Pioneering Neuroscience, 2014, **14**, 15-18.

Increased concentrations of fluoxetine do not affect EPSP amplitude in the crayfish neuromuscular junction via serotonin reuptake inhibition.

HANNAH JANSSEN, DANA HEILBRONNER, and SHANE COMISKEY
Department of Biology, Grinnell College, Grinnell, Iowa

ABSTRACT

This experiment tested the effects of fluoxetine (Prozac) after the addition of serotonin to the saline solution on the EPSP amplitudes of crayfish muscle cells. We tested this using a sharp glass microelectrode as a recording electrode and a filed glass microelectrode as a suction electrode used to draw up nerves to be stimulated. We compared the recorded EPSP amplitudes from crayfish muscle cells exposed fluoxetine and serotonin to the initial control, with no drug, and the experimental control, with just serotonin in the saline solution. We found that serotonin alone amplifies the EPSP amplitude, however the addition of fluoxetine, had no significant effects.

INTRODUCTION

Many studies have tested the effects of various drugs on synaptic activity. Drugs can have varying effects such as either decreasing or increasing synaptic transmission in specifically chemical synapses. Chemical synapses function by receiving a stimulus that propagates down the axon toward the nerve terminal. Once the stimulus reaches the nerve terminal it triggers an influx of Calcium ions, which results in the release of synaptic vesicles containing neurotransmitters into the synaptic cleft (Llinás 1981). In the cleft, the neurotransmitters bind to receptors on the postsynaptic cell. This binding can result in an excitatory postsynaptic potential, EPSP allowing the signal received by the presynaptic cell to travel to the postsynaptic via neurotransmitter release, and cause a response. Synaptic activity can be increased in many ways, for example, by increasing the number of agonists, which will activate more receptors resulting in a greater postsynaptic potential. Increasing the number of synaptic vesicles released also increases synaptic activity because there are more available neurotransmitters for binding. Limiting the number of antagonists that can bind to receptors, but produce no postsynaptic potential, would cause an increase. Lastly, the synaptic activity can be increased by blocking the reuptake of neurotransmitters, which would cause the synapse to remain active for a longer period of time.

Since the majority of antidepressants used today function as reuptake inhibitors, this experiment will work to determine if the antidepressant Prozac functions by inhibiting the reuptake of the neurotransmitter serotonin (. This experiment was derived from a Pioneering Neuroscience article exploring how Fluoxetine (Prozac) acts as an antagonist at 5-HT_2C receptors in the crayfish neuromuscular junction (Mahaffey et al. 2010). Previous research has indicated that Prozac works by inhibiting the reuptake of serotonin (Fitzgerald et al. 2013), but Mahaffey et al's research explores and confirms Prozac's ability to act as an antagonist on 5-HT_2C receptors, which contradicts its ability to function as a reuptake inhibitor (Mahaffey 2010). Our research aims to confirm previous research that serotonin functions via reuptake inhibition instead of as an antagonist at 5-HT_2C receptors.

Serotonin is a neurotransmitter not released by crayfish, but the crayfish do have serotonin receptors (Mahaffey 2010). Since crayfish have these receptors in addition to the glutamate receptors for the neurotransmitter released naturally by crayfish, simply adding serotonin to the saline solution will have an impact of the EPSP amplitude. This happens because not only will the glutamate receptors be active, but the serotonin receptors will also be active.

We intend to compare the amplitude of the EPSP's obtained with two different concentrations of Fluoxetine exposed to serotonin. This will add information to the antidepressant field and aid in the prescription of drugs for specific types of depression such as

based on whether or not Prozac in fact does work as a serotonin reuptake inhibitor (Papakostas et al. 2012). Since Prozac functions by inhibiting the reuptake of serotonin, adding Prozac to the saline solution will cause the EPSP amplitude to increase as the concentration of the drug increases because the synapse will be active for a longer period of time. However, we found no significant correlation between the increase in fluoxetine concentration and the increase in EPSP amplitude.

