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The addition of Methionine Sufoximine, an inhibitor of the Glutamate-Glutamine cycle, decreases excitatory post-synaptic potentials in Orconectes sanbarnii muscle cells.

HEATHER KUBIECK, ANNE ROSS and PUNITA SAHU
Department of Biology, Grinnell College, Grinnell, Iowa

ABSTRACT

The objective of our study was to determine if inhibiting the glutamate-glutamine cycle would increase the amplitude of synaptic potentials in crayfish neuromuscular junctions (*Orconectes sanbarnii*). We based our experiment on previous studies, which showed that the inhibitory chemical *methionine sulfoximine* (MSO) increases the amplitude of synaptic potentials in the neuromuscular junction of crayfish (Bidmon 2008). To replicate this experiment as closely as possible, we measured excitatory post-synaptic potential's (EPSP's) in crayfish muscle cells with a glass microelectrode. We used a two-sample t test and determined that there was a statistically significant decrease between the control and the treated crayfish. This evidence contradicted the study that we based our experiment on, but its results closely resemble another scientific study. In that experiment the glutamate-glutamine cycle was inhibited in rats, and the amplitude of IPSCs significantly decreased following the rats being treated with MSO in the hippocampal area of their brains (Shulman 2006).

INTRODUCTION

A glial cell is a type of cell found in the nervous system that is involved in supporting and protecting neurons. More importantly the networks of glial cells, known as astrocytes, are involved in the modulation of the glutamate-glutamine cycle. During this cycle, enzymes found in astrocytes turn glutamate into glutamine. Glutamine is transported into the cell's extra cellular space and then into the pre-synaptic neuron. After this, phosphate-activated glutaminase produces glutamate, which is then carried into the pre-synaptic vesicle by transporters.

Reuptake, the process by which released neurotransmitters are absorbed to assist in the re-use of glutamate, is a process that is key to the future function of synapses. In the glutamate-glutamine cycle, glutamate released from the neuron is cleared through the synaptic cleft and then participates in the reuptake process to the pre-synaptic cell (Shen 1999).

The role that the glutamate-glutamine cycle plays in influencing the function of synapses was the primary focus of our study. To determine this role we inhibited the shuttle using the chemical *methionine sulfoximine* (MSO). We then measured *excitatory post-synaptic potential* (EPSP) amplitudes in extensor muscle cells of a crayfish tail in both regular saline and in the presence of the chemical MSO. We specifically studied crayfish because they have been found to be a good model organism. In addition the muscle cells in their tails have similar processes to that of the human brain. This is because crayfish have a more centralized nervous system, and their nerves are not distributed throughout body in the *ganglia* like they are in humans (Rdyqvist 2007).

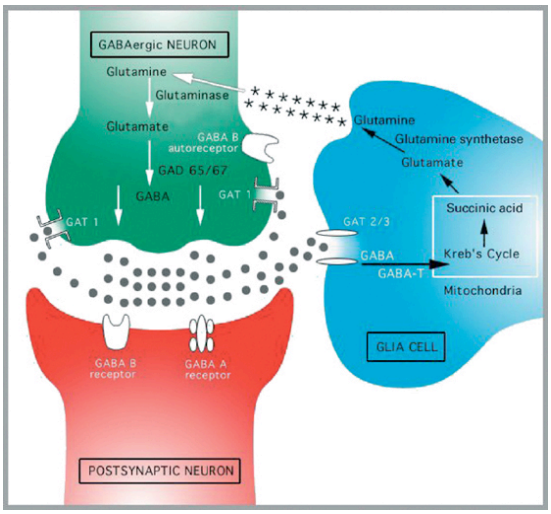

Figure 1. Illustration of the glutamate-glutamine cycle.
Brambilla, P., Perez, J., Barale, F., & Soares, J. (2003). GABAergic dysfunction in mood disorders. *Nature*, *8*, 715.

Furthermore, we initiated this experiment because we were interested in learning more about the potentially dangerous side effects that a shift from

equilibrium of the glutamate-glutamine cycle can have on humans. We wished to know more about these side effects after a study of patients with epilepsy showed that they had increased levels of glutamate and glutamine in their thalamus. This caused their brains to be "overstimulated" (Bidmon 2008).

For our study, we used the chemical MSO to inhibit the glutamate-glutamine cycle. We predicted that by doing so the synapses would be unregulated and there would be a difference in EPSP amplitudes between the cells treated with MSO and those that were not.

