

# Precursor Effects of Glutamate on Frog Skeletal Muscle Contractility: Assessing the Early Signs of Motor Neuron Excitotoxicity

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## Abstract

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that targets motor neurons in the central nervous system. Over-exposure to the excitatory neurotransmitter, glutamate, results in an abnormally high influx of calcium ions into the neuronal cell, triggering incongruous apoptotic events. The death of motor neurons prevents the contraction of their respectively innervated muscles, resulting in muscle atrophy and eventual paralysis. While the detrimental consequences of ALS have been well studied, much still needs to be resolved in understanding the precursor conditions and mechanisms that give rise to neurotoxicity. By exposing intact frog nerve-muscle to varying concentrations of glutamate we determined the force generated by artificially stimulated muscle contractions during twitch threshold and fatigue. Muscle contractility was tested for two control trials, during which the sciatic nerve and the muscle were bathed in Amphibian Ringer's solution, as well as two experimental trials in which the sciatic nerve and muscle were bathed in 0.1  $\mu$ M solution of glutamate (mimicking "normal" levels) and a 1.0  $\mu$ M solution of glutamate (mimicking "toxic" levels), respectively. The predicted observations (initially elevated muscle spasticity during threshold followed by increased muscle fatigue) were consistent with pre-ALS symptoms.

The results of this experiment displayed a trend favoring increased fatigue for increasing concentrations of glutamate. However, twitch threshold tended to be higher in glutamate trials than in control trials containing no glutamate. Based on the data analyzed thus far, no significant or definitive conclusions have been drawn from this study. Continued data analysis is needed before a meaningful conclusion can be drawn. Nevertheless, the results of this experiment could provide insight into early detection of a disease state prior to neuro-degeneration and prompt a more streamlined approach towards drug design and ALS treatment in the future.

## Objective

- To study the effects of varying glutamate levels on the contractility of frog skeletal muscle during twitch threshold and neuromuscular fatigue

- Hypothesis: Increased extracellular levels of glutamate will initially lower threshold voltage and increase contractility. Ultimately, exposure to higher concentrations of glutamate will result in an increase in fatigue and a decrease in contractility.

## Introduction

Glutamate is an amino acid that is an excitatory neurotransmitter. This means that its function is to mediate excitatory signals in the central nervous system as well as to signal other neurons to release their respective neurotransmitters. It is additionally involved in cognition, learning, and memory. Furthermore, regulation is heavily reliant upon glutamate transporters that promote cellular uptake of glutamate. Following receptor activation, glutamate is rapidly removed from nerve cell junctions. Glutamate receptors include ionotropic receptors NMDA, AMPA/kainate and metabotropic receptors (mGluR). The glutamate-glutamine process involves uptake of glutamate and conversion to glutamine by astroglial cells. Glutamine is then secreted into extracellular fluid where nerve terminals convert it back to glutamate. This process enables transport of inactive glutamate to neurons. At resting membrane potential, glutamate and glycine bind NMDA and glutamate additionally binds AMPA, resulting in membrane depolarization. As a result, the magnesium block is displaced from NMDA receptor and the NMDA ion channel opens.

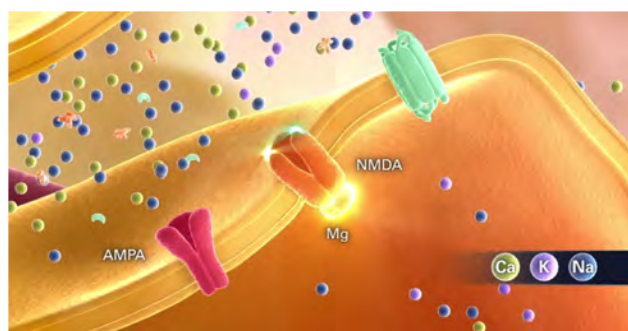


Fig. 1: Glutamate and glycine bound to NMDA with Mg<sup>2+</sup> attached.

