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Long term depression decreased in crayfish chronically exposed to Fluoxetine.

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ABSTRACT

Research is limited on the effects of Fluoxetine on living organisms. Due to that fact, we studied the effects of Fluoxetine, a specific SSRI (Selective Serotonin Reuptake Inhibitors), on crayfish superficial extensor muscles. In this study, excitatory junction potential (EJP) amplitudes were measured using intracellular recording on two different groups of crayfish – one with acute exposure to fluoxetine and another with chronic exposure. We found that although acute fluoxetine exposure did not affect long-term depression (LTD), chronic exposure to the drug reduced LTD.

INTRODUCTION

Prozac belongs to a class of medications known as SSRI or serotonin reuptake inhibitors. Fluoxetine is the active ingredient in Prozac. Fluoxetine and other medications are prescribed for patients diagnosed with depression, Attention Deficit Hyperactivity Disorder, eating disorders, and multiple other disorders. Fluoxetine works by increasing the amount of serotonin in the brain which helps maintain mental balance (Robertson, Jones, Swartzendruber, Yang, & Wong, 1988).These drugs are widely prescribed due to the knowledge that they increase serotonin levels in the brain, and in the synaptic cleft.

Previous research has shown that serotonin increases neurotransmitter release in the superficial extensor muscles of the crayfish. The same study showed that fluoxetine actually decreased excitatory junction potential (EJP) amplitude (Dunnette, Shai, and Shin, 2000). Activity of neurotransmitters which 5-HT (serotonin) transporters normally increase, are inhibited by antidepressants such as fluoxetine (Zhou, FM, Liang T, Salas R, Zhang L, De Biasi M, Dani JA, 2005).

Studies by the Environmental Protection Agency (EPA) found that exposure to SSRIs affected a number of endocrine-mediated processes. They also proved that fluoxetine was toxic and that a concentration of 614 ppb of fluoxetine killed approximately 50% of exposed neonates. The experiments performed by the EPA, showed fluoxetine's potential to disturb and negatively affect the endocrine processes in aquatic organisms. It is apparent that more targeted studies must be done to measure the points at which fluoxetine and other SSRIs harm exposed organisms. It is apparent that the drug has many impacts on aquatic life. Our study was concerned with the effects of long term exposure on the functioning of the neuromuscular junction in a freshwater vertebrate (Black, Rogers, & Henry, 2006).

The primary reason crayfish were used in this experiment was because their nervous system is a simple version of the human nervous system. Crayfish were also cost-efficient and easy to dissect, making our experiment more straightforward and less time-consuming.

Long term depression (LTD) is due to the weakening of synapses after continuous electrical stimulation. The research that we gained from our experiment showed how Prozac affects the LTD of crayfish when applied for a short period of time (20min.) and a long period of time (1 & 2 weeks). This experiment allowed us to figure out how harmful Prozac could be to exposed organisms in their natural habitats. Through our research, we observed whether Prozac increased or decreased long-term depression induced by high-frequency stimulation.

We hypothesized that Prozac would decrease synaptic transmission with an increase in long term depression (LTD) triggered by high-frequency stimulation. To the contrary, we found that acute exposure to fluoxetine did not affect LTD at all, but chronic exposure to the drug inhibited the effects of LTD.

MATERIALS AND METHODS

Solutions.

The crayfish in the short-term exposure experiment was bathed in a standard ringers solution (Table 1), with the addition 0.346g of fluoxetine per 100ml of normal saline ringers. Our long-term exposure group lived in a solution consisting of 100ng of fluoxetine per liter of water. All crayfish were placed in the same concentration of ringer solution during experimentation.

Concentration (mM)	Chemical
205	NaCl
5.4	KCl
13.5	CaCl2
2.6	MgCl2
10.0	Tris Buffer (pH 7.4)

Table 1. Composition of normal saline Ringers

Electrophysiology.

Our data on long-term exposure was collected using intracellular recording using pulled-glass microelectrodes filled with 3M-KCl solution. Also, we used polished-glass micropipettes to stimulate motor axons to fire action potentials. To study long-term depression in our crayfish, we stimulated nerves at a high frequency (5.5Hz, increased from .55Hz) for one minute at a time, which induced synaptic depression in our crayfish as detected by reduced EJP amplitudes. All EJP amplitude recordings were monitored and recorded by Scope v3.6, an oscilloscope monitoring program by ADInstruments.

