EFFECT OF MICROGRAVITY ON NEUROMUSCULAR SYSTEMS DURING PERIODS OF CRITICAL DEVELOPMENT

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Abstract

The ability of the human race to venture beyond our home planet requires many delicate systems to function under extreme conditions, yet perhaps the most delicate system of all is the human body. One of the greatest strains of long-term space travel lies in the absence of gravitational force acting upon the body, which is well known to have consequences specifically on the neuromuscular system. The purpose of our experiment was to identify the consequence of long-term non-use on both the ability of postural muscles to produce a forceful contraction, and the efficiency of the nervous system to achieve muscle contraction via electrochemical stimulation. More specifically, we are investigating whether developing neuromuscular systems face greater consequences of an extended period of muscle unloading compared to fully developed neuromuscular systems. The experimental results confirm that not only does neuromuscular function suffer following long-term periods of non-use, but it confirms that that the loss of neuromuscular function is far more prevalent for neuromuscular systems undergoing growth development compared to those that are already developed.

Introduction

The neuromuscular junction (NMJ) is the physical location where the nervous system reaches and stimulates voluntary muscle fibers. In living organisms under normal circumstances, voluntary contraction of muscles can only be achieved via stimulation from the NMJ. It is already known that the NMJ is directly involved in an organism's ability to generate and sustain muscular force (Ermilov et al., 2007; Johnson and Sieck, 1993; Pagala et al., 1984). Furthermore, restoring normal morphology of the NMJ is connected to recovering muscular strength following injury (Pachter and Eberstein, 1991; Ribaric et al., 1991). Significant changes in muscular activity including disuse from various neuromuscular diseases (Brown and Ironton, 1977; Eldridge et al., 1981; Pachter and Speilholz, 1990) results in remodeling the structures of pre- and postsynaptic areas of the NMJ. Long periods of exposure to the effects of microgravity such as space travel mimic diseases resulting in non-use of voluntary muscles, causing morphological changes at the neuromuscular junction. However, research literature has not addressed how these changes at the neuromuscular junction are affected by the stage of development of the neuromuscular system in question. Our lab aims to understand the consequences of long-term muscle unloading during a period of significant neuromuscular development of adolescence as opposed to fully-developed neuromuscular systems of young adults.

Experimental Design

Due to ethical and logistical restrictions, human subjects could not be used for this study. Instead, rats were used as their neuromuscular systems closely resemble those in humans. 21 two-month old rats, roughly modeling the developmental equivalent of a six-year old adolescent human, and 20 eight-month old rats, roughly modeling the equivalent of a 24-year old adult human, were randomly assigned to one of two groups: hindlimb suspension (HS) and control (CTL), with a total of four treatment groups: juvenile HS (n = 10), juvenile control (n = 11), adult HS (n = 10) and adult control (n = 10). The hindlimb suspension group lived for two weeks[i] in the ground-based hindlimb suspended model (HS) first introduced in 1979 wherein the rats' hindlimbs are rendered non-weight bearing, but they otherwise function normally, using only their front limbs (Morey et al.). The HS model mimics the physiological effects of microgravity on human bodies such as cephalic fluid shift and muscle atrophy (Globus & Morey-Holton, 2016). The weight-bearing control group used all four limbs, and did not undergo any irregular circumstances. Directly following completion of the two-week period, the rats were anesthetized, and the soleus[ii] muscle was surgically removed. The isolated soleus muscle was analyzed using the *ex vivo* muscle testing system procedure wherein one end of the soleus was clamped in place, and the other end was tied with a string attached to a force gauge. On either side of the muscle was an electrode attached to a voltage source, sending electrical current through a specific type of aqueous solution known as Ringer's Solution. The muscle received a sequence of rapid voltages sent through the capacitor over the course of five minutes which regularly alternated between indirect stimulation of muscles via stimulation of nerve terminals on myofibers, and direct stimulation of the muscle via voltage gated-ion channels within the sarcolemma. The timing of voltage sent through the muscle determines indirect and direct stimulation, as shorter periods (0.2 milliseconds) of 37V electrical current only activates the voltage-gated ion channels of the NMJ, and does not directly reach the sarcolemma. Longer voltages (2 milliseconds) stimulate both the voltage-gated ion channels of the nerve and the postsynaptic cells, though the signaling from the nerve becomes redundant since the muscle is directly stimulated from a greater source of electrical stimulation, and the total possible force output of the soleus can rarely be achieved through neural stimulation alone. A two-way ANOVA was conducted with two main effects: 1) treatment (control and hindlimb suspension), and 2) age (juvenile and young adult), and this was used to assess various measures of neuromuscular function. A p-value of ≤ 0.05 was used as a reference to measure significance, and Tukey post-hoc tests were used to confirm and evaluate any statistically significant interactive findings.

