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Muscle fibers and their synapses differentially adapt to aging and endurance training☆

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ABSTRACT

Background: This project aimed to determine the adaptability of the neuromuscular system to the stimuli of exercise training, and aging.

Methods: Young adult, and aged male rats were randomly assigned to either exercise training, or sedentary control groups. Exercise training featured an 8 week program of treadmill running. At the end of the intervention period, neuromuscular function was quantified with ex vivo stimulation procedures on isolated soleus muscles. Morphological adaptations were determined by quantifying myofiber profiles (fiber size and type) of soleus muscles.

Results: Ex vivo procedures confirmed that rested (fresh) young muscles were significantly ($P < 0.05$) stronger than aged ones. By the end of the 5 min stimulation protocol, however, young and aged muscles displayed similar levels of strength. Neuromuscular transmission efficacy as assessed by comparing force produced during indirect (neural) and direct (muscle) stimulation was unaffected by aging, or training, but under both conditions significantly declined over the stimulation protocol mimicking declines in strength. Myofiber size was unaffected by age, but training caused reductions in young, but not aged myofibers. Aged solei displayed a higher percentage of Type I fibers, along with a lower percentage of Type II fibers than young muscles.

Conclusions: The greater strength of young muscles has a neural, rather than a muscular focal point. The loss of strength discerned over the 5 min stimulation protocol was linked to similar fatigue-related impairments in neuromuscular transmission. The two components of the neuromuscular system, i.e. nerves and muscles, do not respond in concert to the stimulus of either aging, or exercise training.

1. Introduction

The neuromuscular is one of several organ systems that are essential to the proper function (physiology), and structure (anatomy) of the mammalian body. This highly integrative system plays an essential role in maintaining health (e.g. regulation of proper blood glucose, protein storage), enabling physical ambulation, as well as making possible the performance of the activities of normal daily living such as climbing stairs, and carrying suitcases, along with the execution of sports and recreational activities. It is clear then that a healthy, fully functional neuromuscular system is integral not only to a normal, healthy life, but also to sustain a desirable quality of life.

Typically, the neuromuscular system is considered to be comprised of skeletal muscle fibers, the motor neurons that excite these myofibers, and the neuromuscular junction (NMJ), which is the synapse that transducts the electrical impulses carried by motor neurons to their associated myofibers (Balice-Gordon, 1997a; Li et al., 2018). This vital system has been shown to possess a remarkable degree of plasticity in response to a variety of factors. Two such factors are changes in neuromuscular activity patterns (Andonian and Fahim, 1988; Fahim, 1997), and aging (Balice-Gordon, 1997b; Willadt et al., 2018). Indeed, our laboratory has previously demonstrated that both decreased activity in the form of muscle unloading, as well as increased activity presented as exercise training, result in morphological remodeling of both NMJs (Deschenes et al., 1993, 2006), and myofibers (Deschenes et al., 1995, 2006). Moreover, it has been found that changes in activity, again both increases and decreases, also induce functional neuromuscular adaptations (Fahim, 1997; Fitts et al., 2010). Of particular interest are the few studies that have examined the interaction between altered activity patterns with aging. In those that have, it has been determined that aged neuromuscular systems were more vulnerable to the detrimental effects of decreased activity patterns than were young

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NMJs (Deschenes and Wilson, 2003; Hepple and Rice, 2016), and myofibers (D'Antona et al., 2003). The purpose of the present investigation was to ascertain whether aging would similarly influence functional and morphological adaptations of the neuromuscular system as a result of participation in an extended endurance exercise training regimen.

2. Materials and methods

2.1. Subjects

Forty male Fischer 344 rats were purchased from the National Institute on Aging breeding colony. Twenty of these animals were considered aged, i.e. 23 months or the approximate equivalent of 70 years of age in a male human's life, while the other twenty were considered young adults at the age of 7 months of age, which is roughly 21 years old in the average lifespan of humans living in the United States (Arias et al., 2017). Rats from each age category were then randomly assigned to either an 8 week endurance training group, or to sedentary, control conditions resulting in 4 experimental groups of 10 rats each. Animals in the endurance trained group participated in a 5 days/week treadmill running program. For both young and aged trained animals, the endurance training regimen began with 2 familiarization trials on the motorized treadmill (Accuscan, Columbus, OH, USA) with a running speed of 5 m/min at a 0% grade for 5 min. After these familiarization trials, the actual training regimen began with a duration of 15 min/session at a 0% grade and a velocity of 7.5 m/min. Over the 8 week course of the training program, the training duration was gradually increased to 60 min/session at a velocity of 15 m/min with the treadmill remaining at a 0% grade. Increments in running speed and duration were determined by the tolerance of aged animals, and were replicated by young ones to ensure that both age groups completed the same training protocol. Exercise tolerance was subjectively determined by visual indications of physical fatigue such as labored strides, heavy panting, and inability to maintain pace. Towards the end of the training program, two of the aged rats suffered toe injuries and were unable to complete the 8 week training intervention resulting in a sample size of eight for that treatment group, i.e. aged-trained. Animals for all four treatment groups were kept two per tub (lined with wood shavings), and were provided with standard rat chow and water ad libitum. The environment in which the animals lived featured a 12/12 hr light-dark cycle with the temperature maintained at 21–22 °C at a relative humidity of ~50%. All procedures were approved beforehand by the Institutional Animal Care and Use Committee which abides by the National Institutes of Health Guide for the Care and Use of Laboratory Animals as revised in 2011. Throughout the project every effort was made to minimize the number of animals used and to alleviate any discomfort.

