Chelerythrine chloride and its effects on phonotactic behavior in female crickets Acheta domesticus

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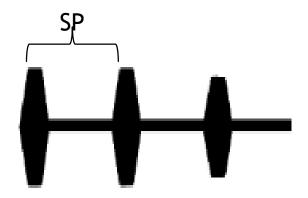
J. N. Andrews Honors Program





Introduction

- Phonotaxis
 - ► Movement in relation to a sound source
 - Female cricket in response to the male crickets' calling song
- Syllable Period (SP)
 - ▶ 30ms~90ms



Introduction

- Selectiveness
 - ▶ Selective: responds to 5 or fewer SPs
 - ► Unselective: responds to 6 or more SPs
- Cricket's selectiveness in relation to age
 - ▶ Presence of Juvenile Hormone III (JHIII)
 - ▶ Stout *et al.* (1976)
- Prothoracic ganglion (PTG)
 - Auditory circuitry
 - ► Atkins *et al.* (2008)

Introduction

- Nanoinjection of JHIII (Choi, et al. 2012)
 - ► Response to narrower range of SPs
 - Protein Kinase C pathway
- Nanoinjection of Chelerythrine Chloride (CC) (Lynch, 2015)
 - ► Potent Protein Kinase C blocker
 - ► Neuronal response
 - ▶ More uniform, consistent physiological response

Current Research

- Evaluate the effects of CC injection on the female cricket's behavioral response
 - ► Gain understanding to a complex yet not fixed phonotactic response of the crickets
 - ► Study the behavioral plasticity and its link to neural circuits involved in controlling the behavior



- Acheta Domesticus
 - ► House cricket
 - ► 2~3 months life



http://bugguide.net

- ► Animal Care
 - ► Temperature: 22-24°C
 - ▶ 12 hour light-dark cycle
 - ► Female crickets separated from male crickets

- 3-7 day old females tested on a non-compensating spherical treadmill
- Computer generated model calls used for auditory stimulus (Audacity 2.0.6) with syllable periods ranging from 30-90 ms

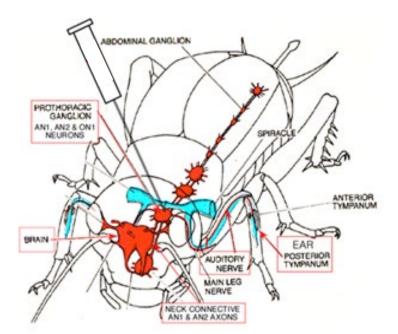
 Optical Kugel application (2008: Version 0.5 beta) used to record and quantify data



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	(Date and time of experiment: 2015-08-07 12:28:21 +02 Length of experiment: 8:50.39 [mm:ss]	00
	(Acoustic stimulus file: /bimac & domest. Chirps/SkHz:75	dB:50ms(CCP)-25msSD copy
)	Description	Value
	1	Number of acquired points:	8699
	1	x-component of centroid vector:	-0.001117
	{	y-component of centroid vector:	0.059611
	}	Length of centroid vector:	0.059622
	1	Average angular orientation	-1.07
	Ĕ.	Total length of path::	259425.58
		Length of path leading towards loudspeaker:	
		Length of path leading away from loudspeak	er: 63910.96

- Pre test
 - > 50ms 90ms 70ms 30ms 60ms 80ms 40ms
 - ▶ 5 minutes song, 2 minutes break

- A small incision made to expose the prothoracic ganglion for injection
- A Drummond Nanoinject II nanoinjector (Drummon Scientific Co., Broomall, Pennsylvania) used to inject 9.2nL of 10⁻⁵ M CC (Sigma) in saline for the experimental group and 9.2nL saline for the control group



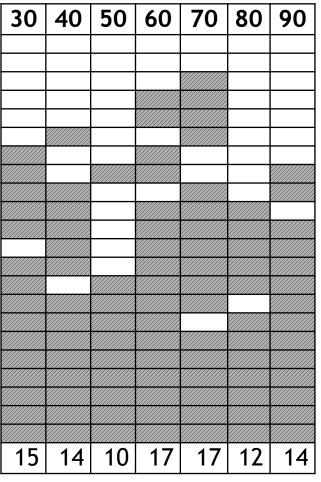
Experimental Group

BEFORE CC SYLLABLE PERIOD (ms)

