Pioneering Neuroscience, 2011, **12,** 29-35.

The Contradictory Effects of *N*-acetylaspartylglutamate and its Products of Hydrolysis on NMDARs in the Crayfish Neuromuscular Junction.

ZEV BRAUN, JAMES KENT, and TAGUE ZACHARY
Department of Biology, Grinnell College, Grinnell, Iowa

ABSTRACT

NAAG is the most abundant peptide neurotransmitter in the mammalian nervous system, but relatively little is known about its function in glutamatergic synapses. Recent research has suggested NAAG to be a neuromodulator of glutamate release, but how it modulates glutamate release is unclear. The objective of this research was to see if NAAG has an effect on NMDARs and to determine if the hydrolyzed products of NAAG aid in modulation of NMDAR function. We did this using a crayfish synapse as a model system, specifically the extensor muscles in the tail, and applied NAAG to the synapse as well as LY341495 (a potent mGluR group II antagonist) to isolate the effect of NAAG on the other probable receptor, NMDAR. We then added ZJ43 along with NAAG onto the synapse to view the effects of pure NAAG on the synapse. Our results suggest that NAAG and its products of hydrolysis have an inhibitory effect on EPSP amplitude, and pure NAAG enhances EPSP amplitude.

INTRODUCTION

Schizophrenia, a mental disorder characterized by hallucinations, paranoia, and other delusions, affects around 1% of the world's population. Previous studies have identified dopamine as the primary neurotransmitter involved in schizophrenic symptoms, but this has yielded little success in creating a psychiatric treatment (Moghaddam 2004). Schizophrenia has been found to correlate with dysfunction of the glutamate system throughout the brain (Javitt 2010). This spurred the development of the 'glutamate theory', which states that insufficient glutamate release exacerbates symptoms of schizophrenia. After the formulation of the 'glutamate theory', scientists began searching for neurotransmitters and neuromodulators that increase the amount of glutamate released into synapses in an attempt to discover a more effective pharmacological treatment of schizophrenia.

N-acetylaspartylglutamate (NAAG), the most abundant peptide neurotransmitter in the mammalian central nervous system (Neale et al. 2005), has effects at glutamatergic synapses. Research has demonstrated that NAAG acts as a neuromodulator by reducing glutamate release (Bergeron et al. 2007; Gafurov et al. 2001). NAAG has been found to activate metabotropic glutamate receptors (mGluR group II), but it is unclear where this agonistic effect takes place. Huang et al. (1999) suggests that activation of postsynaptic mGluR group II yields Long Term Depression. However, Bergeron et al. (2007) describes this interaction presynaptically, stating that through inhibiting adenylyl cyclase and the following second messenger reactions, NAAG hinders glutamate release. Mapping out where NAAG interacts with mGluR group II will help us understand the overall purpose of NAAG in the system.

NAAG has also been shown to affect *N*-Methyl-D-aspartic acid receptors (NMDARs), located on the postsynaptic cell. Some evidence supports the hypothesis that NAAG acts as an antagonist at NMDARs (Gafurov, et al. 2001; Huang et al. 1999; Bergeron et al. 2007). However, at high concentrations of NAAG (666µM), Gafurov et al. (2001) and Huang et al. (1999) observed a weak agonistic interaction with NMDARs. These different actions of NAAG suggest it has a role as a modulator of glutamatergic synaptic transmittance. Urazaev et al. (2001) sought to expose the effect NAAG itself has on glutamatergic synapses as compared to its products of hydrolysis: N-acetylaspartate (NAA) + Glutamate. Given these conflicting findings of NAAG's effects, it is possible that hydrolyzed NAAG (NAA + Glutamate) is responsible for agonistic effects at high concentrations.

