

The Effects of Chronic Nicotine Exposure on Skeletal Muscle Function in *Lithobates pipiens*

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Introduction

- Nicotine affects skeletal muscle by mimicking acetylcholine (ACh) and binding to nicotinic acetylcholine receptors (nAChRs), ion channels found throughout the nervous system that induce muscular contractions, causing an unregulated flow of Ca^{2+} from the sarcoplasmic reticulum. To achieve muscle contraction (Figure 1), ACh binds to receptors at the neuromuscular junction which ultimately results in the release of Ca^{2+} for actin-myosin interaction.
- Research by Chibalin et al. (2012) and Giniatullin et al. (2005) demonstrated that nicotine from cigarettes suppresses Na, K-ATPase activity (Figure 1, box 6), leading to chronic membrane depolarization and reduced excitability of muscle cells.
- Additional studies have shown negative effects of nicotine on muscle function. Nogueira et al. (2018) found that mice exposed to cigarette smoke demonstrated reduced fatigue resistance and impaired calcium (Ca^{2+}) uptake, even when lung damage was not present.
- According to Allen (2008), impaired Ca^{2+} regulation slows the rate of contraction and relaxation, further reducing muscle performance during chronic nicotine exposure.
- Mundel and Jones (2006) reported that transdermal nicotine use increased time to exhaustion in trained cyclists. These findings suggest that acute nicotine exposure may enhance fatigue resistance.
- Studying nicotine's isolated effects is difficult because cigarette smoke contains many harmful carcinogens which could skew observations. As a result, this study aims to observe change in muscle performance when subjects are exposed strictly to nicotine.
- Amphibians, such as frogs, are ideal vertebrates for nicotine studies due to their permeable skin, allowing for controlled nicotine absorption and making them a valuable model for isolating the drug's physiological effects.
- This study hypothesized that nicotine exposure would lead to a decreased difference between the threshold and maximal voltages (the voltages needed to initiate contraction and maximize force, respectively), as well as the time taken to reach fatigue.

