The Dose Dependent Effects of Procaine on Cockroach Electrophysiology

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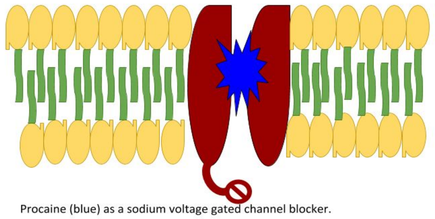
Introduction/Background

- The jewel wasp is known for its unusual reproductive behavior, which involves using a cockroach as a host for its larvae. The wasp stings its venom particularly at the roach's sub-esophageal ganglia (SEG) and induces paralysis (Haspel et al., 2003).
- The jewel wasp venom activates GABAergic chloride channels which leads to central synaptic block and results in paralysis in the host. Procaine is a synthetic drug used as a substitute for jewel wasp venom for pharmaceutical use.

Fig. 1 Procaine's mechanism of action. This figure displays a sodium voltage-gated channel in a lipid membrane being blocked by procaine.



Procaine (blue) as a sodium voltage gated channel blocker.

We hypothesize that increasing procraine concentrations will result in greater loss of motor and sensory input which will be represented by a greater loss of electrophysiological readings.

Main Questions:

-What concentration range of procaine is needed to induce paralysis? What type of electrophysiological effects would this result in? The time it takes to reverse the results?

Methods

Anesthetize Roach by submerging in ice water

Fig. 2 Electrode set up with roach intact. The roach was placed on a styrofoam block to which the electrodes could attach. The recording electrode was placed on the tibia, and the other two placed on the femur as shown.

Setup roach by inserting three electrodes on the leg of attached cockroach

Record RMS for *pre-poke on Spike Recorder

Inject 15 uL Procaine
(0.025%, 10%, 20%, saline as control)

Record RMS for *post-poke

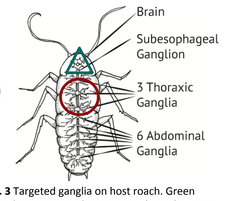
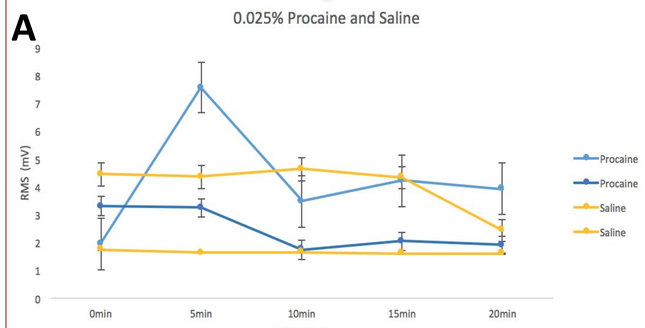


Fig. 3 Targeted ganglia on host roach. Green triangle represents the SEG site where the wasp first stings its prey, followed by a second sting in the thoracic region indicated by the red square. Our methods aimed to inject only at the site of the SEG which has greater paralytic effects on the roach compared to thoracic ganglia [1].

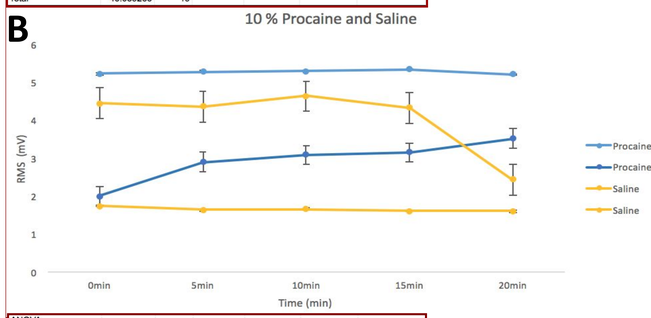
***Pre and post poke involved simply recording for a duration of 30 seconds while performing pokes on the tibia barbs with a glass rod.**
****Some cockroaches were sacrificed and injected with dye after the post poke.**

Results: RMS Values of Neuron Spike Signals



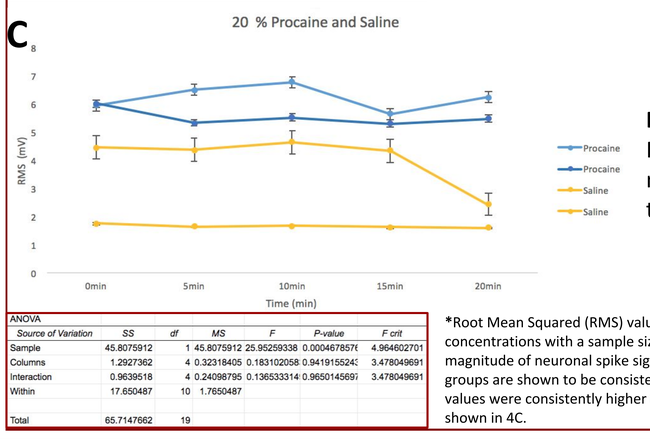
Source of Variation	SS	df	MS	F	P-value	F crit
Sample	1.2172178	1	1.2172178	0.379689906	0.551536889	4.964602701
Columns	6.8758375	4	1.71895937	0.536199457	0.712691191	3.478049691
Interaction	5.9080027	4	1.47700067	0.460724652	0.763231889	3.478049691
Within	32.058208	10	3.2058208			
Total	46.059266	19				