2.2. Neuromuscular performance

Following completion of the endurance training program, neuromuscular performance of the soleus muscle was assessed with an ex vivo muscle stimulation and recording instrument (system 1205A, Aurora Scientific Inc. Aurora, ON, Canada). The soleus was selected for study because it is heavily recruited during locomotor activity (Roy et al., 1991; Yang et al., 1995), and thus could be expected to demonstrate training-induced adaptations. In preparation for performance analysis, muscles were surgically removed from living, anesthetized rats (ketamine/xylazine cocktail of 50/10 mg/kg body mass). Following a 15 min incubation in Ringer solution (137 mM NaCl, 4.7 mM KCl, 3.4 mM $CaCl_2$, 1.2 MM $MgSO_4$, 1 mM NaH_2PO_4, 112 D-glucose, pH = 7.4) that was vigorously aerated with gas (95% oxygen, 5% carbon dioxide) and maintained at 21–22 °C, the muscle was subjected to an electrical stimulation protocol that first established optimal muscle length in order to determine peak isometric force. The stimulation procedures utilized emulated those of Lomo and Rosenthal (Lomo and Rosenthal, 1972) by alternating indirect and direct stimulation so that neuromuscular transmission efficiency could be quantified. The stimulation parameters consisted of a series of sets featuring nine pulses at a constant 37 V and ~25 Hz for a duration of 0.2 msec (indirect or neural stimulation via nerve terminal endings) followed by a single 2 msec duration pulse (direct muscle stimulation) so that the duration of each set was 30 s. Sets were repeated continuously for a total protocol duration of 5 min. Contractile recordings were collected with the software accompanying the stimulation/recording system, and recordings were later analyzed using the same software. Contractile variables quantified were peak tension (highest single measures from indirect and direct stimulation), specific tension (tension/whole muscle wet weight), time to peak tension (onset of force development determined manually until peak force production), and fatigability (% decline in peak tension over the 5 min stimulation protocol). Each of these parameters was quantified during both indirect (nerve) and direct (muscle) stimulation. Finally, neuromuscular transmission efficiency was determined by dividing peak tension produced during indirect (neural) stimulation by that generated during direct (muscle) stimulation of the soleus and multiplying by 100 to be expressed as a percentage.

2.3. Immunofluorescent staining of myofibers

Following the surgical removal of a single soleus for participation in the ex vivo procedure, animals were euthanized by decapitation. The contralateral soleus was then quickly dissected out, cleared of fat and connective tissue, rapidly frozen at resting length in isopentane chilled with dry ice, and stored at −85 °C until analysis.

To determine myofiber profiles (fiber size, fiber type composition, and % area occupied by fiber type), 10 μm thick tranverse sections were obtained from the midbelly of the muscle using a cryostat (Cryocut 1800, Reichert-Jung, Nußloch, Germany) which was set at −20 °C. Once placed on a microscope slide, sections were rinsed in PBS with 1% BSA for 5 min. All primary antibodies used to ascertain myofiber type were obtained from the Developmental Hybridoma Bank at the University of Iowa. These immunogens were originally isolated by Dr. Stefano Schiaffino (Smerdu et al., 1994) and donated to the Hybrodoma Bank. The antibody BA-D5 diluted to a concentration of 1:10 in PBS with 1% BSA was used to identify Type I myofibers, while SC-71 used at a concentration of 1:1 reacted specifically to Type IIA myofibers. With this staining procedure, Type IIX myofibers can be identified by their lack of immunofluorescence (although still visible). Type IIB myofibers are not expressed in the soleus of mature rats (Delp and Duan, 1996), and thus were not subject to staining. After adding properly diluted primary antibodies, muscle sections were incubated in humidified chambers at 37 °C for 1 h at room temperature. Following this, sections were rinsed three times for 5 min each in PBS with 1% BSA and then incubated with fluorescently labelled secondary antibodies purchased from Molecular Probes (Eugene, OR, USA) at a concentration of 1:500 in PBS containing 5% goat serum. The secondary antibodies were conjugated to Alexafluor 555 to identify Type I myofibers, and Alexafluor 350 to identify Type IIA myofibers (see Fig. 1). Following incubation for 30 min at 37 °C in humidified chambers, sections were rinsed three times, 5 min each, in PBS with 1% BSA before receiving a 3 min rinse in deionized water. Excess water was gently blotted off and Prolong Gold (Molecular Probes, Eugene, OR, USA) was applied to muscle sections before applying cover slips and storing the slides at −20 °C in the dark.

2.4. Microscopy

To quantify myofiber profiles, an Olympus BX41 microscope equipped with fluorescence capacity (X-Cite, Excelitas Technologies Corporation, Waltham, MA, USA) was used. A random sample of