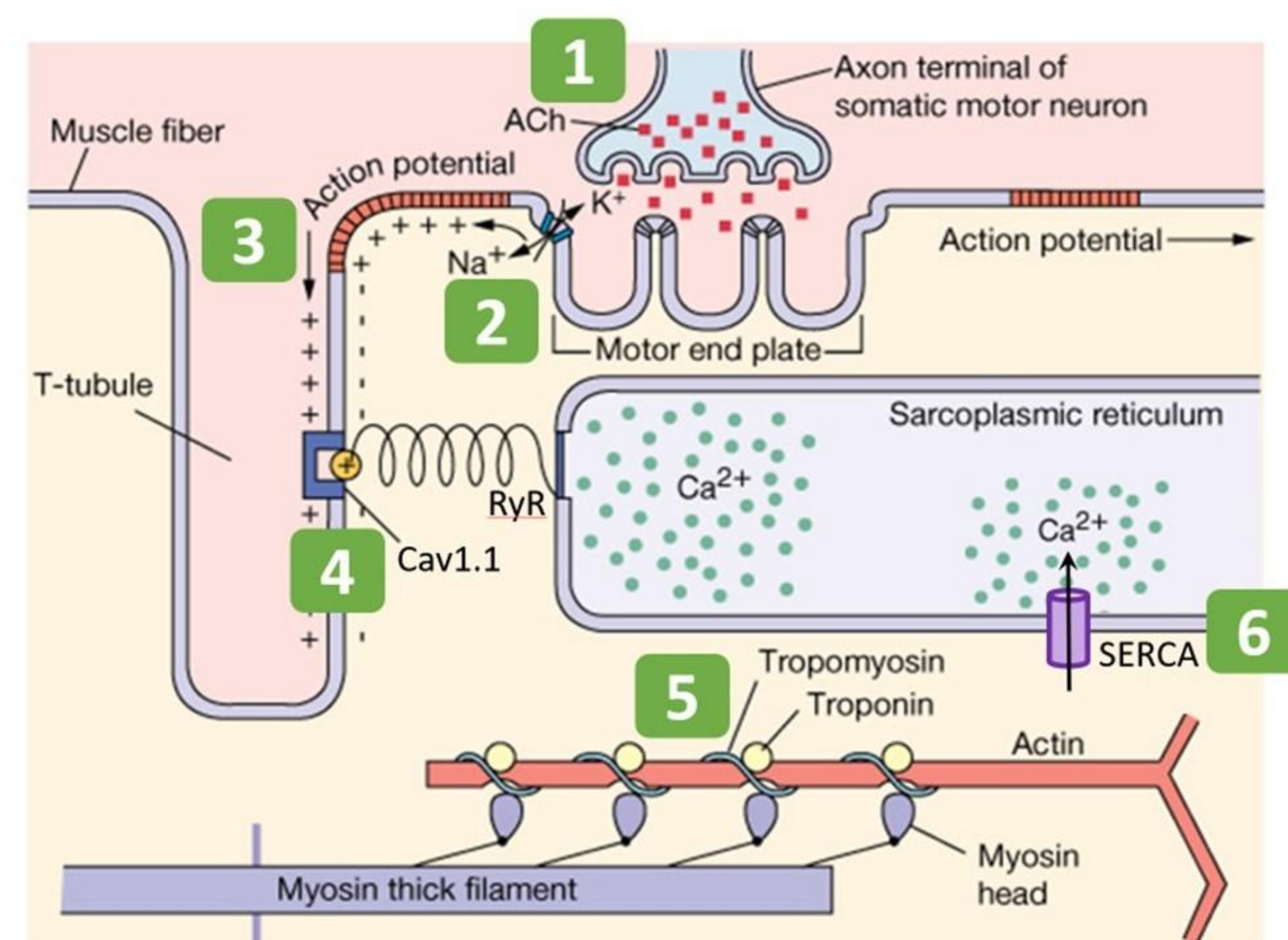


Figure 1. Image displaying the process of muscle contraction. (Asbury et al., 2023)

Methods

Twelve Northern leopard frogs (*Lithobates pipiens*) were randomly assorted into 3 groups: control (0 μ g nicotine), medium exposure (5 μ g nicotine), and high exposure (10 μ g nicotine). Each group received their assigned treatment once a day for 14 days. The percent change in metabolic rate due to nicotine exposure was used to determine dosage for the groups. Values of 3-8% increase in oxygen consumption were elicited with a 5 μ g/mL dose, and a value of 10 μ g/mL was determined by doubling that determined dose. During administration, 1 mL of treatment solution was added dropwise onto the dried seat patch of the frog. Using a protocol adapted from Sheafor, (2025), the frog's gastrocnemius muscle was stimulated to measure the threshold (lowest voltage required to reach contraction) and maximal response (voltage needed for maximum force of contraction). The threshold and maximal voltages, as well as the difference between the two, was compared between both legs to ensure consistency. All but one frog demonstrated a threshold difference of 1 V or less between each leg. Following this, the muscle was stimulated in order to measure time to reach fatigue. This was achieved by stimulating the muscle with half of the maximal voltage repeatedly, with the frequency of each stimulation being increased every 2 seconds until the muscle began losing tension despite continual stimulation. A BIOPAC BSL voltage stimulator was used to stimulate the muscle, and a BIOPAC SS12LA (force transducer) measured the force of each muscle contraction. To evaluate statistical significance, t-tests assuming unequal variance were performed.

Results

Statistically significant differences were found between the control and 5 μ g/mL voltage difference values (Figure 2), as well as between both treatment groups and the control when measuring time taken to fatigue (Figure 3).

