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Chronic exposure of crayfish to ethanol reduces EPSP depression due to sudden change in ethanol concentration

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ABSTRACT

In this experiment, the difference in the values of EPSPs between crayfish chronically exposed to ethanol and non-exposed crayfish in the presence of ethanol was observed. The study tested the ESPS values in the crayfish extensor muscles when placed first in crayfish saline, and then again when the same muscles were introduced to a 2% and 10% solutions of ethanol in crayfish saline. The EPSPs of both exposed and normal crayfish decreased between the crayfish saline and the 2% ethanol solution in crayfish saline. However, in the chronically exposed crayfish, the EPSPs were not as greatly affected by the presence of the 10% ethanol solution as the non-exposed crayfish had less than 10% reduction of EPSP amplitude. These results indicate that crayfish with chronic exposure to ethanol may process it and react differently react to the presence of ethanol than normal crayfish.

INTRODUCTION

The crayfish neuromuscular junction has been a major model for studying action potentials, excitatory and inhibitory responses, synaptic facilitation, presynaptic inhibition and a multitude of other neural properties. The crayfish neuromuscular junction is widely used among neuroscientists because it is an ideal preparation in the sense that crayfish are inexpensive, have a fast and simple dissection, and exhibit many of the same neural properties and cellular functions as humans (Liu, Killilea and Ames, 2002). Similarly to humans, crayfish possess glutamate receptors. These receptors serve as the primary excitatory receptor in the central nervous system, especially within the mammalian brain. Studies suggest that chronic ethanol exposure disrupts glutamate receptors' excitatory response (Nagy, 2004). Thus, we are trying to discover if chronic exposure to alcohol changes the glutamate receptors. If we find that exposure to ethanol indeed changes the glutamate receptors, then we will better understand the underlying neurophysiology of alcohol addiction, which would lead to better treatment.

As found in previous experiments, the extensor muscles in the tails of Native American crayfish, *Procambarus clarkii*, can be used to measure many neural properties including EPSP's. The measured differences in the amplitudes of EPSPs of the chronically exposed and non-exposed crayfish will vary slightly when initially placed in crayfish saline. However, with time, the values of the EPSP's will begin to vary increasingly and will vary even more when the same crayfish muscles are introduced to a solution of 2% ethanol, and even more when exposed to a 10% ethanol solution. We predict that the EPSPs of the on-exposed crayfish will decrease more rapidly than those of the chronically exposed crayfish because in the chronically exposed crayfish, the glutamate receptors will have changed in response to exposure. Thus, if we observe a difference between the values of the EPSP of the chronically exposed crayfish and the non-exposed crayfish, then the glutamate receptors in the chronically exposed crayfish have been altered in someway. We plan to use suction electrodes in combination with standard intracellular recording at the crayfish neuromuscular junction in the extensor muscles to answer questions regarding the effects of ethanol on glutamate receptors. We found that ethanol similarly decreased the EPSP's of both normal and chronically exposed crayfish in 2% ethanol solution, but the pre-exposed population was significantly more resistant to EPSP depression in the 10% ethanol solution.

MATERIALS AND METHODS

Ethanol Exposure

We modified the ethanol exposure technique proposed by Friedman, Bittner, and Blundon (1988) to prepare our crayfish for dissection. The exposed crayfish were kept in a solution of 0.75% ethanol in aerated tap water for 138 hours in a ten-gallon aquarium. The ethanol water solution was changed daily. In other respects, the care and feeding schedule was the same as the control crayfish.

Preparations

The preparations used were the extensor muscles of a Native American crayfish, *Procambarus clarkii*. One group was used as a control, and the other was a variable group. The variable group was chronically exposed to ethanol. The crayfish muscles were put on ice 30 minutes

prior to being dissected and used for our experimental purposes.

Dissection

The preparation used was the extensor muscle of a Native American crayfish, The crayfish tail was separated from the chilled body of the crayfish and incisions were made along the lines close to the lateral sides of the tail. The incisions were carefully made along the ridges as to avoid damaging the muscle tissues need for the experiment. The legs and under-carriage of the crayfish were removed and the ganglia was then gently pushed out leaving only the extensor muscles of the crayfish exposed. The preparation was then pinned to a sylgard lined specimen dish.