MATERIALS AND METHODS

Preparing Solutions and Making Microelectrodes

We diluted a 10 mM solution of both fluoxetine and serotonin into a saline solution. We mixed 250 µL of fluoxetine into 250 mL of saline to make a solution with a fluoxetine concentration of 10 µM. The same was done to prepare the serotonin solution. We also made a fluoxetine solution of 20 µM by mixing 500 µL of the 10 mM stock solution into 250 mL of crayfish saline. The saline was composed of 5.4 mM KCl, 200.7 mM NaCl, 12.3 mM $MgCl_26H_2O$, 5 mM Sodium Hepes Buffer, and 6.5 mM $CaCl_22H_2O$. When we were testing the crayfish exposed to both solutions, we mixed together 75 mL of each solution. We made two glass microelectrodes using a PUL World Precision Instruments electrode puller. We filled one with a 3 M KCl solution and the other was filled with normal saline and filed with sandpaper so it could draw a nerve into it. After all the air bubbles were removed from the electrode filled with 3 M KCl, we positioned it so that it would be in an optimal position for penetrating a crayfish muscle cell and the suction electrode was positioned on the other side of the dissection dish.

Preparation

We cooled the crayfish in an ice bath prior to dissection to allow it to become numb and unable to move. Once the crayfish reached this state, we cut its tail and discarded the remainder of the organism. We cut the tail on both sides along the ventral part of the abdomen and then we peeled the legs off of the crayfish. We were left with the large mass of muscle in the dorsal part of the abdomen; we used our thumb to push the remainder of muscle out of the crayfish, leaving the thin layer of abdominal extensor muscles attached directly to the shell. We then pinned the crayfish onto a dish for testing and observation, and poured the prepared saline solutions over the tissue.

Taking Measurements

We poured the solution, either with serotonin, with serotonin and 10µM fluoxetine, with serotonin and 20 µM fluoxetine, or with just the saline solution over the crayfish. We continually checked the resistance to ensure that our microelectrode was useable; the resistance was at least 13.33 MΩ. After that, we located a nerve along the edge of the crayfish tail and positioned the suction electrode; we pulled a nerve into the electrode that could be stimulated by the stimulator. After we found a nerve and drew it into the suction electrode, we began inserting the microelectrode (filled with 3 M KCl) into crayfish muscle cells below the nerve we were stimulating. We recorded the data using A-D Instruments and LabChart.

RESULTS

We tested the effects of the presence of fluoxetine after the addition of serotonin to the saline solution on EPSP amplitudes of crayfish muscle cells, and the impact of increased concentrations of the drug. The effect of fluoxetine at 10 µM and 20 µM concentrations was measured with sharp glass microelectrodes and filed glass suction electrodes. We sampled various nerves along the crayfish tail and sampled multiple different cells for each nerve.

Shown in figure 1, we found that serotonin significantly increased the EPSP amplitude, however the addition of fluoxetine had no significant impact. Since crayfish have serotonin receptors but do not release serotonin, the addition of serotonin should amplify the EPSP amplitude because more receptors will be active. However, although the addition of fluoxetine should positively affect the EPSP amplitude as a serotonin reuptake inhibitor, the data shows no significant correlation between the addition of fluoxetine and an increase in EPSP amplitude compared to just the addition of serotonin.

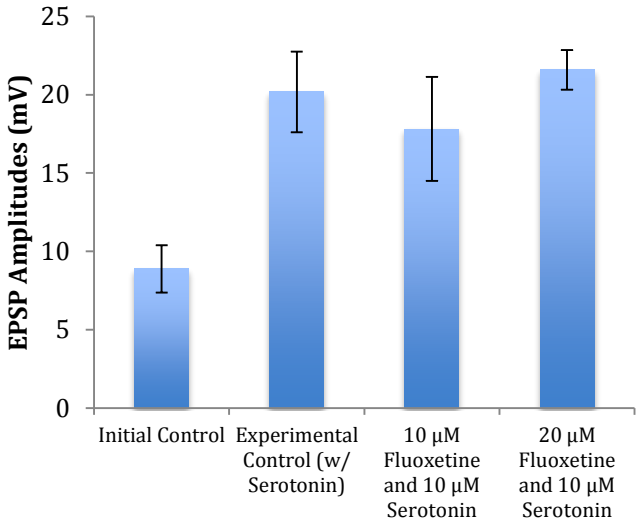

Figure 1: The effect of fluoxetine in 10 µM concentrations and 20 µM concentrations compared to the control (with serotonin) in crayfish muscle cells. P = 0.00189 between the initial control (without any drug or serotonin) and the experimental control (with just serotonin). P= 0.58106 between the experimental control and 10 µM fluoxetine. P = 0.62967 between the experimental control and 20 µM fluoxetine. The error bars represent the standard error. N = 6 (no drug or serotonin), 10 (serotonin), 13 (10 µM fluoxetine and serotonin), 19 (20 µM fluoxetine and serotonin).