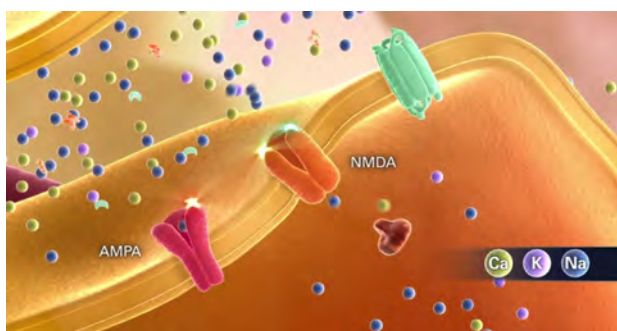


Fig. 2: Glutamate binds to AMPA causing membrane depolarization and resulting in removal of Mg<sup>2+</sup> from NMDA.



Fig. 3: The Mg<sup>2+</sup> block has been removed from NMDA, opening the cation channel.

The aforementioned mechanisms are involved in glutamate excitotoxicity due to the fact that elevated levels of extracellular glutamate cause over-activation of NMDA receptors. Ultimately, prolonged exposure to calcium ions, due to the open ion channel, results in apoptosis. Glutamate levels known to elicit such a response are those reaching 2-5  $\mu$ M and are thus considered toxic to cells.

These mechanisms resulting in glutamate toxicity are associated with Amyotrophic Lateral Sclerosis (ALS), a progressive neurodegenerative disease that targets neurons in the CNS. The disease is characterized by the death of motor neurons, leading to eventual paralysis due to the fact that muscles that no longer receive signals from said neurons begin to atrophy. Elevated levels of glutamate have been detected in cerebrospinal fluid surrounding motor neurons and it is thought that mutations affecting EAAT2 may be involved through interference in glutamate uptake.

## Methods

### Preparation

- Glutamate stock solution (to be used in testing fatigue as outlined below) was prepared using 4.413 mg of glutamate in 50 mL of Amphibian Ringer's Solution.
- 0.5 g of MS-222 in 500 mL of DI water was used to anesthetize the frog being studied.
- iVox recording and analysis software was calibrated and prepared for use in the outlined conditions for testing the responsiveness of the nerve.



