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Low Concentrations of Ethanol Applied to Glutamatergic Synapses in Crayfish Decreases Synaptic Transmission

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ABSTRACT

In the course of our experiment, we exposed the glutamatergic synapses of crayfish to 44 mM and 88 mM ethanol and obtained excitatory post-synaptic potentials (EPSP) readings. These readings were compared to baseline EPSP readings which were taken before the ethanol was added. It was expected that the amplitudes of the EPSP's would decrease after the addition of ethanol, resulting in decreased synaptic transmission. The experiment revealed that when 44 mM ethanol was introduced to the synapses of crayfish, the EPSP amplitude did in fact decrease, but the 88 mM solution saw the amplitude initially drop and then increase.

INTRODUCTION

Ethanol, the intoxicating ingredient in alcoholic beverages, can produce euphoria, drunkenness, coma, or death in humans. It also acts as a depressant on the central nervous system (Special 2004). Ethanol's effect on glutamatergic synapses is particularly important since these synapses are prevalent in the brain, as are those found in the extensor muscles of the tail of a *Procambarus Clarkii,* commonly known as the crayfish. Thus any effect of ethanol on synaptic transmission in these neuromuscular junctions of the crayfish will be similar to its effect on the glutamatergic synapses found in the human brain. The results from this experiment could help to further understand glutamatergic synapses and synaptic transmission in the brain under the influence of ethanol.

In one prior study, the changes in behavior and resting membrane potential in crayfish were observed after the application of ethanol. This study was preformed by immersing a living crayfish in an ethanol
solution and recording the changes over a period of two weeks. The crayfish's behavior eventually adapted to the environment of constant alcohol. Resting membrane potential in the crayfish initially decreased below the control resting membrane potential and then rose above the control resting potential (Faust, 2000). This shows that ethanol can cause fluctuations in resting membrane potential, which would affect EPSP's as well.

In another study, a low concentration of ethanol was found to inhibit N-methyl-D-aspartate which further inhibits NMDA-activated ion current. NMDA receptors help facilitate synaptic transmission, as they bind with glutamate (Weight, 1989). Therefore, interference with NMDA receptors would inhibit glutamatergic synapses. Acute ethanol treatment has been found to cause the NMDA receptors to become blocked, which results in a restricted glutamate-stimulated calcium influx. Ethanol also obstructs the NMDA receptor's electrical current. Ethanol has been shown to affect glutamate receptor-gated ion channels as well (Tsai 1998). Another study focused on the effect of ethanol on pre-synaptic components, and in its presence EPSP's were found to decrease. This study shows a result comparable to the one we expected in our experiment (Strawn 2002). Therefore, we hypothesized that the amplitudes of the EPSP's would decrease under the influence of ethanol, thereby decreasing synaptic transmission. The results indicated that a 44 mM concentration affected a decrease in EPSP amplitude, while the 88 mM showed an initial decrease followed by an increase in amplitude.

MATERIALS AND METHODS

Dissection of the Crayfish

After being anesthetized in ice for at least twenty minutes, the crayfish's tail was cut from its body and the ventral portion of the crayfish exoskeleton was removed. The tail was then dissected to expose the extensor muscle fibers necessary for obtaining a resting membrane potential and stimulation of the nerve. The crayfish was then pinned to the Petri dish, and Ringer's solution (Table 1) was added to maintain the internal chemical composition of the crayfish. This was repeated on three additional crayfish.

Chemical	***Concentration (mM)***
NaCl	205.0
KCl	5.4
$CaCl_2$	135.0
$MgCl_2$	2.6
Tris (pH 7.4)	10.0

Table 1. Ringers solution chemical composition

Preparation of the Microelectrode

Several electrodes were made using a microelectrode puller, which heated and pulled apart 1.2 mm glass capillary tubes to create a fine tip. They were filled with 3 M KCL solution to maintain the electrical current when recording membrane potential, and then put in an electrode holder. The microelectrodes were used to measure membrane potentials.