Results

Prior to the two-week period, average body mass was collected as follows: CTL = 161.9g and HS = 162.2g; and directly following the two-week period, the weights were: CTL = 244.1g and HS = 233.1g. To clarify, there was no significant difference for either of the time periods, however following anesthetized removal of the soleus muscle, the average mass for the isolated soleus muscle showed a significant difference between the HS and CTR groups ($p = 0.0003$) with the average soleus from CTL equaling 119.3mg, and HS weighing only 83.5mg. The significant change in isolated soleus weight confirmed that the HS group experienced greater overall muscle atrophy relative to the control. The maximum force output in newtons, referred to as "peak tension," from both direct and indirect stimulation was compared from the first minute of the five-minute protocol, and the final minute of the protocol. Many parameters of

[i] The time period of two weeks cannot be translated into "human time" in the same way age can be translated. The period of two weeks in "rat time" should be considered roughly equivalent to two weeks in "human time." Impairment over time roughly follows exponential decline, and levels of impairment that occur in long-term unloading are largely represented in periods of two weeks.

[ii] The soleus was chosen as it is a postural muscle with largely type I fiber composition. It also has a high-usage pattern, meaning under normal conditions, it is used regularly for long periods of time. The soleus muscle is consistent with the goal of understanding effects of muscle unloading on frequently-used voluntary muscles.

neuromuscular functioning can be derived using these measurements. First, the percent of muscle stimulation from the first minute that could be achieved during the fifth minute is referred to as "fatigue." Fatigue was calculated for both direct and indirect muscle stimulation. The juvenile HS group experienced significantly greater fatigue for both indirect ($p = 0.0006$) and direct ($p = 0.0101$) stimulation, meaning that by the fifth minute of the protocol, juvenile HS could only achieve 15.380% of what they originally achieved via indirect nerve stimulation, and 8.650% of what they originally achieved via direct muscle stimulation. The other groups (adult CTL, adult HS, and juvenile CTL) experienced fatigue, but to a far lesser extent, experiencing a range of only 67.109%-68.580% fatigue for indirect and 42.200%-53.780% fatigue for direct stimulation. Another parameter of interest is referred to as "neuromuscular efficiency." Neuromuscular efficiency is defined as the percent of peak tension that can be achieved via indirect nerve stimulation alone versus that which is generated via direct stimulation of the sarcolemma. Within the first minute, HS juveniles experienced reduced neuromuscular efficiency compared to the other groups ($p = 0.0916$), as nerve stimulation only achieved 48.200% of the peak tension from direct stimulation. Though a p value less than or equal to 0.05 was not achieved for neuromuscular efficiency within the first minute, the trend for loss of neuromuscular efficiency for HS juveniles remains consistent with other demonstrations of neuromuscular loss specific to juveniles. The other three groups maintained a higher ability of the NMJ to achieve muscle stimulation, as 71.009% efficiency was achieved for juvenile CTL, 82.720% efficiency was achieved for adult CTL, and 83.890% efficiency was achieved for adult HS during the first minute. A final parameter of interest is "specific force," which is a ratio of peak tension relative to each individual soleus muscle mass. Even when controlling for the size of the soleus, the juvenile HS continued to demonstrate lower specific force relative to the other groups for indirect stimulation ($p = 0.0464$). The interaction was also evident for direct stimulation ($p = 0.081$) meaning that the specific force achieved by the muscle itself suffered the greatest for the juvenile HS group, even when controlling for relative soleus mass. The p value for specific tension of direct stimulation ($p = 0.081$) does not fall within the limit of $p \leq 0.05$, however the findings show a strong trend that is consistent with the rest of the data and with the hypothesis.

Discussion

Based on our findings, we know that the many functions of the neuromuscular system, including neuromuscular efficiency, fatigue, and specific force, suffer after periods of muscle unloading, and we know that the loss of neuromuscular function is far more prevalent when periods of non-use occur during periods of growth development. There remains a question of why: what are the specific morphological origins behind neuromuscular inefficiency, fatigue, and specific force, and how does this differ over the course of an organisms' lifespan? Our research lab will follow up with this question by staining muscle fibers using immunofluorescence in order to observe morphological components of the NMJ for the mentioned groups: juvenile CTL, young adult CTL, juvenile HS, and young adult HS. The immunofluorescent staining allows for comparison of pre- and post-synaptic components of the NMJ such as bassoon, a protein of the active zone on the presynaptic nerve, voltage-gated presynaptic calcium channels, vesicles of the presynaptic nerve cell containing acetylcholine, and nicotinic acetylcholine receptors at the postsynaptic membrane. The goal is to isolate individual components of the NMJ to identify the origin of neuromuscular function loss occurring during muscle unloading, and uncover why these losses are more prominent during periods of non-use for developing neuromuscular systems as opposed to developed neuromuscular systems. Furthermore, our lab continues to investigate NMJs of different muscle fiber types, usage types, and different sexes, with the hopes of uncovering many of the mysteries and nuances about neuroplasticity at the NMJ. With the help of other researchers, we hope to have a better understanding of the limitations in using rat models, and understand the extent to which the data gathered from those models can be accurately applied to human bodies. Nevertheless, the findings from our study have great implications on long-term muscle unloading that a neuromuscular system would undergo in the case of long-term space travel. Juvenile neuromuscular systems that are undergoing normal development suffer far greater consequences from long-term muscle unloading compared to fully developed neuromuscular systems. The consequences of these findings are relevant for colonization on a planet with reduced gravitational force, such as Mars, and are relevant for long-term microgravitational travel involving children. For normal neuromuscular development to occur as we know it on Earth, there would need to be extra measures taken to combat loss of neuromuscular function that youth experience more severely than adults.

Sources

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