Solutions

The first bath in which the preparation was placed comprised of crayfish saline (Ringer's) solution. The second bath in which the preparation was placed was that of 2% ethanol solution in crayfish saline. The ration for this solution was 98mL:2mL, 98mL being the crayfish saline and 2mL being 95% ethanol. We were able to use 95% ethanol solution because we did not know the initial volume of Ringer's in a preparation to within 5%. Our preparations were first observed in normal crayfish saline and EPSP values were obtained. Then, we introduced the same muscles (whether of chronically exposed or non-exposed crayfish) to a solution of 2% ethanol. We then proceeded to observe our preparations using a program called *Scope* for 10 minutes to gather data points (using the height values of respective EPSPs) at minute intervals. After 10 minutes, 7mL of 95% ethanol was added to make the solution 10% ethanol, and per minute readings was made for two minutes.

Normal Crayfish Saline Composition
205 mM NaCl
5.4 mM KCl
13.5 mM CaCl2
2.6 mM MgCl2
10.0 mM Tris (pH 7.4)

Table 1. Standard Ringer's solution.

2% (10%) Ethanol Solution Composition
98mL of Crayfish Saline:
205 mM NaCl
5.4 mM KCl
13.5 mM CaCl2
2.6 mM MgCl2
10.0 mM Tris (pH 7.4)
2mL (11mL) of 95% ethanol to 98mL Crayfish Saline

Table 2. 2% Ethanol solution. Modifications for 10% ethanol are in parentheses.

Recording and Stimulation

Multiple measurements were made within the extensor muscles in both solutions. Measurements were later observed and graphed for comparative purposes. Intracellular resting membrane potentials were measured through use of intracellular recording with microelectrodes. Glass microelectrodes, with resistance of 20-100 MΩ and filled with 3M-KCL, were used to enter the cell and used in combination with a ground electrode to optain resting potentials (mV). Both the microelectrode and ground electrode were connected to an amplifier that then transmitted voltages to *Scope*, which gathered our data and displayed our stimulus artifact and EPSP. Nerves controlling the crayfish extensor muscle were sucked into a fire polished capillary suction electrode. A voltage was applied, with reference to the Ringer's solution, at approximately 1Hz while the voltage was varied until an ESP was observed in Scope.

Data Analysis

EPSP amplitude was recorded in scope and time denoted by the second hand of a wall hanging clock. All EPSP's are given as a decimal percent of the average EPSP value of each preparation before the addition of ethanol.

RESULTS

In this experiment, the EPSPs of chronically exposed to ethanol and non-exposed crayfish were compared. It was hypothesized that the EPSPs of the non-exposed crayfish would not vary greatly when placed in crayfish saline. It was also predicted that the EPSPs of the non-exposed crayfish would decrease more rapidly than those of the chronically exposed crayfish when introduced to 2%% and 10% ethanol solutions. Our chronically exposed and non-exposed crayfish in crayfish saline and 10% ethanol assay maintains that there is a difference between the EPSPs of the chronically exposed and non-exposed extensor muscles when introduced to a 10% ethanol bath. Due to the varying nature of EPSP's within a preparation, it was impossible to distinguish chronically exposed crayfish from their normal counterparts when in observed in standard Ringer's solution.

EPSP values for the chronically exposed and non-exposed crayfish extensor muscles in 2% ethanol solution did vary in comparison with the readings in normal crayfish saline with decreases of 41% and 23%, and 13% and 28% respectfully (figure 1). As seen in figure 1, exposed crayfish EPSP's initially decreased approximately 30% less than those of normal crayfish. This trend did not continue as the first control slightly recovered and second control fully recovered from the ethanol exposure, while both pre-exposed crayfish continued to have a decrease in EPSP amplitude.

However, despite these reductions of EPSP values, there is no significant difference between these populations as a T-test yielded a P value of 0.44 (figure 3). Similarly, there was no difference between EPSP depression between normal Ringer's and 10% ethanol solution for both chronically exposed and normal preparations. Nevertheless, the change in EPSP after the addition of 10% ethanol solution is clearly significantly different between the two populations. T-tests produced a P value of 0.004, a full order of magnitude smaller than the required 0.05. These findings are summarized in Figures 2 and 3. Whereas the second control had an initial decrease of 48% after one minute of exposure, dropping to 54% a minute later, both pre-exposed crayfish had an initial depression of less than 3% after one minute, and less than 8% after two.

