AGE-SPECIFIC STRUCTURAL ADAPTATIONS TO THE NEUROMUSCULAR SYSTEM IN RESPONSE TO MICROGRAVITY

Max Rackley
William & Mary Program in Neuroscience
Advisor Michael Deschenes, PhD
Virginia Space Grant Consortium

Abstract

Every human body system is subject to changes without a gravitational force, especially components of the neuromuscular system. However, most of our current understanding comes from fully developed adult systems, neglecting those from developing adolescents. Thus, this study aimed to identify how vital structures among different aged neuromuscular systems adapt to microgravity. The plantaris muscle, responsible for forceful contractions during locomotor activities imperative for the success of space missions, was sectioned from young and mature rats, which were randomly assigned to either a weight-bearing control or hind-limb suspension group to mimic microgravity conditions. Muscles were stained using immunofluorescent procedures to visualize structural components of the neuromuscular system, imaged using confocal and fluorescence microscopy, and measured digitally. Results reveal an age-specific response in which juvenile muscles saw greater atrophy in response to microgravity conditions; however, the opposite effect was observed when looking more microscopically, where mature muscles saw a significant redistribution of synaptic structures. Given that different structures of the neuromuscular system responded differently despite being exposed to the same intervention, age-specific countermeasures should be developed to ensure astronauts of all ages are healthy and safe in space.

Introduction

Before we can take the next giant leap for mankind and explore planetary bodies, it’s crucial that we take one small step to understand the consequences microgravity has on human bodies of all ages. In space, every body system is subject to harmful changes, especially the neuromuscular system[1]. One of the most integral structures to the neuromuscular system is the neuromuscular junction (NMJ) which, as illustrated in Figure 1, is the structure located between a motor neuron and muscle fiber[2]. The NMJ is directly responsible for each forceful muscle contraction and has been known to be extremely susceptible to changes in the environment.

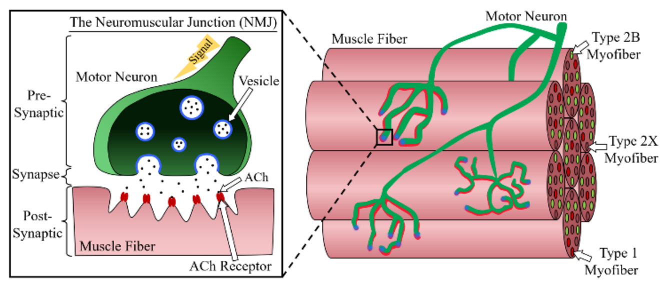

Figure 1*: Neuromuscular system structure and function. Once an electrical signal reaches the end of a motor neuron, vesicles release Acetylcholine (ACh), which diffuses across the synapse and binds to ACh receptors on the muscle fiber, resulting in a muscle contraction. Each fiber contains different types of myofibers that have unique force-output and resistance to fatigue. Adapted from Castets et al., 2020.*

Whether the body is experiencing extended bed rest or long-duration spaceflight, the lack of NMJ stimulation and muscle use causes the structures to deteriorate in an effort to conserve valuable resources and energy[3]. As the NMJ decays, the involved muscle also responds, decreasing the size and composition of its contractile units, known as myofibers. Astronauts notice this disuse response within just a few days in a microgravity environment despite engaging in the most current, rigorous form of exercise countermeasures[4]. Neuromuscular remodeling is dangerous and if not managed appropriately can cause serious, irreversible consequences that will impair or even prevent the success of missions in space.

Given that extraterrestrial colonization is on the horizon, not only will astronauts be exposed to an unforgiving microgravity environment for extended periods, but children will also inevitably be space explorers too. Most of our current knowledge surrounding the risks of microgravity comes from fully developed neuromuscular systems, and it is still unclear how a child's developing neuromuscular system might react to the unknowns of space[1].

Since components of NMJ can undergo remarkable adaptations to the environment and exhibit significant control over its myofibers, this study assesses whether developed or developing systems experience more severe structural adaptations in response to low gravity. Investigating how different aged neuromuscular systems respond to muscle disuse experienced in space contributes to the improvement of protective measures, ensuring both adult and children extraplanetary explorers can operate to the best of their ability. In addition to aiding NASA astronauts, results from this study will also benefit people on Earth. By increasing our understanding of the fundamental mechanisms and contributing structures behind neuromuscular plasticity, our study fortifies the prevention and treatment of those who suffer from debilitating neuromuscular disorders with no cure like ALS or myasthenia gravis[5].