Organisms.

For our short-term experiments, we used six acutely exposed crayfish, from which we managed to induce depression twice. We started our long-term experiments with seven crayfish, five of which we had time to test. Out of those five, only one produced strong EPSPs and reliable examples of long term depression.

RESULTS

In order to understand the effects of fluoxetine on long-term depression in the neuromuscular junction of crayfish, we examined the reactions of crayfish after short-term and chronic exposure to fluoxetine. We spent the first day of our project preparing the fluoxetine bath where our long-term group lived for two weeks. While the long-term crayfish lived in their bath, our next few class periods were devoted to the acutely exposed group of crayfish. Once the long-term group was sufficiently exposed, we spent the rest of our time in lab alternating between studying the long-term depression in both our acute and chronically exposed crayfish.

Comparing our acutely and chronically exposed crayfish, we found no difference between the average EJP baseline amplitudes ($F= 0.30973$, $p> 0.05$). Although the EJP amplitudes were not fundamentally different, the reaction of our chronically exposed crayfish was far different than that of the acutely exposed crayfish. However, we observed that compared to the baseline EJP, the long-term depression decreased amplitude in our acutely exposed group of crayfish (n=4) by 50%, and remained the same in chronically exposed crayfish (n=1).

Furthermore, Figures 1 and 2 show the differences in voltage in our crayfish before and after prolonged, high-frequency stimulation of the nerves which caused LTD. We found that after we induced long-term depression in acutely exposed crayfish, the electrical stimulation caused the EJP amplitude to decrease, which is expected of long-term depression. However, the chronically exposed crayfish did not react to long-term depression. Instead of decreasing, the EJP amplitude was minutely increased. Therefore, long-term depression was minimized with chronic exposure to fluoxetine in this sample.

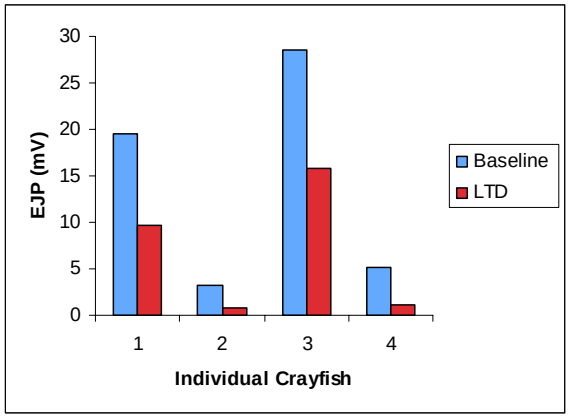

Figure 1. A comparison between the EJP before (at baseline) and after LTD was induced in crayfish (n=4) acutely exposed to fluoxetine. The average ratio of baseline EJP to post-LTD EJP was about 2:1.

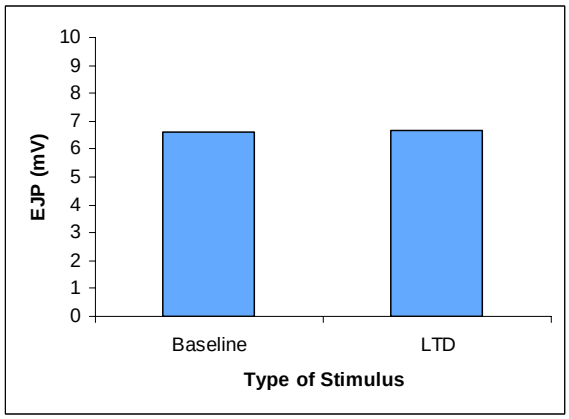

Figure 2. A comparison between the EJP before (at baseline) and after LTD was induced in a crayfish (n=1) chronically exposed to fluoxetine. The ratio of baseline EJP compared to post-LTD EJP was about .99:1.