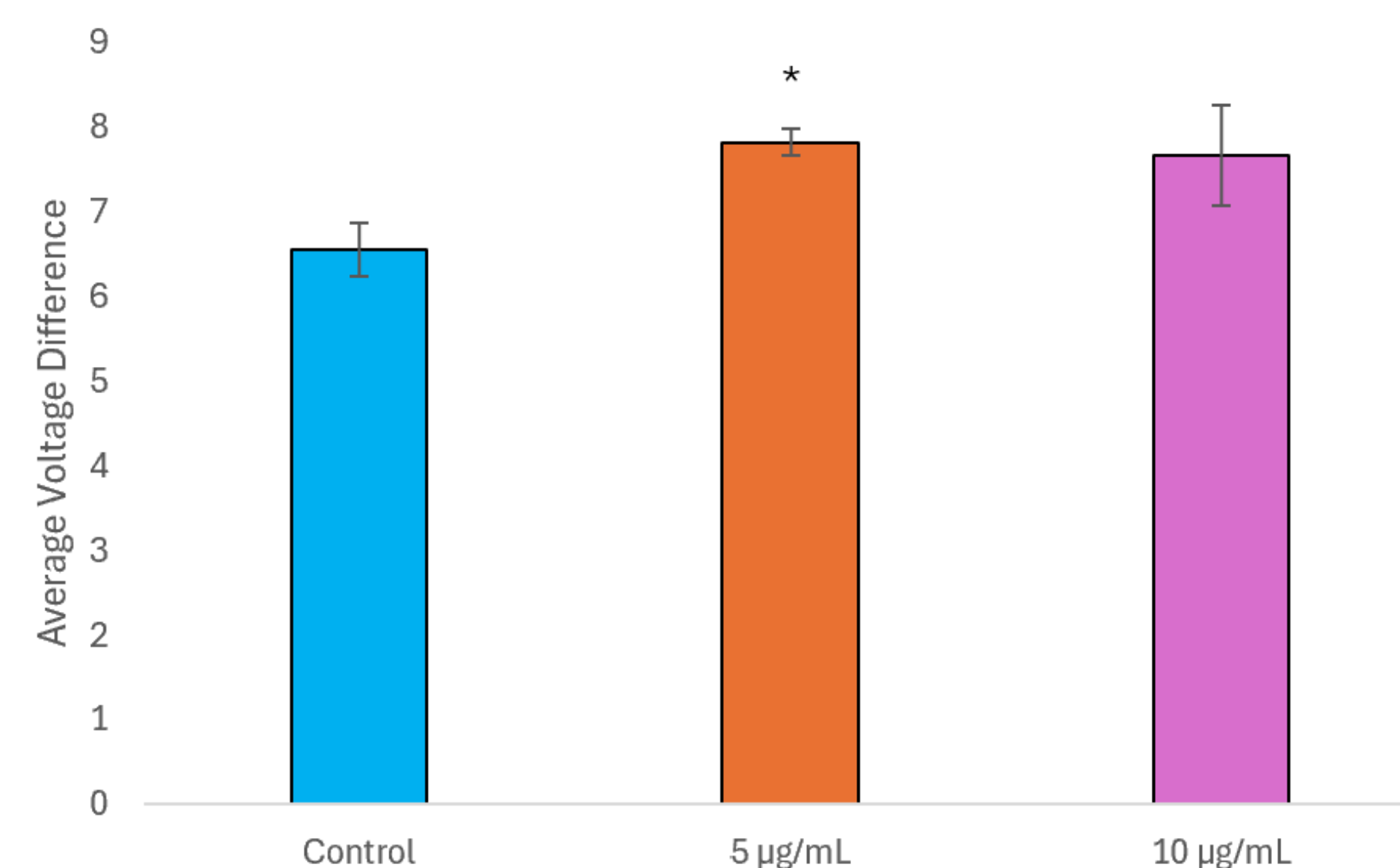


Figure 2. Average difference between threshold and maximum voltage in frog gastrocnemius muscles exposed to 0.0 μ g/mL (control), 5.0 μ g/mL, and 10 μ g/mL nicotine. Values are means \pm standard error. *($p \leq 0.05$) indicates a significant difference between the respective treatment and control.