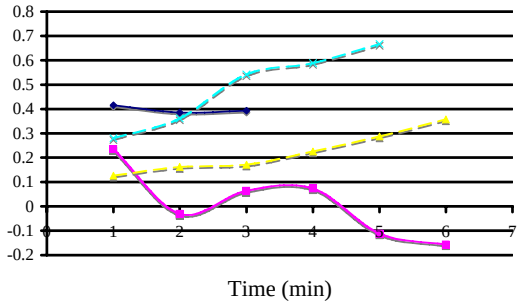

Figure 1. Change in EPSP ratio after addition of 2% ETOH solution. Vertical axis is the ratio with respect to normal and the horizontal axis is time in minutes. Blue diamonds are control 1, pink squares are control 2, yellow triangles are ethanol exposed 1, and light blue x's are ethanol exposed 2. Aside from control 2, all preparations had a decrease in EPSP amplitude.

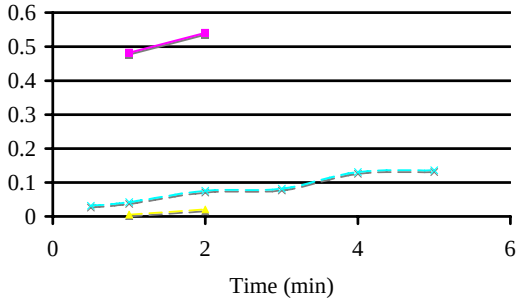

Figure 2. Change in EPSP ratio after addition of 10% ETOH solution. Vertical axis is the ratio with respect to normal and the horizontal axis is time in minutes. Solid lines are normal crayfish; dashed lines are ethanol-exposed crayfish. Pink squares are control 2, yellow triangles are ethanol exposed 1, and light blue x's are ethanol exposed 2. It is clear that non-exposed crayfish have much greater decrease of EPSP after application of 10% ethanol solution.

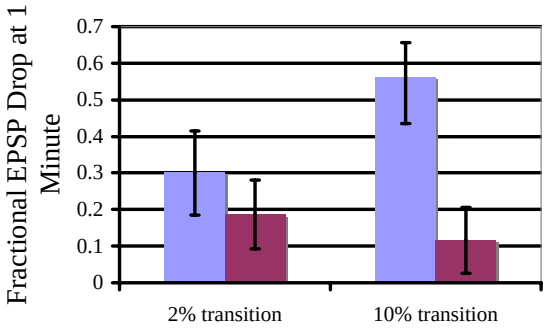

Figure 3. Fractional EPSP drop 1 minute after addition of 2% and 10% ethanol solution. N = 2 for all cases. Error bars are standard error for each population. Light blue represents control crayfish readings while magenta is ethanol-exposed crayfish. The difference in EPSP drop between populations after addition of 2% ethanol solution is not significant (P=0.44). Pre-exposed crayfish had significantly less EPSP depreciation after addition of 10% ethanol solution (P=0.004).

DISCUSSION

EPSP's of chronically exposed and control preparations showed a significant decrease in amplitude upon exposure to both 2% and 10% ethanol solutions. Despite variation in control 2, it is clear that decreases occurred after both ethanol exposures. Likewise, decreases were observed in the chronically exposed crayfish. Our results do not suggest that crayfish become accustomed to low levels of ethanol exposure as chronically exposed crayfish had similar drops in EPSP amplitude to those of normal crayfish, upon the addition of both 2% ETOH solution. However our results support the idea that chronic exposure to ethanol minimizes EPSP depression after sudden increases of ethanol concentration. The exposed crayfish had a much smaller drop of EPSP amplitude after changing from 2% to 10% ethanol solution (figure 2). This strongly suggests that the glutamate receptors in the extensor muscle region have been modified in some way, though the mechanisms behind this remain unclear. It was later determined that ethanol may have reduced the number of release sites (vesicles) and the probability of release (Strawn, JR., Cooper, RL., 2002).

Future studies are needed to determine the long-term effects of ethanol on glutamate receptors and verify our results. Tsai, Gastfriend, and Coyle suggest that the N-methyl-D-aspartate (NMDA) glutamate subgroup is affected, however the mechanisms behind this are unclear.

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