### Testing Twitch Threshold

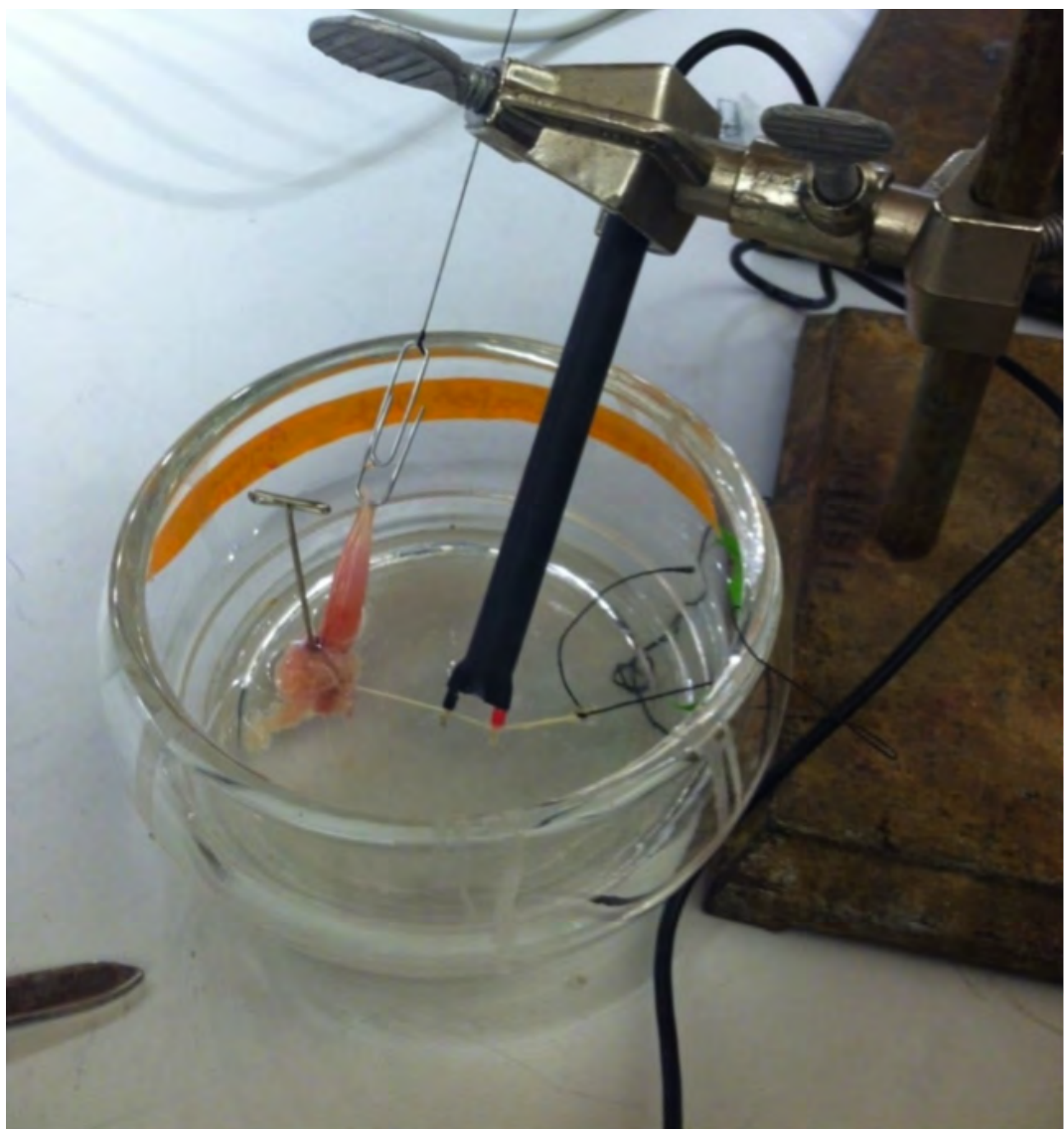
- The sciatic nerve was stimulated by increasing the Amps delivered by the electrodes to the nerve in 0.1V increments, starting at 0.0 V and ending at 1.0 V.
- Threshold voltage was recorded for each trial.
- The amplitude of each wave generated by a muscle contraction at and post-threshold was measured and recorded.
- The force generated by each contraction was obtained by dividing its amplitude by the sensitivity of the force transducer, FT-302, which produced an output of 100 mV per gram of weight.

### Testing Fatigue

- The sciatic nerve was stimulated continuously for 2 minutes at 10 Hz at 1.0 V.
- Time lapses (defined as a period in which there is no response to continued stimuli) between major contractions were recorded. A major contraction was defined as any contraction equal to or greater than the minimum contraction generated during the evaluation of twitch threshold for each respective condition.
- The wave generated immediately preceding and immediately succeeding each time lapse was recorded.
- The maximum wave generated at tetanus, and the duration of tetanus was recorded.

**For each frog leg, four conditions were tested for twitch threshold and fatigue. The conditions were as follows:**

- In the first experimental condition, the nerve was bathed in 100 mL of Ringer's Solution.
- In the second experimental condition, the nerve was bathed in 100 mL of Ringer's plus 100 microL of the stock glutamate solution. The solution was mixed by pipetting up and down. This represented "normal" glutamate levels.
- In the third experimental condition, the nerve was bathed in 100 mL of Ringer's plus 900 microL of the stock glutamate solution. The solution was mixed by pipetting up and down. This represented the "toxic" glutamate levels.
- For the fourth experimental condition, the content of the dissection dish was removed by pipetting it out and disposing of the solution into a designated waste container. The dissection dish was rinsed by pipetting with approximately 50 mL of Ringer's. The dish was refilled with 100 mL of Ringer's and the trial was repeated.