DISCUSSION

As stated above, Prozac is used to treat patients who suffer from depression, ADHD, eating disorders as well as many others. The active ingredient,

Fluoxetine, is classified as SSRIs (selective serotonin reuptake inhibitors). This drug works by increasing the amount of serotonin in the brain which helps maintain mental balance (Benfield, Heel, and Lewis, 1986). According to a study that observed the effects of increased levels of serotonin due to exposure to SSRIs on two model aquatic organisms, they—Black, Rogers, & Henry of the EPA (2006)—found that because of the exposure to SSRIs, a number of endocrine-mediated processes were affected.

We simulated the natural habitat of the crayfish by putting them in a pool of water with a 100ng/L concentration of fluoxetine. After the respective time periods, we observed the effects that the amounts of fluoxetine had on the various crayfish. The results of our research showed how Prozac affected the crayfish when it was applied for a short period of time (20min.) and a long period of time (1 & 2weeks). Our experiments on crayfish helped us to understand the negative effect of Prozac (fluoxetine) on organisms, including humans, if larger doses leaked into main water supplies, such as the rivers and lakes in the United States. We feel this research is important because trace amounts have already been found near large cities (Benotti & Brownawell, 2006).

Some difficulties and limitations we found as our experiment progressed included the variability of the resting potentials since different crayfish were used. Another problem which we saw in our preliminary experiments was the technical difficulty of creating an action potential and long-term depression in our crayfish. Other difficulties we faced included the unexpected unhealthiness of our long-term crayfish. Whether due to sickness, old age, or exposure to fluoxetine, the muscles of the crayfish became scarred, making it difficult to find a reliable resting potential. As always, we were limited by time and the number of crayfish on which our experiments were performed.

Through our research, we observed that when long-term depression was induced by increasing frequency for repeated stimulation, the voltage noticeably decreased in our acutely exposed group of crayfish (n=5) when stimulated. However, in the only chronically exposed crayfish which produced a reliable EPSP, long-term depression caused little change in the voltage of the cell; other crayfish with long-term fluoxetine exposure were difficult to stimulate. Because we experimented on crayfish, this research helped us determine how much of a negative effect the concentrations of Prozac (fluoxetine) had on an organism if it leaked into a main water supply that was frequently used by the organisms, such as the rivers and lakes in the United States.

Future research could include determining the actual cause of our difficulties by studying crayfish chronically exposed at different concentrations of fluoxetine, to see if the scarring would reoccur. Also, a study with more varied time spans of chronic exposure to fluoxetine as well as a longer time to work with addicted crayfish would prove interesting.

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REFERENCES

Benfield, P., R.C. Heel, S.P. Lewis. 1986. Fluoxetine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in depressive illness. *Drugs.* 32:481-508. (abstract)

Black, M.C., E.D. Rogers, & T.B. Henry. 2006. Endocrine effects of selective serotonin reuptake inhibitors (SSRIs) on aquatic organisms. 5 Nov 2006. http://es.epa.gov/ncer/publications/meetings/8-23-2005/abstract/black1.html. (abstract)

Brooks, B.W., C.M. Foran, S.M. Richards, J. Weston, P.K. Turner, J.K Stanley, M. Slattery, T.W. La Point. 2003. Aquatic ecotoxicology of fluoxetine. *Toxicol Lett.*142: 169-183.

Dunnette, B., A. Shai, J. Shin. 2000. The effects of serotonin and fluoxetine on neurotransmission in crayfish superficial extensor muscle. *Pioneering Neuroscience* 1: 57-63.

Benotti, M.J., B.J. Brownawell. 2006. Occurrence and fate of high volume pharmaceuticals in wastewater impacted environments. 5 Nov 2006. http://es.epa.gov/ncer/publications/meetings/8-23-2005/Benotti_1055_d1.pdf. (abstract)

Robertson, D.W., N.D. Jones, J.K. Swartzendruber, K.S. Yang, D.T. Wong. 1988. Molecular structure of fluoxetine: a highly selective serotonin-uptake inhibitor. *J. Med. Chem.* 31: 185-189.

Zhou, FM, Liang T, Salas R, Zhang L, De Biasi M, Dani JA. (2005) Corelease of dopamine and serotonin from striatal dopamine terminals. *Neuron.* 4: 65-74.