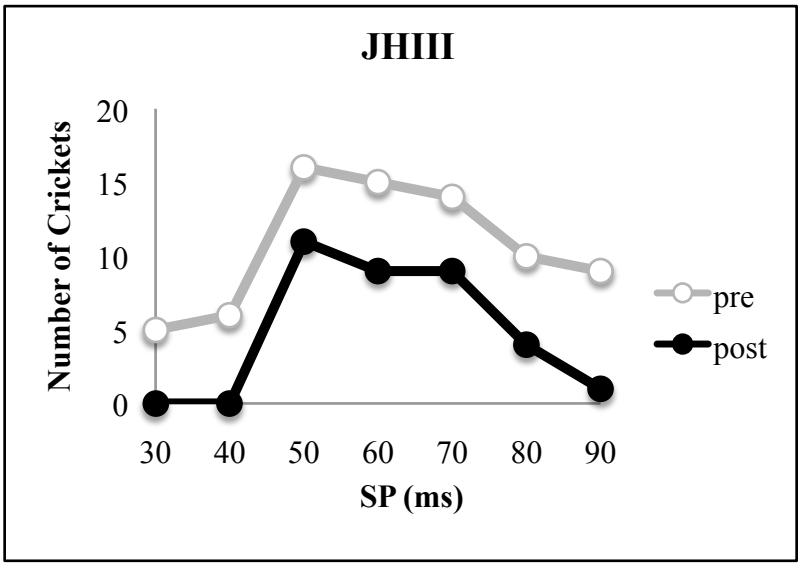

Figure 4: Data from JHIII shows similarities with data from Pyrilamine (antihistamine) with a peak in positive phonotactic behaviour at the 50 ms syllable period. Also, the number of crickets responding to syllable periods, on average, is greater in the pre-test (Markovic, 2010).