MATERIALS AND METHODS

Electrode Preparation

The glass electrodes used in this experiment were made from Borosilicate Glass Capillaries, item 1B120F-4 made by KWIX Fil™ Precision Instruments (Sarasota, Florida). They were split and sharpened using a pipette puller (WPI) on settings level two and heat level 7.5 with the cover closed. They were filled with 3 M KCl with a ¾ ounce B-D syringe. Their resistance was between 5-20 mega ohms. Before placing the electrode in the electrode holder, the tip was dipped in regular ringer solution to remove traces of KCl, which prevents altering the KCl concentration in the standard solution.

Dissection

The specimen of crayfish used in this experiment was *Orconectes sanbarnii*, provided by the Carolina Biological Supply Company. They were covered in ice for at least 15 minutes before we cut at the cephalothorax, which represents the major body we were interested in preserving (the tail). We cut laterally along both telsons, the last parts of the abdomen segments. They were recognizable because they were characterized by disc-shaped rigid bumps running laterally parallel on both sides of the tail's shell. We kept the remainder of the abdomen center, and removed the first layer of muscle and fat gently using a thumb to preserve the nerves. The tail was then placed with interior turned up into a small round Petri dish with diameter 2 inches.

Making Measurements

We placed two pins at opposite ends of the tails and filled the Petri dish with 10 mL of regular ringer solution, which was replaced every fifteen minutes for the control group. Then using suction electrode we acquired a nerve. Six cells were then located in the same segment and pierced with the microelectrode in order to record an EPSP. We repeated this process after applying the treatment with 40 mM of MSO after removing excess saline.

Measurements were taken using the same nerve as the untreated crayfish. We attempted to relocate and the cells used in the untreated crayfish in order to control any variation that cell location could have on our results.

To recheck the EPSP for each cell the tail was rinsed off with distilled water and regular saline was added again. The same steps of measurements were repeated in order to determine if the crayfish tail returned control conditions or if the MSO had induced a change on the facilitation of EPSP's.

Standard Crayfish Solution

KCL 5.4 mM
NaCl 19.6 mM
MgCl 2.6 mM
CaCl 13.5 mM
Sodium Hepes Buffer 10 mM

Activating a Synapse

To stimulate the crayfish, we used a GRASS SD9 Stimulator (Grass Instrument Co.), keeping the settings constant for each trial; frequency at 0.4 pps, delay at 2 ms, and duration at 0.4 ms, with only the volts varying to obtain an EPSP. The stimulation was initiated and caused the nerve in the suction electrode to react accordingly.

Data Collection

Two reference electrodes were placed in the ringer solution of the dissection dish. The reference wire provides a current that pulls the electrical flow past the nerve. The ground electrode gives a reference point for where the ringer solution is, and thus creates a baseline for measuring all data. We used the program Scope 4 to measure and analyze the EPSP's.

RESULTS

The main objective of our experiment was to discover if the chemical *methionine sulfoximine* (MSO) causes the amplitude of EPSP's in a crayfish's neuromuscular junction to significantly change. We predicted that the chemical MSO would cause an increase in EPSP amplitudes. This idea was tested through measuring synaptic potentials in four crayfish by using sharp microelectrodes and intracellular recording.

We based our experimental procedures largely on a study conducted by the University of California, San Francisco. In the San Francisco study, MSO caused a significant increase in EPSP amplitudes for crayfish. It

was predicted that MSO causes deregulation of synapses and consequently results in larger EPSP's (Rdyqvist 2007). However, the data in our experiment illustrates just the opposite-- MSO causes a statistically significant decrease in EPSP amplitude ($t=3.29$, $p<0.05$).

We performed a Students T-Test in order to determine if there was statistically significant difference between the values collected from the control solution data and those collected from the experimental data. We found that when tested using an alpha level of 5%, there does in fact exist a difference between the data that suggests more than simply chance as the corollary variable.

The following diagram shows that the EPSP between the two trials is significantly reduced, from control data to experimental we can see a reduction of ½. This gives that there was a statistically large decrease with the addition of MSO.