## Results

### Twitch Threshold

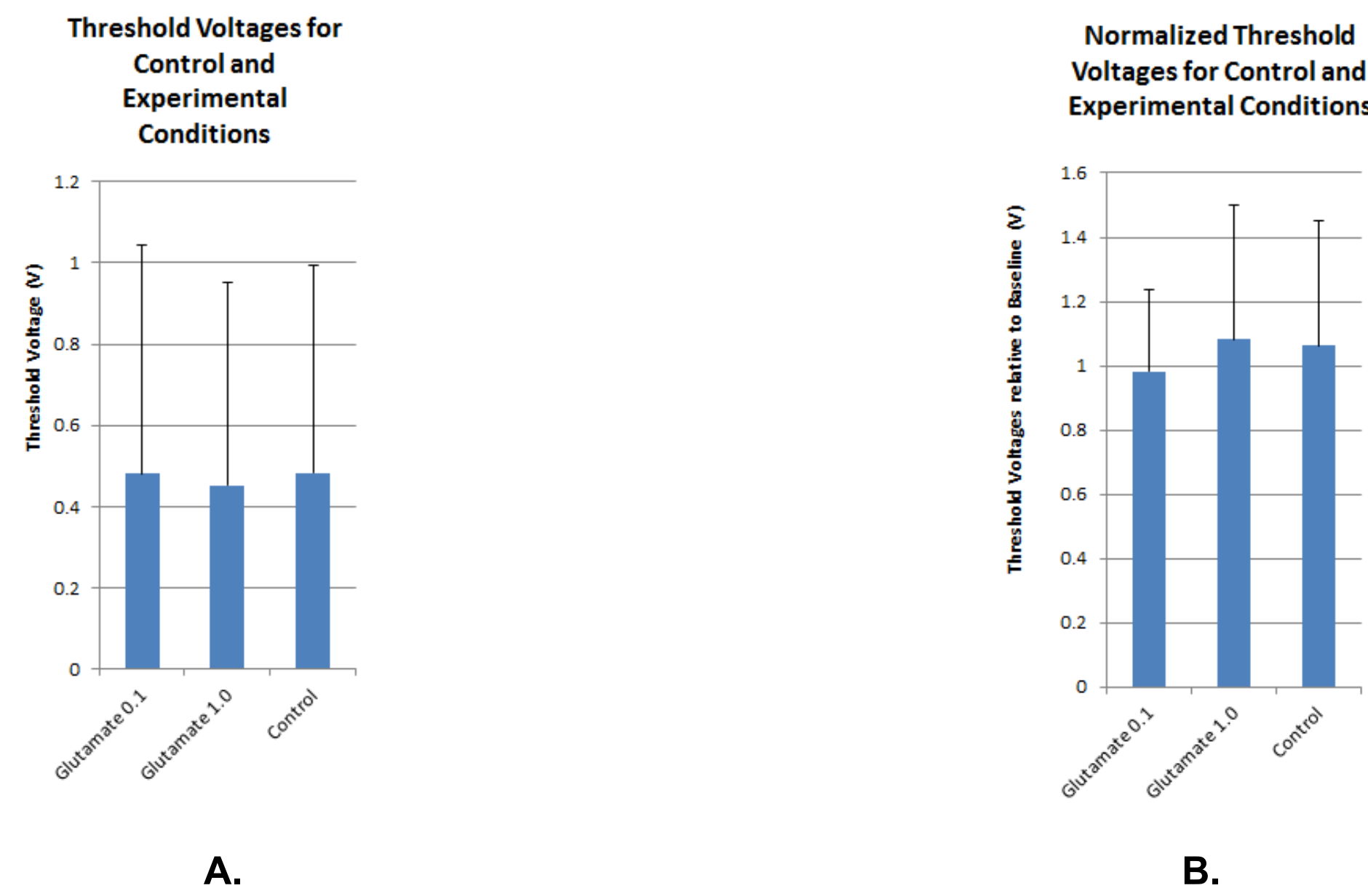


Figure 1: **A.** Results of t-test performed for determination of twitch threshold up to present; **B.** Normalized t-test values for twitch thresholds.