Our research sought to alleviate the inconsistencies of previous studies and add to the discussion of NAAG's role on NMDAR. We conducted two sets of experiments, employing the crayfish neuromuscular junction (NMJ) as our model glutamatergic synapse. The preliminary experiments sought to determine the presynaptic and/or postsynaptic effects of NAAG at the NMJ. Using a paired pulse ratio, we compared two closely

stimulated excitatory postsynaptic potentials (EPSPs) to see if there were residual effects by presynaptic Ca^{2+} influx. We hypothesized in the preliminary experiments that exogenous NAAG would have exclusive postsynaptic effects (e.g. binding with the NMDAR).

Our main experiments sought to isolate the effect of NAAG on NMDAR. We manipulated the NMJ by adding ZJ43 to the synapse, an inhibitor of the enzyme Glutamate carboxypeptidase II (GCP II). Because GCP II naturally breaks down NAAG into NAA and Glutamate, by adding ZJ43 we could observe the effect of pure NAAG. In order to nullify any presynaptic effects that NAAG would have on the level of glutamate in the system, we added LY341495, an inhibitor of mGluR3. In our main experiments we hypothesized that hydrolyzed products of NAAG (NAA + Glutamate) bind to NMDAR with a higher affinity than NAAG, thus eliciting an EPSP, whereas NAAG has an antagonistic effect.

We found that NAAG acts postsynaptically, supporting our preliminary hypothesis; NAAG with its products increase EPSP and pure NAAG increases EPSP, showing contradictory results for our main experiments.

MATERIALS AND METHODS

Microelectrode Preparation

Creation of the measurement electrode required a hollow glass tube (borosilicate glass capillary) of 1.2mm in diameter to be pulled apart by a PUL-1 electrode puller (WPI, Sarasota FL). We filled the microelectrode with 3M KCl, making sure no air bubbles were inside the capillary. Similarly, a microelectrode holder was filled in the same manner. The microelectrode was then attached to the holder and then attached to the micromanipulator (Fine Science Tools Inc., Foster City CA). To ensure accurate measurement, only electrodes with a resistance of 4-12 Mega-ohms were used.

The suction electrode was prepared in advance and attached to a SD9K stimulator (Astro-Med Inc., Warwick RI) for stimulation of nerve fibers. Crayfish saline was sucked into the electrode, followed by a nerve, by means of an attached syringe, filling both the electrode and its holder. The suction electrode was connected to another micromanipulator.

Preparation and Function of Chemicals

90-95mL of Crayfish Saline was used to bathe the crayfish depending on the experimental prep. Crayfish Saline (5.4mM KCl, 196mM NaCl, 2.6mM $MgCl_2 \times 6H_2O$, 10mM sodium HEPES buffer, and 13.5mM $CaCl_2 \times 2H_2O$). If there were calcium potentials when recording EPSPs, then a mixture of no calcium ringer was added with a maximum ratio of 9mL no calcium solution: 1mL calcium solution. The no calcium ringer is the same as the calcium ringer except there was 0mM $CaCl_2 \times 2H_2O$ and 16.1mM MgCl2×6H2O. Both solutions had a pH of 7.4. All end concentrations were determined by applying the drug to 100mL of crayfish solution.

ZJ43 inhibits GCP II. ZJ43 was kept in a stock solution with distilled water at a concentration of 1mM. 10µL of 1mM ZJ43 was added to 100mL of crayfish saline, when dictated, having an end concentration of 0.1µM.

NAAG, the peptide neurotransmitter of interest, was kept in a stock solution with distilled water at a concentration of 10mM. Two different concentrations of NAAG were used in the experiment; the first concentration was to determine the effect of NAAG by itself on the synapse and the second concentration was used for the hydrolyzed products of NAAG. 200µL of NAAG was applied to 100mL of crayfish saline, when dictated, having an end concentration of 20µM. The second concentration of NAAG used 4µL of stock solution applied to 100ml of crayfish saline with an end concentration of 0.4µM.

LY341495 is a specific mGluR group II antagonist suspended in a solution of DMSO at a concentration of 5mM. 20µL of LY341495 was applied to 100mL of crayfish saline, when dictated, having an end concentration of 1µM.