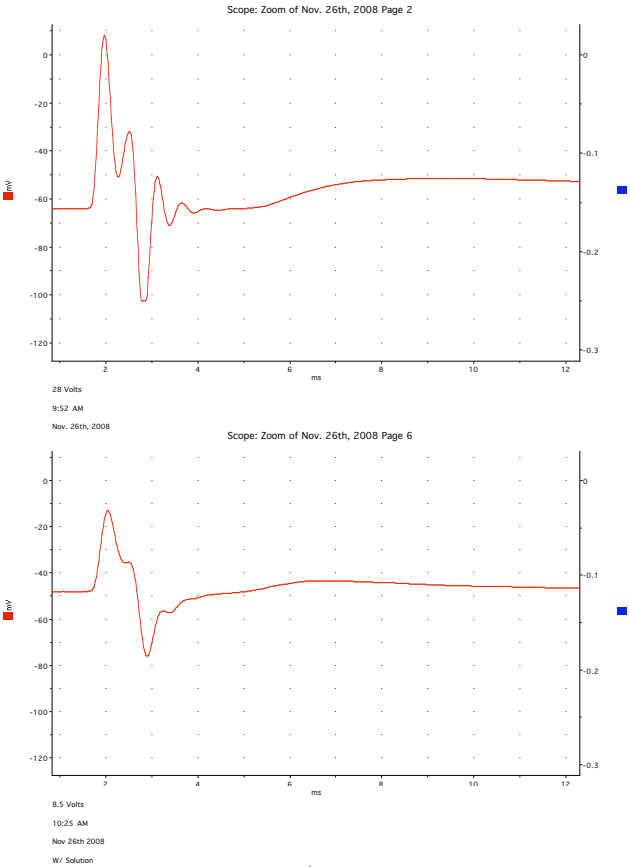

Figure 2. Data taken November 20th from the same crayfish, one is control data, the other experimental. **a)** The top diagram shows the EPSP of the crayfish in the regular saline. Max-min for the EPSP of the top is 10.794. **b)** The diagram graph on the bottom represents the EPSP with the addition of MSO. The max-min is 5.612.

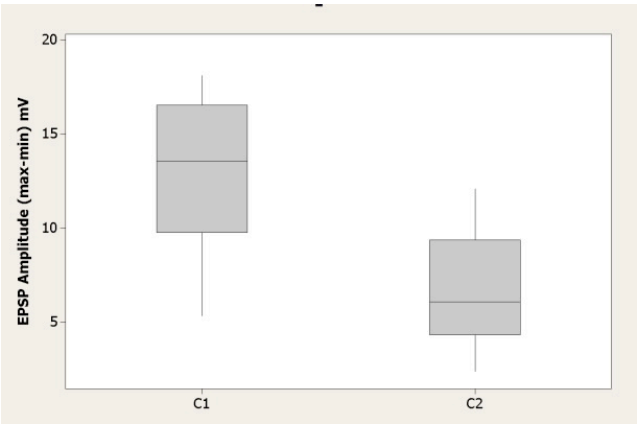

Figure 3. (C1) is the control data. (C2) is the experimental data. Because both distributions were approximately normal, and 2 sample pooled t-test was used. 14 measurements were taken for the control, while 10 measurements were taken for experimental group over the course of five weeks. There was a statistically significant difference in EPSP voltage between the crayfish in saline versus the crayfish treated with MSO ($t=3.29$, $p<0.05$). A two-sample t-test was used to obtain the results. We removed one outlier point from the graph (from C1) in order to more clearly illustrate the distinct difference between C1 and C2 data. The midline of each box plot represents the median.

DISCUSSION

In this experiment, the data we collected contradicted the University of California, San Francisco study, in which *methionine sulfoximine* (MSO) causes a significant increase in excitatory post-synaptic potential (EPSP) amplitude. Instead, we discovered that the MSO caused a significant decrease in EPSP amplitude in our experiment (Rdyqvist 2007). Our results closely mirror the results of a study conducted by the University of Pennsylvania School of Medicine. That study measured the effects of inhibition of the glutamate-glutamine cycle on IPSC amplitudes in rats.

Our results are inconclusive for a number of key reasons. First, we did not control for time because the cell location process proved inconsistent in terms of success and therefore time became an uncontrollable variable. In addition, the expense of our chemical limited the number of trials we could afford to run. We wanted our results to be comparative from the same crayfish, we eliminated all control data that was obtained without accompanying results in the MSO. Gathering a large quantity of data for each sample group ($n>30$) would have made our results more generalizable to the actual population, however this was not a possibility given our time constraints.

Our data illustrates that blocking the glutamate-glutamine cycle likely inhibits the recycling of neurotransmitters thus causing a decrease in EPSP amplitude. To determine how profound of an effect

inhibition of this cycle has on EPSP amplitude, varying levels of frequency for both the treated and untreated crayfish should be tested. We believe that varying the frequency as described would result in a more substantial difference in the EPSP amplitudes between the two test groups. The results of this initial study are only preliminary and need to be replicated in order to strengthen the validity of its results.

The replication of this experiment would not only allow for more decisive data, but it would create a more controlled atmosphere in which to conduct further experiments.

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