Obtaining Baseline EPSP's

EPSP's or excitatory post-synaptic potentials are used to measure synaptic transmission between neurons. Baseline EPSP's were recorded in ringers solution to compare the results obtained with ethanol in the solution. To obtain EPSP's from the crayfish in the Ringer's solution, a Grass D9 stimulator was employed. The nervous fibers in the crayfish tail were stimulated with a two-pronged stimulator which was placed in a muscle segment of the crayfish tail. The EPSP's were then measured by MacLab and organized into graphs through *Scope v.3.6.3*. Further analysis of data was done with Microsoft Excel.

Application of Ethanol

The crayfish was submerged in Ethanol after taking control readings with it in Ringers solution. The concentrations were made with a pipetter by diluting 1 M ethanol in Ringer's solution. We tested both 44 mM and 88 mM concentrations in 50 mL solutions. Each concentration was tested on two different crayfish. EPSP's were then recorded over thirty minutes at random time intervals. Random time intervals were used because the nature of the setup did not allow for specific time intervals.

RESULTS

The amplitude of the EPSP's decreased as a result of the introduction of ethanol over time. Figure 1 shows the results of two day's of experimenting with 44 mM ethanol. EPSP's taken prior to time zero are baseline EPSP's that were measured before ethanol was added. There is a drop in the amplitude of the EPSP's after twenty minutes.

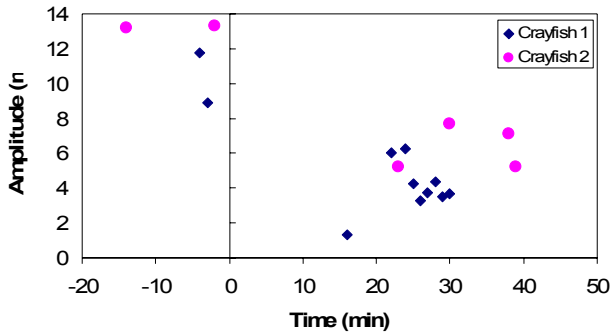

Figure 1. This figure displays the amplitude of the EPSP's taken before and after a 44 mM Ethanol concentration was added to two different crayfish over a thirty minute time period. The amplitudes decrease during this time period.

Figure 2 shows the averages for pre-ethanol EPSP's and the averages for EPSP's after ethanol has been in the solution for twenty minutes. This is the average of the EPSP's from twenty to thirty minutes in the solution. The figure shows a decrease in amplitude of EPSP's for the crayfish after ethanol has been added for twenty minutes. The average decrease for Crayfish 1 is 5.94 mV in amplitude and the average decrease for Crayfish 2 is 6.82 mV.

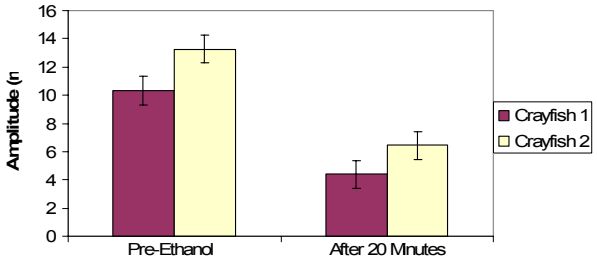

Figure 2. This figure shows the averages for the amplitudes of the EPSP's for the crayfish taken while submerged in 44 mM solution of ethanol. The amplitude of the EPSP's taken for baseline readings and the amplitude of the EPSP's taken after twenty minutes show a decrease. In the pre-ethanol state n=2 for both crayfish. After twenty minutes, n=8 for crayfish 1 and n=4 for crayfish 2.

Figure 3 represents experiments with 88 mM ethanol solution on two crayfish. Application of 88 mM ethanol produced mixed results. Some of the EPSP's were larger than baseline EPSP's. These EPSP's show an immediate affect on the amplitude, in that it gradually increases to a higher amplitude than previously recorded.

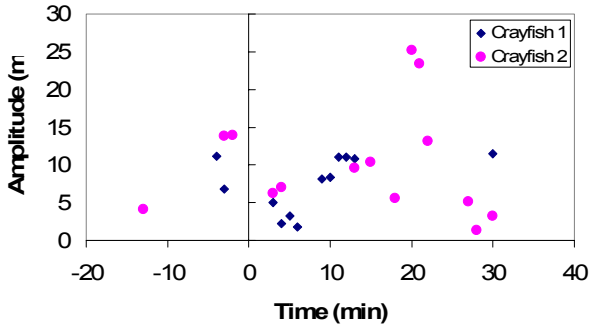

Figure 3. This figure displays the EPSP's taken before and after an 88mM Ethanol concentration was added to two different crayfish over a thirty minute time period. The amplitudes seem to decrease initially then increase.