Glutamate was used in this experiment as a control to determine its effect on EPSP amplitude when applied at a concentration twice the amount present in purchased NAAG. Glutamate was kept in a stock solution with distilled water at a concentration of 10mM. 2µL of glutamate was applied to 100mL of crayfish saline, when dictated, producing an end concentration of 0.2µM.

Crayfish Dissection

The crayfish were purchased from Ward's and were 3-5 inches in length. We first cooled the crayfish in an ice bath at least 15 minutes prior to dissection. The tail was removed and cuts were made down the lateral ridges of the tail. Most of the ventral tissue was removed exposing four underlying dorsal muscle fibers that ran along the length of the tail. We secured the tail with pins to the dissection plate under the microscope and immersed it in 90-95mL of normal crayfish saline.

General Experiment Assembly

The dissected crayfish tail was bathed in 90mL of crayfish ringer solution if the experiment prep required two different drugs to be added or 95mL of crayfish ringer solution if the experiment prep required one drug to be added. The preparation of the drug was kept separate in a 15mL Falcon tube (Corning, Corning NY) containing 5mL of crayfish ringer solution. After finding a viable nerve fiber, we used the suction electrode to suck up the nerve and hold it in place. Then we impaled the microelectrode into a muscle fiber in the same segment of the tail. Subsequent stimulation of the nerve fiber through the suction electrode resulted in the recorded EPSP using the program Scope 4 (AD Instruments, Colorado Springs CO). The frequency of stimulation for single pulse and paired pulses was 0.25Hz. The separation of the paired pulse EPSP's was 50ms. The duration of the stimulus was 5ms. We used suprathreshold voltage to elicit an EPSP. The drug(s) was applied by pouring the contents of the Falcon tube on the crayfish tail near the recorded synapse. Mixing the drug(s) in the solution was accomplished by slowly pulling and pushing solution in and out of a syringe. This general outline provided the basis of our seven experiments.

Data Measure and Analysis

The data measured were EPSP amplitude and paired pulse ratio (PPR). We only used data measuring a resting potential of -40mV or lower for analysis, all other data points were discarded. All comparisons of data were classified as average pre-drug application EPSP amplitude, average post-drug application EPSP amplitude, and in two cases, average washout drug EPSP amplitude. Two-sided independent samples non-pooled variance t-tests were ran on all data comparisons. Some of our hypotheses were one sided, and the data is interpreted accordingly.

The following equation was used in order to calculate the paired pulse ratio:

$$\frac{(1^{st}\text{ EPSP Amplitude} - 2^{nd}\text{ EPSP Amplitude})}{1^{st}\text{ EPSP Amplitude}}$$

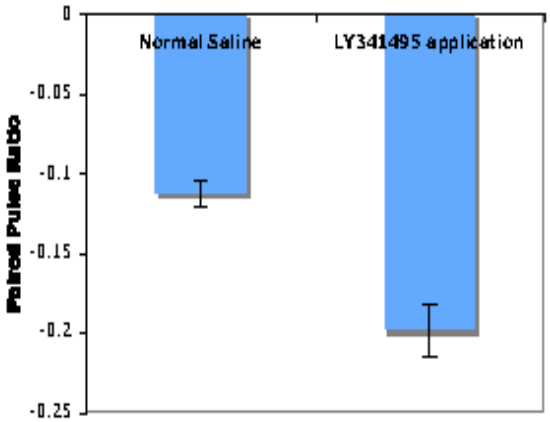
All experiments were preformed once on a single crayfish tail, so n refers only to the number of recordings from a single crayfish tail.