<u>Experimental Design</u>

Instead of using human subjects, rats were leveraged since their neuromuscular systems closely resemble those of humans. An outline of the procedure for this study can be seen in Figure 2. To determine the effects microgravity has on the development of neuromuscular systems, 20 juvenile and 20 mature rats were randomly assigned to either a weight-bearing control group or a hindlimb-suspended experimental group (Step 1). These 3- and 8-month-old rats roughly translate to 9- and 24-year-old humans. Rats within the control group used all four limbs during normal, daily activities, whereas those in the experimental group mimicked the physiological effects of microgravity by being subjected to the widely used hindlimb-suspension model[6,7]. Rats with their hindlimbs suspended experienced muscular atrophy and fluid shift to the head, similar to that experienced in space. Simply immobilizing the limbs does not result in the same observed effects, and therefore could not be used as a model to accurately simulate a microgravity environment.

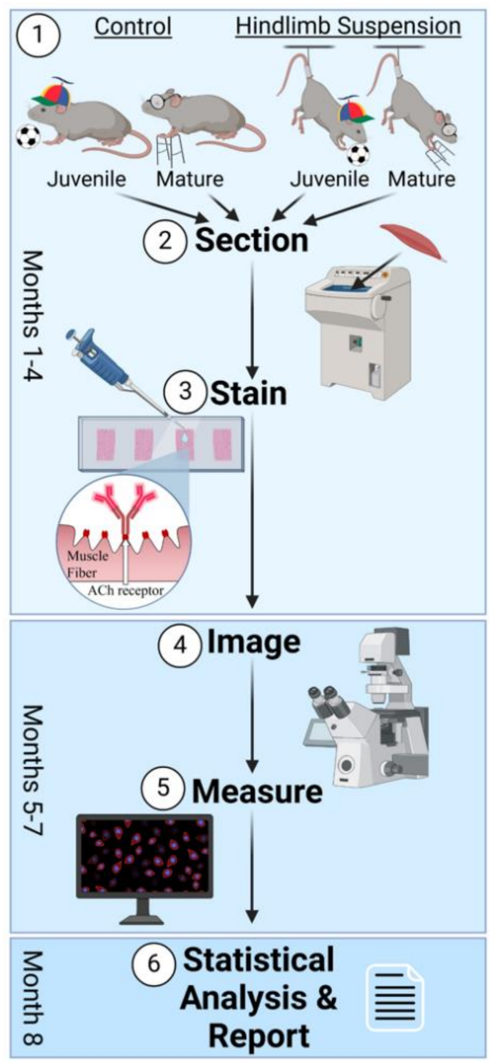

Figure 2: *Project summary and timeline.*

After 2 weeks within their treatment group, the 40 rats were anesthetized and their plantaris muscles were obtained according to proper protocol. Although not an antigravity muscle with an abundance of slow-twitch (type 1) fibers, the plantaris muscle normally contains a high composition of fast-twitch (type 2B and 2X) fibers, responsible for producing forceful, voluntary muscle contractions that would be imperative for the success of NASA mission-specific tasks[8].

Juvenile and mature plantaris muscles were stored at -80 °C to ensure the samples were well preserved. Then, muscles were sectioned (Step 2) and stained using immunofluorescent procedures (Step 3) to visualize variables vulnerable to remodeling. This included myofiber types as well as pre- and post-synaptic components of the NMJ which can be seen in Figure 3.