### Fatigue

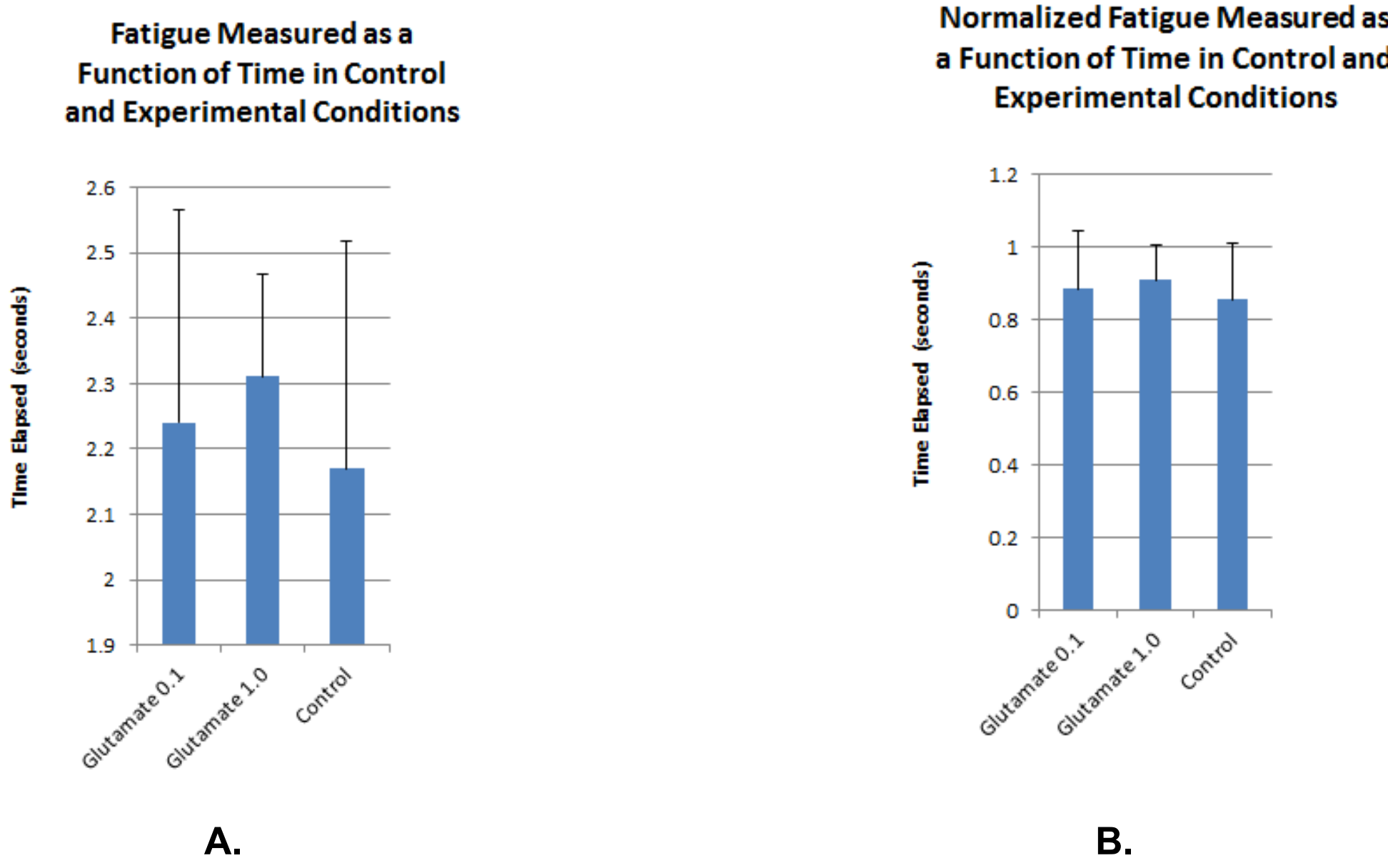


Figure 2: **A.** Results of t-test performed for determination of fatigue up to present; **B.** Normalized t-test values for fatigue.