RESULTS

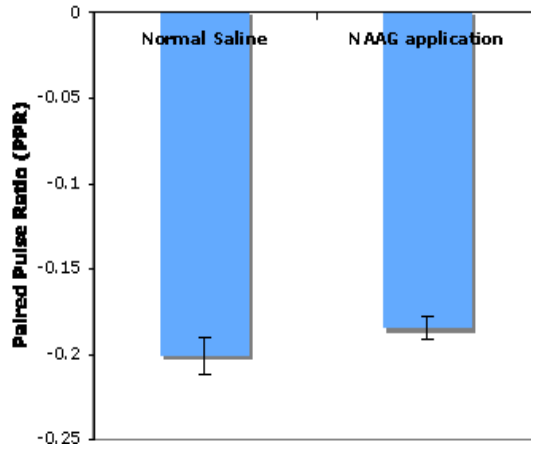
Our primary goal for this experiment was to examine the effect of the hydrolyzed products of NAAG and glutamate by itself on the NMJ. In order to get to this step, however, we had to test five assumptions: 1) whether NAAG had an effect on the crayfish NMJ; 2) whether the effect of NAAG on the NMJ was presynaptic, postsynaptic, or a combination of both; 3) whether mGluR group II- a receptor NAAG has affinity for- exists on the synapse presynaptically, by inhibiting the receptor with LY341495; 4) whether DMSO- the solution LY341495 is dissolved in- had any independent effects on the NMJ; 5) whether NAAG still has an effect on the synapse after mGluR group II has been inhibited.

Preliminary Experiments

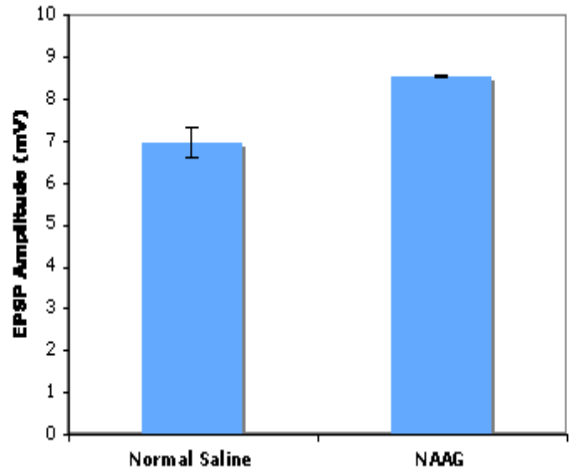
A)



B)



C)



D)

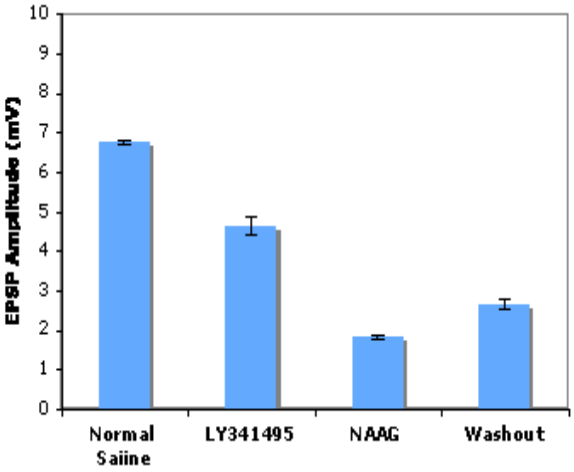


Figure 1. A and B represent paired pulse ratio as a function of experimental condition. Error bars represent one standard error of the mean. C and D represent EPSP amplitude as a function of condition. The error bars represent one standard error of the mean.

Experiment 1:

We hypothesized that NAAG would have an effect on EPSP amplitude in the NMJ. This experiment served a duel function, the first as stated previously, and second as a comparison to NAAG + ZJ43 application. To test this we used one drug application with single pulse stimulation. After establishing a baseline EPSP amplitude, 200µL of 5mM NAAG was added to the synapse and subsequent EPSP amplitudes were measured.

The results supported our hypothesis $p<0.001$. Normal saline n=23, NAAG n=155 with means of 6.93mV and 8.52mV, respectively (Fig. 1C).

Experiment 2:

We hypothesized that the application of NAAG on the NMJ would not significantly change the PPR. To test this, we ran the same set-up as the first, except we used paired pulse stimulation.