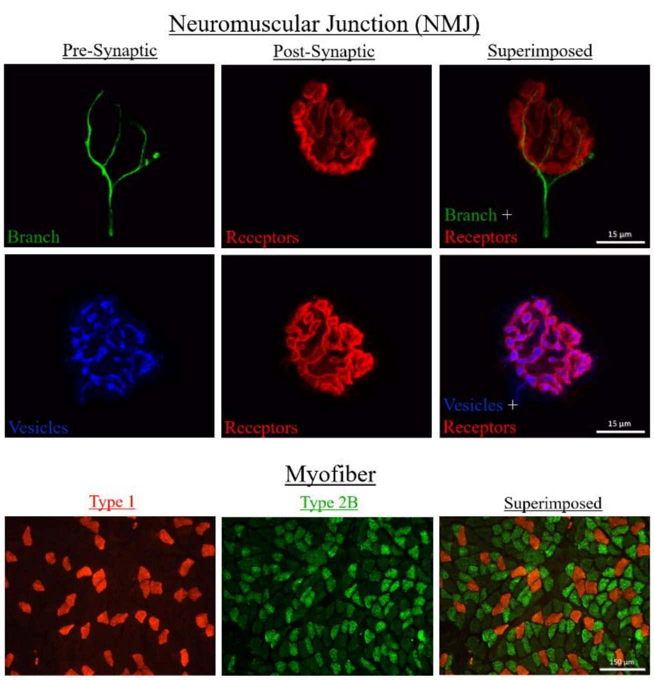

Figure 3: *Representative NMJ and myofiber images from the plantaris muscle. The scale bar for NMJ images is 15 µm and 150 µm for myofiber images.*

Blinding procedures were performed to eliminate any possibility of bias while imaging and measuring. Pre- and post-synaptic components of the NMJ were imaged using a confocal microscope, whereas myofiber profiles were imaged with a fluorescence microscope (Step 4). Pre-synaptic NMJ variables that were assessed include the length and number of motor neuron branches as well as the area and perimeter of vesicle clusters. The post-synaptic variable of interest was the area and perimeter of the stained acetylcholine receptors on the muscle fiber. Myofiber types were analyzed based on area and percent composition. Once each variable was measured digitally (Step 5), an ANOVA statistical analysis was performed (Step 6), and in the event of a significant effect ($p \leq 0.05$), a Tukey post-hoc test was utilized to observe pair-wise differences.

Results

Data from myofiber profiles can be seen in Table 1, where the only statistically significant effect lies between age groups (juvenile vs mature) rather than treatment (muscle use vs muscle disuse). Despite being the least abundant fiber type in plantaris muscle, type 1 fibers experienced the most severe reduction in area in response to muscle disuse. However, the degree of type 1 fiber's physiological adjustments revealed an age-specific trend. In response to microgravity conditions, mature muscles experienced a 20% reduction whereas juvenile muscles experienced a more severe 33% reduction in type 1 area. Type 2B fibers were by far the most prevalent fiber type present across both age groups and were slightly more resistant to the effects of microgravity (12% reduction in mature muscles vs. 27% area reduction in juvenile muscles). As for the most resistant fiber type in this experiment, type 2X myofibers in mature muscles saw only a 3% reduction in area and 17% for juvenile muscles. Regarding fiber type composition, there was a reduction in the percentage of type 1 fibers in

	Variable	Juvenile Control	Juvenile Hindlimb Suspension	Mature Control	Mature Hindlimb Suspension
Area	Type 1 (µm²)	1503.55 ± 112.68	1130.95 ± 117.31	1795.86 ± 110.00	1486.95 ± 120.40
	Type 2B (µm²)	1080.36 ± 70.54	853.11 ± 53.16	1603.25 ± 84.66	1428.47 ± 138.79
	Type 2X (µm²)	1679.77 ± 98.73	1429.74 ± 71.62	2555.65 ± 104.68	2480.02 ± 297.07
	Overall (µm²)	1421.22 ± 87.26	1137.94 ± 47.12	1984.92 ± 74.30	1798.48 ± 173.47
Percent	Type 1 (%)	11.3 ±1.6	17.4 ± 4.9	16.8 ± 2.1	15.1 ± 1.7
	Type 2B (%)	47.0 ± 2.0	47.4 ± 1.6	46.6 ± 3.0	52.7 ± 3.5
	Type 2X (%)	41.7 ± 2.5	35.2 ± 5.1	33.4 ± 3.0	32.2 ± 4.1