### Tetanus

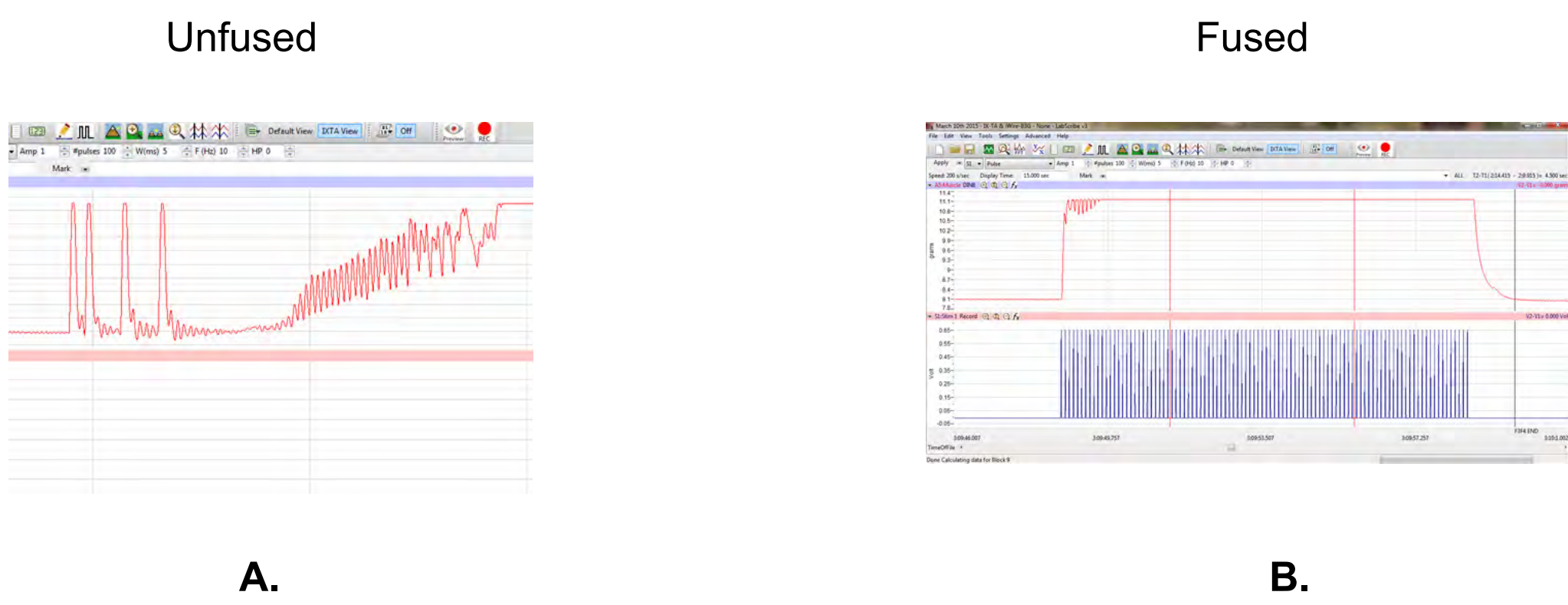


Figure 3: **A.** Unfused tetanus for F1 upon completion of 0.1 microM glutamate; **B.** Fused tetanus for F3 upon completion of control after wash.