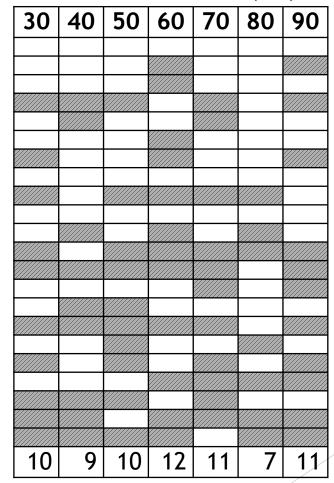
AFTER CC			
SYLLABLE PERIOD	(ms))	

Results

Paired t-test, P=0.015 n=22



NUMBER OF FEMALES/SP



NUMBER OF FEMALES/SP

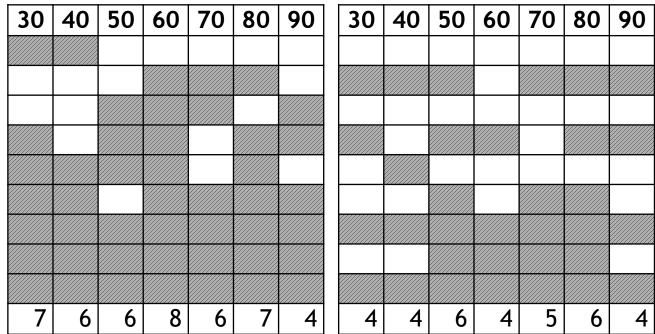
Control Group

BEFORE INCISION/SALINE SYLLABLE PERIOD (ms)

AFTER INCISION/SALINE SYLLABLE PERIOD (ms)

Results

Paired t-test, P=0.102 n=9



NUMBER OF FEMALES/SP

NUMBER OF FEMALES/SP

Statistical Analysis

- Selective crickets remained "selective"
- Unselective crickets became "selective"
- "Selective" => "Less responsive"
 - No significant change in responds to 50-70ms
 - Paired t-test, P=0.061

Paired T test,

P = 0.807

Selective Crickets Pre test		
Before CC	After CC	
0	0	
0	2	
1	1	
2	5	
2	2	
2	1	
2	3	
4	0	
4	5	
5	0	
5	6	

Paired T test,P=0.00126

Statistical Analysis

- ► 30~40ms range (p=0.038)
 - ▶ Pre test: 1.3 SPs
 - ► Post test: 0.86 SPs
- > 50~70ms range (p=0.061)
 - ▶ Pre test: 2 SPs
 - ► Post test: 1.5 SPs
- ▶ 80~90ms range (p=0.042)
 - ▶ Pre test: 1.2 SPs
 - ▶ Post test: 0.82 SPs

Statistical Analysis

Experimental Group

	Post responded	Post not responded	Total
Pre responded	54	46	100
Pre not responded	17	37	54
Total	71	83	154

McNemar's test P<0.001

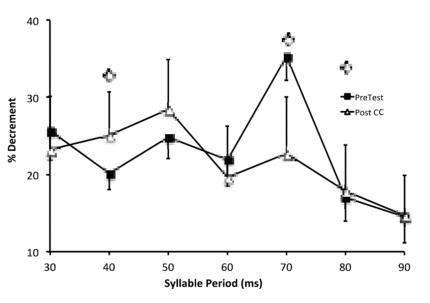
Control Group

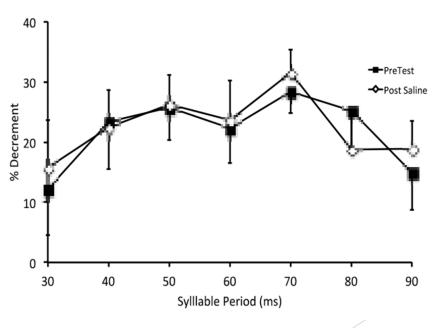
	Post responded	Post not responded	Total
Pre responded	28	18	46
Pre not responded	5	12	17
Total	33	30	63

McNemar's test P<0.01

Discussion

- Physiological Response graph (Lynch, 2015)
 - ► CC injection flattens SP-selective decrement by L3 without Increasing the overall decrement.





Experimental Group

Control Group

Conclusion

► CC, a Protein Kinase C blocker, may have blocked the affect of JH III which led to a less responsiveness in the female crickets

Further Study

- Increase the sample size for both groups
- Behavioral response after the injection of CC then JHIII
- Use count data with the with values given by the Optical Kugel

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Thank you