Table 1: *Myofiber data. Values are means ± SE. N = 10 per group.*

		Variable	Juvenile Control	Juvenile Hindlimb Suspension	Mature Control	Mature Hindlimb Suspension
Pre-Synaptic	**Nerve Branching**	Number of Branches	3.85 ± 0.21	3.53 ± 0.24	5.46 ± 0.49	4.67 ± 0.32
		Total length (µm)	58.85 ± 2.93	56.14 ± 4.06	79.02 ± 5.19	72.43 ± 5.42
		Branch Complexity (%)	2.47 ± 0.17	2.28 ± 0.33	4.97 ± 0.70	3.95 ± 0.48
	Vesicles	Total Area (µm²)	331.91 ± 23.81	286.99 ± 15.21	473.92 ± 25.11*	374.36 ± 24.18*
		Total Perimeter (µm)	78.24 ± 2.66	72.15 ± 2.24	88.57 ± 3.02	82.64 ± 1.87
		Stained Area (µm²)	84.21 ± 6.58	77.01 ± 2.83	130.36 ± 13.17*	98.80 ± 6.80*
		Stained Perimeter (µm)	186.16 ± 13.50	168.06 ± 6.77	228.64 ± 9.84	219.06 ± 13.86
Post-Synaptic	**ACh Receptors**	Total Area (µm²)	367.49 ± 15.08	318.52 ± 11.76	522.18 ± 19.37*	433.7 ± 25.46*
		Total Perimeter (µm)	82.44 ± 1.46	75.79 ± 1.76	92.09 ± 2.08	86.67 ± 2.01
		Stained Area (µm²)	182.12 ± 7.27	168.59 ± 5.16	238.25 ± 7.93	225.69 ± 12.00
		Stained Perimeter (µm)	198.54 ± 7.70	167.89 ± 7.64	262.00 ± 11.03*	213.33 ± 10.78*

Table 2: *NMJ data. Values are means ± SE. N = 10 per group.*
Branch Complexity = (Number of Branches × Total Length)/100
** indicates significant (p ≤ 0.05) difference between control and hindlimb suspension intervention.*

mature muscles, whereas juvenile muscles actually saw a slight increase. Finally, the fiber type that was the same despite intervention was type 2X fibers in mature muscles, but type 2B fibers were constant in juvenile muscles.

NMJ data depicted in Table 2 also reveal a significant difference between age groups; however, there was a significant difference in intervention for only mature muscles. Comparing muscles exposed to microgravity conditions and those that did not, there was a significant reduction in total and stained vesicle areas. Additionally, the ACh receptor total area and stained perimeter saw a significant reduction. This effect was only apparent in mature muscles, not juveniles. Although not meeting statistical significance, mature muscles also saw a greater reduction in the other pre-synaptic variable of interest: nerve branching. Branch complexity, for example, which considers both the number and length of branches saw a 26% reduction in mature muscles but only a 9% reduction in juveniles.

Discussion

Although it has been more than 50 years since humans have set foot on the moon, humanity has always kept its eye on the sky and vowed to return and develop a sustained presence with our children. Before we can establish a long-term presence in space, it is imperative that results from this study are considered in order to fully understand the underlying mechanisms of how developed and developing neuromuscular systems respond to long-term space travel.

Analysis of myofiber results suggest that adult and juvenile muscles showed similar, but different degrees of atrophy. Across every myofiber type, juveniles' developing muscles saw more severe atrophy in response to microgravity. Additionally, mature muscles saw a slight decrease in type 1 fiber expression, whereas juvenile muscles saw an increase in the percentage of these antigravity fibers. This unique redistribution of type 1 fibers found only in the developing neuromuscular system may be an attempt to compensate for the reduction of type 1 fiber area. These myofiber trends suggest juvenile plantaris myofibers may be more sensitive to the harmful effects of microgravity than those found in adults.

Interestingly, when looking more microscopically, the opposite effect is observed where mature muscles were more susceptible to microgravity conditions. There has been data supporting this disconnect between the NMJ and its myofibers[9]. Here, mature muscles experienced significant remodeling of the vesicle area containing chemical messengers as well as the receptor area to receive those messengers. This suggests that microgravity impairs the microscopic communication before and after the synapse in mature muscles, but not juveniles. According to the data, it is still true that neither juvenile nor mature muscles are spared from the harmful effects of microgravity; however, mature muscles appear to experience more severe remodeling to various pre-synaptic vesicles and post-synaptic receptors. Therefore, the opposite effect is occurring in which a developing neuromuscular system may be more resilient to the disrupted cellular communication experienced in microgravity environments.

Although one of our greatest strengths on Earth, the human body's fascinating ability to adapt to its environment becomes a major limitation as we turn toward our final frontier for human exploration. Astronauts train rigorously to prevent the neuromuscular disuse response experienced during space travel. However, given the direction space tourism is heading, traveling to space will become available not only to well-trained astronauts, but also to the general public and children. Results from this study highlight that different aspects of the neuromuscular system respond differently, despite being exposed to the same intervention. Thus, before anyone without extensive training can become a modern explorer, it is vital that improved countermeasures are developed to combat the different age-specific responses to long-term microgravity.

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