The results supported our hypothesis $p=0.211$. Normal saline n=29, NAAG n=126 with means of -0.201 and -0.185, respectively (Fig. 1B).

Experiment 3:

We hypothesized the application of LY341495 on the NMJ would significantly alter the PPR. To test this we used one drug application with paired pulse stimulation. After a baseline was established 20µL of 5mM LY341495 was applied to the synapse subsequent EPSP amplitudes were measured.

The results supported our hypothesis $p<0.001$. Normal Saline n=29, LY341495 n=145 with means of -0.113 and -0.199, respectively (Fig. 1A).

Experiment 4:

We hypothesized that DMSO would not have any independent effects on the NMJ. To test this we used the one drug application with single pulse stimulation. We established a baseline EPSP amplitude and then applied 20µL of 1mM DMSO onto the synapse. We then measured the subsequent EPSP amplitude.

Due to experimenter error, the number of recordings in each condition for this experiment is too small to run statistical analysis. The results are inconclusive.

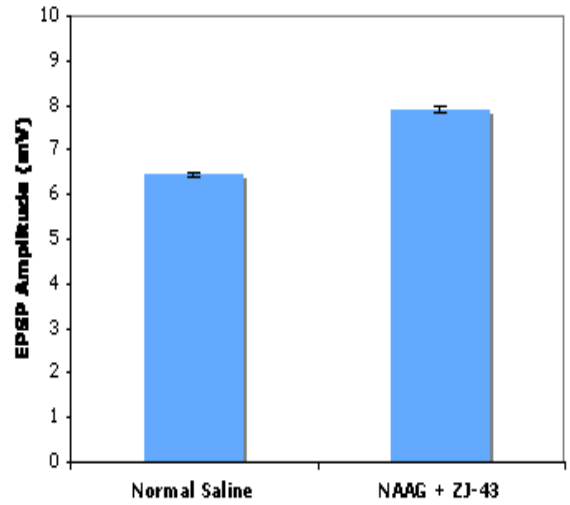
Experiment 5:

We hypothesized that the application of NAAG on the NMJ when LY341495 was present would significantly increase EPSP amplitude. To test the fifth question we used the two drug application with single pulse stimulation. A baseline EPSP was established, then 20µL of 5mM LY341495 was applied to the synapse and subsequent EPSP amplitudes were measured. Another baseline was established with LY341495 still in the synapse. 200µL of 10mM NAAG was applied to the synapse and the subsequent EPSP amplitude was measured.

The results refute our hypothesis $p<0.001$. LY341495 application n=89, NAAG application n=105, with means of 4.630mV, and 1.814mV, respectively. Washout of NAAG and LY341495 significantly increased EPSP amplitude not supporting our hypothesis $p<0.001$. Post application of NAAG n=105, Washout of NAAG and LY341495 n=38 with means of 1.814mV and 2.647mV, respectively. The washout did not bring the EPSP amplitude to baseline showing less support for our hypothesis $p<0.001$. Normal saline n=31, washout n=38 with means of 6.74mV and 2.65mV, respectively (Fig. 1D).

Main Experiments

A)



B)

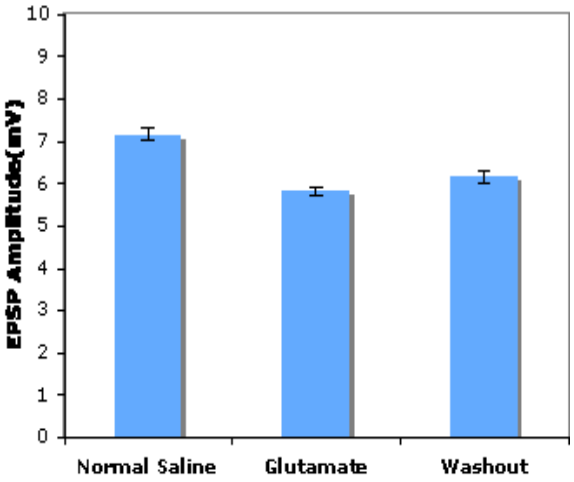


Figure 2. This data characterizes EPSP amplitude as a function of experimental condition. Error bars represent one standard error of the mean.

