Inhibitory effects of imidacloprid on crayfish (Procambarus spp.) neuronal action potentials

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Abstract

The objective of this study was to determine what effect the neonicotinoid imidacloprid has on crayfish neuronal action potentials. Different amounts of imidacloprid were added to an exposed crayfish nervous system. The action potentials before and after the treatment were recorded using a home-made suction electrode and PowerLab software. After data analysis, we found out that exposure to imidacloprid causes an inhibitory effect on the neuronal action potentials of crayfish, being the extent of inhibition related to the concentration of imidacloprid to which the crayfish nervous system was exposed.

Introduction

Imidacloprid is a neurotoxin exogenous to the invertebrate nervous system that is selectively more toxic to insects than to warm-blooded animals. Imidacloprid interferes with the transmission of stimuli in the nervous system by causing a blockage in the nicotinergic neuronal pathway, which is a neuronal pathway that is more abundant in insects than in warm-blooded animals. This blockage leads to the accumulation of acetylcholine, resulting in the animal's paralysis, and eventually death in some cases. Because of its mode of action, imidacloprid affects mainly the motor cells of animals.

Previous research on clothianidin, a neonicotinoid similar to the neonicotinoid used in this experiment (imidacloprid) shows that crayfish exhibited reduced responsiveness to stimulus with increasing clothianidin concentration (Miles et al, 2017). That being considered, we expect the imidacloprid treatment to reduce the responsiveness to stimulus in crayfish, which should be

seen as a decrease in the firing rate of neurons. Additionally, this experiment also attempts to explore any other effects that imidacloprid may have on neuronal firing rates in crayfish.

For this experiment, crayfish were used because their nervous system has relatively few neurons and their firing rates can be recorded easily. Additionally, there is previous research on neonicotinoids done on crayfish, so using this animal as a model for this experiment allows for a comparison of the results obtained here with those available in the existing literature.

Materials and Methods

Animal care

Female and male crayfish, *Procambarus spp.,* were used. They measured 7-10 cm in total body length. The crayfish were procured from Carolina Biological Supply (Burlington, NC), but they were farmed and harvested in Louisiana. They were kept in plastic tubs filled with tap water (up to 3 individuals per tub, for no longer than 1 week), fed dry food daily, and kept on a 12-hour light cycle.

Saline

The saline solution used in this experiment is Standard Saline (modified after van Harreveld, 1936), and its components were: 12.0 g/L NaCl; 0.4 g/L KCl; 1.5 g/L CaCl2 (anhydrous); 0.5 g/L MgCl2* 6H2O; 0.17 g/L NaHCO3. The solution was aerated and mixed thoroughly. Drops of dilute HCl or additional NaHCO3 may have been added to bring the solution to pH 7.4.

Dissection

The crayfish were ice-anesthetized for no less than 1 hour before dissection. After the crayfish were

anesthetized, they were sacrificed by an abdomen separation. After the separation, the cephalotorax was put into a trash bin inside the freezer compartment of a refrigerator. As described in Strawn et al., 2000, to gain exposure of the ventral nerve cord with the nerve roots, the midline of the ventral articular membrane was cut. An additional cut lateral to the midline was needed to remove the membrane and thus expose the superficial flexor muscles, the ventral nerve cord, and segmental ganglia to the saline bath solution.

Electrode Construction

Suction electrodes were constructed in-home by using colored wiring with plugs and caps, a silver wire, aluminum tape, and other materials. For step-by-step instructions and a more detailed list of materials, see Johnson et al., 2007.

Recording

Throughout the experiment, the saline added was chilled and kept chilled to avoid having changes in temperature that could interfere with our results. The recordings for the control group were taken from nerve root 1, from possibly different ganglia. In order to obtain a recording, the tip of the electrode lightly sucked the nerve root for which the action potentials were being recorded. The action potentials were recorded by using the hardware PowerLab and an amplifier, as needed, and then shown on a graph using the software LabChart.

Experimental Design

For the control group, the crayfish remained in conditions as similar as possible as those in the treatment group, with the exception of the imidacloprid added to those in the treatment group. For the treatment group, 5 doses of treatments were added to each crayfish, and then the change in action potentials was recorded for the following 60 seconds. Each treatment consisted of 1 mL

of an imidacloprid solution with a concentration of 10ng per liter. As a result of the experimental design, the concentration of imidacloprid increased as each treatment was added.

Only the recordings that were seen as quality recordings were analyzed in this experiment. These recordings had low noise and the action potentials were at least twice the magnitude of that of the noise.

Results

The results obtained show that the median value for each set of data (i.e. each treatment and control group) decreased consistently with an increasing concentration of imidacloprid. Each group, with the exception of treatment 5, shows a similar distribution of data points that consists of a cluster made up of most data points within each group. As an observation, after treatment 1, there is an outlier neuron showing a significant drop in its normalized percentage change.

Figure 1: Box plot for change in action potential frequency indices (n control=32, n imidacloprid = 6). The Y-axis represents the normalized percentage change and the X-axis indicates the treatment group that each set of data belongs to.

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control group) decreased consistently with an increasing concentration of imidacloprid. Each group, with the exception of treatment 5, shows a similar distribution of data points that consists of a cluster made up of most data points within each group. As an observation, after treatment 1, there is an outlier neuron showing a significant drop in its normalized percentage change.

Additionally, the normalized percentage change of each treatment group became more negative after each treatment was added. These consistent negative changes may be evidence of potentially dose-dependent trends. Lastly, there was, from treatment 4 to treatment 5, a dramatic increase in how far apart from each other the data points were.

Discussion

There seems to be a negative correlation between action potential frequency and concentration of imidacloprid. This observation would go along with our initial predictions regarding an existing negative correlation between both variables.

Additionally, this negative correlation agrees with the documented effects of clothianidin (a neonicotinoid similar to imidacloprid): crayfish showed reduced responsiveness to stimulus with increasing clothianidin concentration (Miles et al, 2017). Thus, by having a similar neonicotinoid producing similar effects on the action potentials of crayfish neurons, the results obtained in this experiment may help make clearer what general effects neonicotinoids have on crayfish, and, to some extent, aquatic invertebrate animals. Lastly, the exhibited reduced responsiveness may be explained by the mode of action that imidacloprid has, which involves causing a blockage in the

nicotinergic neuronal pathway that leads to the accumulation of acetylcholine, resulting in paralysis, and eventually, in some cases, death.

For future research, performing this experiment with treatments at higher concentrations may determine the point at which action potential propagation ceases altogether. Additionally, considering that imidacloprid as a pesticide is implicated in the rise of colony collapse disorder among honeybees, it would be worth to consider how the methods of this study could be transferred and modified to investigate imidacloprid's effects on the firing rates of honey bee neurons.

References

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