DISCUSSION

The outcomes of this experiment do not support our hypothesis that increasing the concentration of fluoxetine would result in an increase in EPSP amplitudes. We had expected that result since the serotonin would be active for a longer period of time because of Prozac's ability to inhibit serotonin reuptake.

These results contradict Fitzgerald et al's (2013) research finding that Prozac functions via serotonin reuptake inhibition, since we found no significant correlation between the addition and increase in concentration of fluoxetine on the EPSP amplitude. However, although contradicting Fitzgerald et al's (2013) research, the lack of significant results under these conditions may indicate that Mahaffey's (2010) research finding that fluoxetine does not work as a serotonin reuptake inhibitor, but as an antagonist at 5-HT_2C receptors may be correct. This cannot be confirmed since the EPSP amplitudes did not decrease, as an antagonist would cause them to. Further research and experimentation such as not adding serotonin to the saline allowing Prozac to function as an antagonist would need to be conducted to confirm Mahaffey's 2010 findings. An explanation of our results could be that the concentrations of fluoxetine we used may not have been great enough to have an effect on the EPSP amplitude. Further research with increased concentrations could be conducted to confirm our results.

ACKNOWLEDGEMENTS

We would like to thank Professor Clark Lindgren, our professor for helping us develop our research question. We would also like to thank Sue Kolbe, our lab assistant and Jason Parks, our lab technician for assisting with the set-up and crayfish dissection. Lastly we would like to thank Talora Martin and Alexandra Byrne, our class mentors for advising us on techniques and helping us develop our final conclusions.

REFERENCES

Béïque, J., E. Chapin-Penick, L. Mladenovic, & R. Andrade (2004). Serotonergic facilitation of synaptic activity in the developing rat prefrontal cortex. *The Journal of Physiology, **556:*** 739-754.

Fitzgerald, K. T., & A.C. Bronstein (2013). Selective serotonin reuptake inhibitor exposure. *Topics in Companion Animal Medicine, **28*(1):** 13-17.

Llinás R, IZ, Steinberg, K Walton (1981). Relationship between presynaptic calcium current and postsynaptic potential in squid giant synapse. *Biophysical Journal*1981; **33(3):** 323-351.

Mahaffey, P.J., Vu, Mai Hai, Ritter, Eric (2010). Fluoxetine Acts as an antagonist at 5-HT_2C Receptors in the Crayfish Neuromuscular Junction. *Pioneering Neuroscience,* **11:** 27-30.

Paiezanis, Eleni, T. Renior, V. Lelievre, F. Saurini, M. Melfort 2010. Behavioural and Neuroplastic Effects of the New-Generation Antidepressent Agomelatine Compared to Fluoxetine on Glucocorticoid Receptor-Impaired Mice. *International Journal of Neuropsychopharmacology,* **13:** 759-774.

Papakostas, G. I., H. Fan, & E. Tedeschini (2012). Severe and anxious depression: Combining definitions of clinical sub-types to identify patients differentially responsive to selective serotonin reuptake inhibitors. *European Neuropsychopharmacology : The Journal of the European College of Neuropsychopharmacology, 22*(5): **347-355.**

Pitchot, W., M. Polis, S. Belachew, & M. Ansseau (2008). Depression and neuroplasticity]. *Revue Médicale De Liège, **63*(5-6):** 372-377.

Spinks, D., & G. Spinks (2002). Serotonin reuptake inhibition: An update on current research strategies. *Current Medicinal Chemistry, **9*(8):** 799-810.

Wong, D. T., F.P. Bymaster, & E.A. Engleman (1995). Prozac (fluoxetine, lilly 110140), the first selective serotonin uptake inhibitor and an antidepressant drug: Twenty years since its first publication. *Life Sciences, **57*(5):** 411-441.