### Spontaneous Contractions

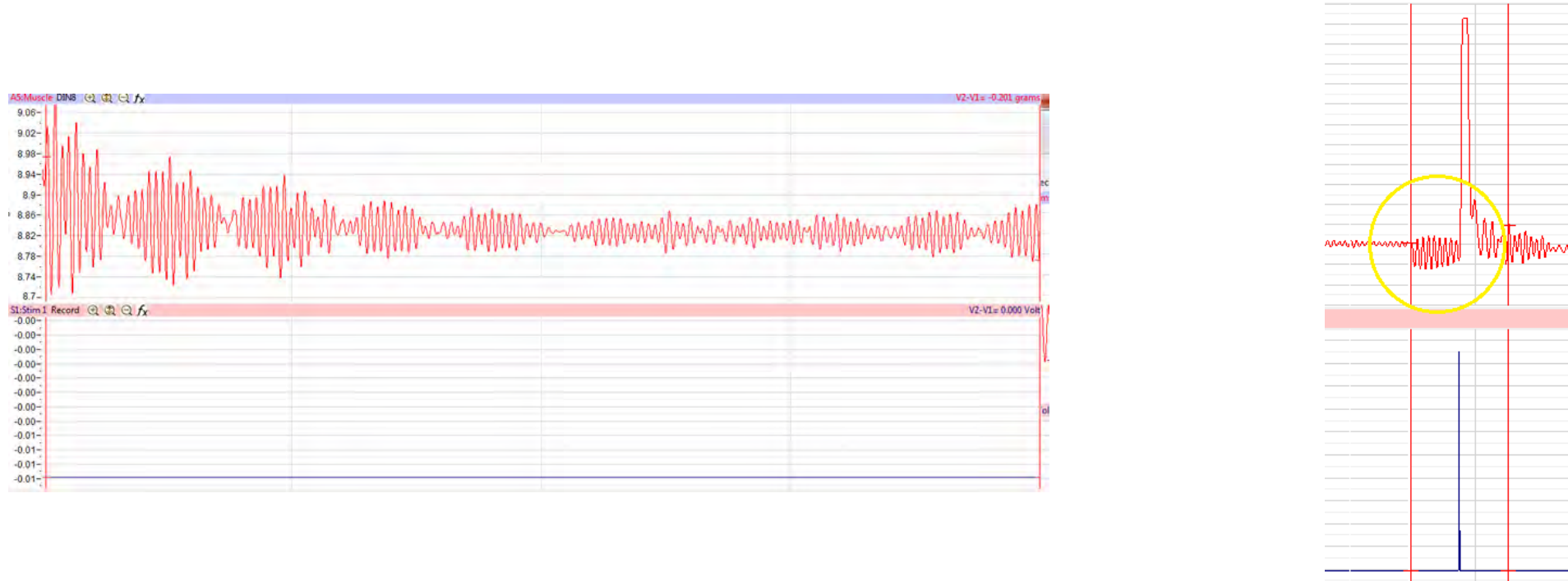


Figure 4: Spontaneous contractions elicited during exposure to 1.0 microM glutamate solution.

## Conclusions

Data analyzed until the present time suggests that despite what was initially expected, twitch threshold tends to increase with increasing concentrations of glutamate. In most instances, force generated during twitch threshold determination increased with increasing concentrations of glutamate. The time elapsed between muscle contractions during determination of fatigue tends to increase with increasing concentrations of glutamate. Spontaneous contractions were observed in trials involving glutamate exposure. Yet, these contractions remain to be quantified. Despite the trends noted thus far, further data analysis, coupled with statistical analysis, is required before any significant conclusions can be inferred from experimentation.

### In further studies:

Fatigue should be tested over a longer period of time. It is possible that fatigue was observed during too short of a time frame for glutamate toxicity to produce observable effects. The prep could be bathed in glutamate for several hours before artificial stimulation to allow time for the sciatic nerve to be affected by the overwhelming presence of glutamate. This may result in a data set that is more comprehensive and yields more significant results.

Tension should be standardized. The tension applied to the muscle prep could have varied for each experiment. It is possible that in some experiments, tension was too high while in others tension was too low. This would affect the readings for contractility on the LabScribe software, leading to erroneous calculations.

The control after wash needs to be optimized. It is possible that trace amounts of glutamate remained in the dissection dish during the control trials. This could have accounted for skewed results since the control and baseline values did not match up well. To circumvent this issue in the future, all experimental conditions should be randomized. Instead of progressing in order from baseline, to glutamate, and returning to control, trials should be randomized for each experiment.

A wider range of glutamate concentrations should be employed to better gauge the effects of glutamate toxicity on the nerve-muscle prep.

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## References

- Glutamate. (n.d.). Retrieved April 12, 2015, from <http://www.alsa.org/research/about-als-research/glutamate.html>
- Glutamate Animation Scene02 NMDA Activation 072312. <https://login.medscape.com/login/sso/getlogin?urlCache=aHR0cDovL3d3dy5lZWZWRzY2FwZS5vcvdmld2FydGlibGUvNzY5MTA4&ac=401>
- Leighton, Mark P. "Pictorial Review of Glutamate Excitotoxicity: Fundamental Concepts for Neuroimaging." American Journal of Neuroradiology. N.p., n.d. Web.
- Pinton, P., C. Giorgi, R. Siviero, E. Zecchini, and R. Rizzuto. "Calcium and Apoptosis: ER-mitochondria Ca<sup>2+</sup> Transfer in the Control of Apoptosis." Oncogene. U.S. National Library of Medicine, n.d. Web. 12 Apr. 2015.
- What is ALS? (n.d.). Retrieved April 12, 2015, from <http://www.alsa.org/about-als/what-is-als.html>