Experiment 6:

We hypothesized that the application of glutamate on the NMJ would significantly increase EPSP amplitude. The setup for the glutamate control experiment followed one drug application with single pulse stimulation. After establishing baseline EPSP amplitude, glutamate was applied to the synapse and subsequent EPSP amplitude was measured.

The results refuted our hypothesis $p<0.001$. control n=31, glutamate n=125 with means of 7.12mV and 5.81mV, respectively. Washout did not bring EPSP amplitude back to baseline $p<0.001$. Washout n=26 with a mean of 6.15 (Fig. 2B)

Experiment 7:

We hypothesized that the application of NAAG and ZJ43 on the NMJ would significantly decrease EPSP amplitude. ZJ43 was required to inhibit GCP II. ZJ43 and NAAG were mixed and agitated prior to application. The setup for the pure NAAG experiment followed one drug application with single pulse stimulation. After a baseline EPSP amplitude was established, NAAG and ZJ43 were applied to the NMJ and subsequent EPSP amplitude was measured.

The results refuted our hypothesis $p<0.001$. Normal saline n=29, NAAG + ZJ43 n=86 with means of 6.44mV and 7.89mV, respectively (Fig. 2A).

DISCUSSION

We investigated the effect NAAG has on crayfish neuromuscular junctions, the location where this occurs, and if its products of hydrolysis, not NAAG itself, are the chemicals causing these changes in EPSP amplitude. The paired pulse NAAG experiment showed us that NAAG acts postsynaptically, which supports previous research by Gafurov et al. (2001) and Huang et al. (1999). Because of this, after conducting the paired pulse NAAG experiment we tested the effect of LY341495 (shown to be a potent mGluR group II inhibitor by Bergeron et al. (2007)) on EPSP amplitude because in some literature mGluRs group II were thought to be postsynaptic (Huang et al. 1999). Using paired pulse, we saw LY341495 acts presynaptically. However, due to this method of assessing the location of drug action, its explicit presynaptic activity could hide additional postsynaptic processes. Our main experiments, dealing with NAAG's products of hydrolysis, did not provide any significant information, as we could not base them on our inconclusive single pulse NAAG experiment. Thus we still do not know if NAAG itself has an intrinsic effect on EPSP.

However, even though our main experiments were inconclusive, our preliminary experiments pertaining to NAAG did show that NAAG exists in the crayfish NMJ. Since NAAG repeatedly altered EPSP amplitude, the receptors and/or enzymes must already be present for NAAG to interact with. This is consistent with research done by Athena Carlson, Shanice Webster, and Meredith Kalkbrenner (unpublished), who used immunofluorescence to discover if NAAG exists in the crayfish NMJ.

Additionally, we found NAAG and its products lowered EPSP amplitude based on the single pulse experiment where LY341495 and NAAG were added, respectively (Fig. 1D). NAAG and its products decreased amplitude further from the

antagonistic effect of LY341495, and after washout return EPSP to baseline, this observation does support our finding that NAAG and its products lower amplitude. Because NAAG and its products decreased EPSP after mGluRs group II were blocked by LY341495, NAAG and its products probably partially antagonized another receptor in the synapse, namely NMDAR for our purposes, since NMDAR also affects amplitude.

Based on our LY341495 and NAAG experiment, LY341495 decreased EPSP amplitude. This contradicts research from previous studies, which found that because LY341495 blocks mGluR group II, mGluR group II cannot reduce glutamate release (which would diminish EPSP amplitude). Due to our unreliable DMSO control experiment, DMSO (the solvent of LY341495) might have its own effect on the synapse independent of LY341495. Alternatively, postsynaptic mGluR group II may elicit a different response in the postsynaptic cell than lowering EPSP, so by inhibiting mGluR group II, amplitude is reduced.