Fig A. 0.025% Injection of Procaine (2) or Saline (2). n=4. Repeated measures two way ANOVA.



ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Sample	7.8675968	1	7.8675968	2.61502879	0.136927038	4.964602701
Columns	0.622712	4	0.155678	0.051744193	0.994158068	3.478049691
Interaction	1.8048192	4	0.4512048	0.149971277	0.958760087	3.478049691
Within	30.086081	10	3.0086081			
Total	40.381209	19				

Fig B. 10 % Injection of Procaine (2) or Saline (2). n=4. Repeated measures two way ANOVA.



ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Sample	45.8075912	1	45.8075912	25.95259338	0.000467857	4.964602701
Columns	1.2927362	4	0.32318405	0.183102058	0.941915524	3.478049691
Interaction	0.9639518	4	0.24098795	0.136533314	0.965014569	3.478049691
Within	17.650487	10	1.7650487			
Total	65.7147662	19				

Fig C. 20 % Injection of Procaine (2) or Saline (2). n=4. Repeated measures two way ANOVA.

*Root Mean Squared (RMS) values measured at different procaine concentrations with a sample size of 2 for each. Here, RMS measures the magnitude of neuronal spike signals over a period of 30 seconds. Control groups are shown to be consistent at all 3 concentrations (4A-4C). RMS values were consistently higher than control at a concentration of 20% as shown in 4C.

Results: Average Percent Firing Over Time

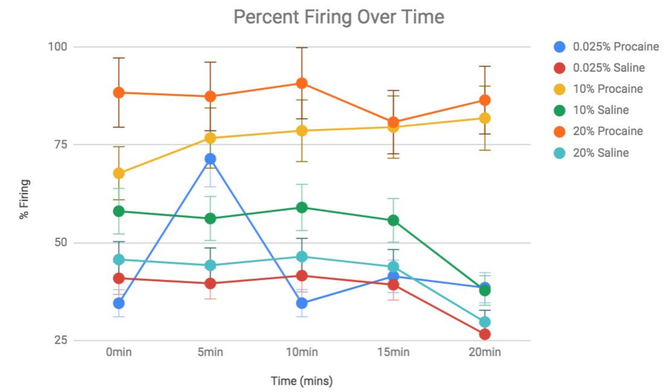


Fig. D Average percentage of Procaine and Saline across concentrations. RMS values were converted to percent based on the largest value yielded per roach. Neuronal firing for 20 minutes over increments of 5 minutes for all concentrations are graphed.
-No significance between group and time (P>0.05).

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	459.4838003	1	459.4838003	0.8487931788	0.3678744213	4.351243331
Columns	487.527158	4	121.8817896	0.2251492469	0.9211299751	2.866081402
Interaction	299.498805	4	74.87470236	0.1383142052	0.9660895737	2.866081402
Within	10826.7552	20	541.3377625			
Total	12073.2650	29				

Conclusions

- Procaine being the pharmaceutical equivalent of the jewel wasp venom has demonstrated to take effect at relatively low doses compared to what's been used as per literature [2]. However, the variation in RMS values indicates that procaine may not be the best alternative for jewel wasp venom.
- Dissections after dye injections gave inconclusive results if the procaine reached the SEG or dispersed into other surrounding tissue instead.

Future Directions

- Determining a more effective means of administering the drug (Referring to the dissection after dye injections).
- Procaine may not be a suitable alternative for wasp venom, however since it is a reversible Na+ voltage gated channel blocker, it can be used as a non-invasive means to study paralysis as well as other inhibitory disorders.
- Focus on the effects of procaine on roach legs severed from the body to compare effects in individual limbs as opposed to affecting the central nervous system.

References

1. Gal R., Libersat F. 2010. A Wasp Manipulates Neuronal Activity in the Sub-Esophageal Ganglion to Decrease the Drive for Walking in Its Cockroach Prey. *PLoS One*. 4:e10019.
2. Kaiser M., Libersat F. 2015. The role of the cerebral ganglia in the venom-induced behavioral manipulation of cockroaches stung by the parasitoid jewel wasp. *J. Exp. Biol*. 218:1022-1027.