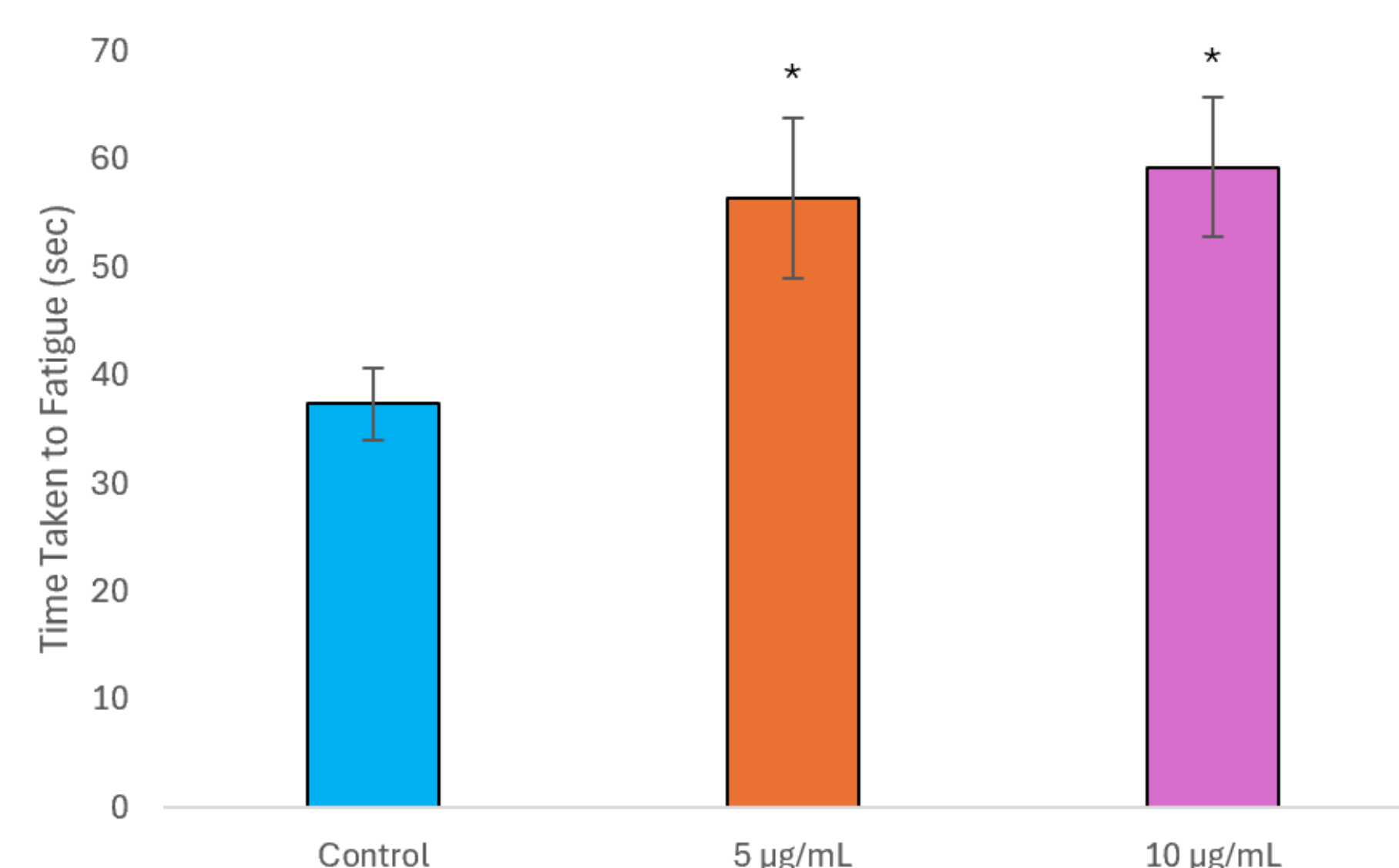


Figure 3. Average time taken to reach muscle fatigue for each treatment group, 0.0 μ g/mL (control), 5.0 μ g/mL, and 10 μ g/mL nicotine. Values are means \pm standard error. *($p \leq 0.05$) indicates a significant difference between the respective treatment and control.

Discussion & Future Directions

The difference between the threshold voltage and maximal response voltage was statistically different between the control and medium dose treatments, but not between the control and high dose group (Figure 2). When compared to the control, the medium dose group had lower thresholds, with significance between these groups being represented by a p-value of 0.02. These lower thresholds contributed to a greater overall voltage difference. A lower threshold indicates that the muscles of frogs exposed to a 5 μ g/mL treatment required less stimulation to initiate contraction. The chronic membrane depolarization observed by Krivoi et al. (2006), Chibalin et al. (2012), and Giniatullin et al. (2005), may explain the lower threshold values. Chronic membrane depolarization may lead to increased intracellular calcium content as observed by Takahashi et al. (2020), which could prompt leakage of calcium from the sarcoplasmic reticulum (Figure 1, box 6), perhaps increasing excitability. For the high dose treatment, there were two sets of outliers that greatly skewed the data. In one, the threshold voltage was at or above the maximal voltage of the other frogs. Given that we could not stimulate the muscle beyond 10 V with the BSL voltage stimulator, we were unable to attain a maximal voltage or voltage difference. The other set of outliers was in a frog that had a difference of 2 V in the threshold values between the two muscles, but no difference in the maximal voltage, something not observed in any other frog. There was a statistically significant difference in the time taken to reach muscle fatigue when comparing both the medium and high groups to the control (Figure 3). This increased fatigue resistance was similar to the study by Mundel and Jones (2006), which demonstrated increased time taken to exhaustion, perhaps indicating that this time frame was too acute to reveal the hypothesized long-term effects. We propose that future research takes place over chronic time periods. We hypothesize that studies lasting over 21 days, a time frame used by Chibalin et al., 2012, may begin to demonstrate the chronic changes initially hypothesized.

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