The pure glutamate experiment caused the EPSP to drop, disproving our hypothesis that glutamate increases EPSP amplitude. Washout of glutamate from the synapse raised the EPSP somewhat, supporting our unexpected results. This contradicts previous studies, which found glutamate to be an agonist of NMDAR. Also, because this experiment was conducted on the same crayfish as the pure NAAG experiment (where ZJ43, an inhibitor of GCP II, was added), endogenous NAAG could not be hydrolyzed and thus further diminish EPSP. Because of this, glutamate might be even more potent at antagonizing NMDAR. However, it is possible that ZJ43 had a different, confounding effect on the pure glutamate experiment. Also, adding 0.2⌈M glutamate might have diffused out of the cleft, interacting with satellite cells, or inducing desensitization. This sudden abundance of exogenous glutamate in solution is not normal for neuromuscular junctions, so extraneous effects like those mentioned could have occurred. These flaws in the experiment make the results inconclusive.

ZJ43, an inhibitor of GCP II, was added with NAAG to observe NAAGs pure effect on NMDAR. If we accept that NAAG itself raised amplitude here, the partial antagonistic effect of its products contrasted with the agonistic effect of NAAG might explain its role as neuromodulator of the NMJ, congruous with findings by Bergeron et al. (2007) and Gafurov et al. (2001). When little glutamate enters the cleft, GCP II may increase activity to yield more glutamate from NAAG available for interaction with NMDAR, therefore decreasing EPSP. of NAAG EPSP increased. Although washout did not Adversely, when excess glutamate is present, GCP II may diminish activity so that more NAAG molecules can agonize NMDAR to raise EPSP.

Analyzing data from LY341495 and NAAG paired pulse experiments provided data on how these drugs modify EPSP post-hoc. However, amplitude was not the original intent of these experiments (as they dealt with location), so conclusions drawn from them in this way may be susceptible to Type 1 Error. However, in an attempt to limit confounding variables, we took only the first pulse from these experiments, as these amplitudes should mimic those under the conditions of a single pulse experiment as well as decreased our alpha level to 0.025.

After graphing NAAG first pulse amplitudes, NAAG was shown to significantly reduce EPSP ($p<0.001$ with saline baseline n=29 and NAAG application n=126, and with means of 6.08mV and 5.66mV respectively). This is consistent with results from the LY341495 and NAAG experiment, and the pure glutamate experiment.

Unfortunately, our research contained several weaknesses. When we measured the EPSP produced by a single pulse in the presence of NAAG, we did not determine a baseline EPSP before its addition. Because the EPSP amplitude steadily decreased during the time for baseline (as can be inferred by the large standard error), we could not find an average to compare to NAAG's effect, rendering it inconclusive (Fig. 1C). EPSP amplitude decreased because the recording microelectrode gradually fell out of its cell, impairing its ability to accurately measure EPSP. Creating bounds for the membrane potential can exclude inaccurate data points and would allow us to avoid this issue in the future, as simply disregarding data points greater than -40mV was useful in our experiments but did not address this issue.

The LY341495 and NAAG graph exposed some errors in that experiment. After application of LY341495, EPSP immediately lowered but just as rapidly rose during that time period. This could be caused by the suction electrode failing to stimulate the nerve properly at first but then returning to normal efficiency later on. Later, when NAAG was introduced, this problem may partially illustrate why EPSP instantly decreased instead of declining at a visible rate (Fig. 1D).

When we added LY341495 and measured the paired pulse ratio of EPSP amplitude, we noticed that after immediately adding this drug the first pulse of the paired pulse decreased in amplitude but the second remained near the value found during the

baseline. This leads us to believe we may be enervating multiple axons, since in that instance the drug may not have reached the synapse for the axon of the second pulse, but had reached the synapse for the axon of the first pulse. Measuring EPSP while stimulating multiple axons limits consistency of results taken from the NMJ in question (as several synapses could be competing to elicit a response), which reduces their accuracy.