Figure 4 shows the averages of the EPSP amplitudes taken at time intervals of ten minutes after the ethanol was added as compared to pre-ethanol EPSP's. The figure shows that an initial drop in the amplitude then led to an increase in amplitude that was higher than pre-ethanol levels. Crayfish 2 shows falling EPSP amplitudes after twenty minutes, while Crayfish 1 stays at relatively the same level as it was in the second ten minutes of ethanol treatment. The initial drop for Crayfish one in the first ten minutes averaged to be 4.2 mV and the initial drop for Crayfish 2 was 3.42 mV. Both crayfish's averages then exhibit an increase in EPSP amplitude. Crayfish 1's amplitude increased by 6.29 mV, while Crayfish 2 increased by 6.06 mV. Then the crayfish differ in results. Crayfish 1 stays roughly the same, while Crayfish 2 decreases 3.43 mV.

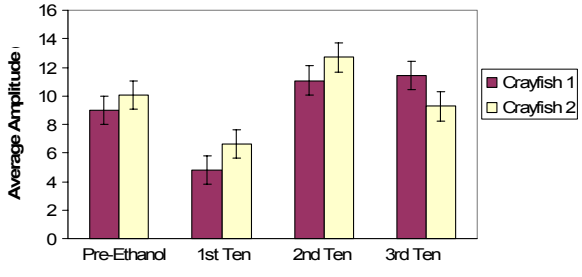

Figure 4. The figure shows the averages of the amplitudes of the crayfish taken at time intervals of ten minutes. The bars represent averages of EPSP's taken over these time intervals for thirty minutes. The EPSP's show an initial decrease followed by an increase in amplitude. In the pre-ethanol stage, n=2 for crayfish 1 and n=3 for crayfish 2. In the first ten minutes n=6 for crayfish 1 and n=2 for crayfish 2. In the second ten minutes n=3 for crayfish one and n=4 for crayfish 2. In the third ten minutes n=1 for crayfish 1 and n=5 for crayfish 2.

DISCUSSION

In our experiment, the amplitudes of EPSP's decreased, especially when the 44 mM ethanol solution was added. This shows that ethanol possibly has a debilitating effect on synaptic transmission at this concentration. The 88 mM ethanol solution seemed to cause an initial drop and then an increase in EPSP amplitude. This experimental concentration may have mimicked a chronic application of ethanol, which has been shown to increase the amplitude of EPSP's (Tsai 1998).

One of the possible reasons for the drop in EPSP amplitudes under the 44 mM concentration is that ethanol has been found to inhibit the NMDA-activated ion current (Weight 1989). It has also been found to cause a blockage in the NMDA receptor, which in turn restricts the glutamate-stimulated calcium influx (Tsai 1998). Since the NMDA receptor facilitates synaptic transmission, this could explain why the 44 mM ethanol concentration decreased the amplitude of EPSP's. There are some other possibilities that could be causing the decrease in EPSP amplitude after 44 mM ethanol is introduced. For example, it is possible that ethanol could retard Ca^{2+} release in the pre-synaptic membranes of the junctions, since blockage of the NMDA receptor reduces calcium influx at glutamatergic synapses. Another possibility is that receptors other than NMDA receptors in the post-synaptic membrane could be affected. Thus EPSP's would not be generated as effectively as they would under normal conditions.

The 88 mM concentration of ethanol caused an initial decrease and then increase in the amplitude of the EPSP's. Chronic conditions have been shown to cause an increase in EPSP amplitude. It is possible that the experiment simulated a crayfish held under chronic conditions. Synapses exposed to chronic alcohol treatments have been known to increase in EPSP amplitude (Tsai 1998).

More extensive tests of the same experiment could be preformed in order to gain enough data for statistical testing, which would help to determine whether the results were significant. Further experiments could be done to test different alcohols and different concentrations. These could both use the same basic method, while just changing the type or concentration of alcohol. Additional experiments could also test to see whether the ethanol specifically affects the NMDA receptor, calcium channels, or some other receptor.

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