Because of our limited accessibility to specimens, we conducted our experiment on the effect of pure glutamate after washing out chemicals from our experiment on pure NAAG. We suspect that minute concentrations of NAAG and ZJ43 still existed in solution, which could have caused unknown changes to the results we recorded following that experiment.

In general, our experiments could not yield precise results because we could not administer drugs efficiently. We were forced to pour drugs into the solution inside the dissecting dish but could not properly agitate these chemicals because this would disturb the microelectrodes, thus making us readjust the experimental assembly. Then, if we could not induce EPSP again, we would be forced to terminate the experiment before witnessing any effects the drug might have had. In advanced laboratories, systems are used that can disperse drugs into the synapse under experimentation as they would naturally, making data much more reliable and reflective of a realistic environment.

Our observations on NAAG's excitatory effect on EPSP and its possible role as modulator of the glutamatergic synapse support the theory that this drug may help alleviate schizophrenia. Javitt (2010) found that glutamate hypo-function leads to schizophrenia, so our findings of NAAG's agonistic effect on NMDAR support this research. However, many questions have rose from our research, as to the mechanism by which NAAG's products of hydrolysis decrease EPSP, if NAAG itself does have an effect of its own, where glutamate interacts on satellite cells, and what specific interactions NAAG has with the postsynaptic cell.

ACKNOWLEDGMENTS

We would like to thank Chris Kaiser-Nyman, Ashley Millet, and Sue Kolbe for the generous amount of time and effort that they put into our project. We would like to thank Clark Lindgren for his insightful analogies and for pointing us in the right direction. We also want to give a big hug to Grace Hazeltine and Balyssa Bell for their editing. Lastly, we would like to remember all the grateful crayfish that laid down their lives for life, liberty and the pursuit of a good grade.

REFERENCES

Bergeron, R., Y. Imamura, J.V. Frangioni, R.W. Greene, and J.T. Coyle. 2007. Endogenous N-acetylaspartylglutamate reduced NMDA receptor-dependent current neurotransmission in the CA1 area of the hippocampus. *Neurochemistry*. 100: 346-357.

Fricker, S. M., de la Flor, Shah, Woodley, Dawson, & Kew. 2009. Effects of N-acetylaspartylglutamate (NAAG) at group II mGluRs and NMDAR. *Neuropharmacology*. 56: 1060-1067.

Gafurov B., A.K. Urazaev, R.M. Grossfield, E.M. Lieberman. 2001. *N*-acetylaspartylglutamate (NAAG) is the probable mediator of axon-to-glia signaling in crayfish medial giant nerve fiber. *Neuroscience* 106: 227-235.

Huang, L., M.J. Rowan, R. Anwyl.1999. Induction of long-lasting depression by (+)-alpha-methyl-4-carboxyphenylglycine and other group II mGlu receptor ligands in the dentate gyrus of the hippocampus in vitro. *European journal of pharmacology* 366: 151-158.

Javitt, D.C. 2010. Glutamatergic Theories of Schizophrenia. *The Israel Journal of Psychiatry and Related Sciences* 47.1: 4-16.

Maghaddam, B. 2004. Targeting metabotropic Glutamate Recoptors for Treatment of the Congnitive Symptoms of Schizophrenia. *Psychopharmacology* 174: 39-44.

Neale, J.H., R.T. Olszewski, L.M. Gehl, B. Wroblewska, and T. Bzdega. 2005. The Neurotransmitter N-acetylaspartylglutamate in models of pain, ALS, diabetic neuropathy, CNS injury and schizophrenia. *Pharmacological Sciences* 26: 474-484.

Trombley and Westbrook. 1990. Excitatory synaptic transmission in cultures of rat olfactory bulb. *Neurophysiology* 64: 598-606.