Published in final edited form as:

J Neurosci Res. 2015 March ; 93(3): 504–513. doi:10.1002/jnr.23495.

Effect of Resistance Training on Neuromuscular Junctions of Young and Aged Muscles Featuring Different Recruitment Patterns

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Abstract

To examine the effects of aging on neuromuscular adaptations to resistance training (i.e. weight lifting), young (9 mo old) and aged (20 mo old) male rats either participated in a 6 week ladder climbing protocol with additional weight attached to their tails, or served as controls (N=10/group). At the conclusion, rats were euthanized and hindlimb muscles were quickly removed and frozen for later analysis. Longitudinal sections of the Soleus and Plantaris muscles were collected and pre- and post-synaptic features of neuromuscular junctions (NMJs) were visualized with immunofluorescent staining procedures. Cross-sections of the same muscles were histochemically stained to determine myofiber profiles (fiber type and size). Statistical analysis was performed with a 2 way ANOVA (main effects of age and treatment) with significance set at $P = 0.05$. Results revealed that training-induced remodeling of NMJs was evident only at the post-synaptic endplate region of Soleus fast-twitch myofibers. In contrast, aging was associated with pre- and post-synaptic remodeling in fast- and slow-twitch myofibers of the Plantaris. Although both the Soleus and Plantaris muscles failed to display either training or aging-related alterations in myofiber size, aged Plantaris muscles exhibited an increased expression of Type I (slow-twitch) myofibers in conjunction with a reduced percentage of Type II (fast-twitch) myofibers suggesting early stages of sarcopenia. Overall, these data demonstrate the high degree of specificity of synaptic modifications made in response to exercise and aging, and that the sparsely recruited Plantaris is more vulnerable to the effects of aging than the more frequently recruited Soleus muscle.

Keywords

synapse; acetylcholine; bungarotoxin; nerve terminal; exercise

INTRODUCTION

Important national and international health organizations such as the Centers for Disease Control and Prevention (CDC), the American College of Sports Medicine (ACSM), and the World Health Organization (WHO) have released recommendations for adults and older individuals to participate in a regular program of exercise training (American College of Sports Medicine et al., 2009; Pate et al., 1995; World Health Organization, 2010). These statements and position stands laud the ability of exercise training to effectively prevent or manage a host of non-communicable maladies including cardiovascular disease, stroke, Type II diabetes, obesity, and arthritis.

In addition to endurance, or aerobic style exercise, it is strongly recommended that health-related exercise programs include resistance training, or weight lifting. Resistance training is especially valuable among the aged as this mode of exercise has been found to be successful in treating or preventing chronic health conditions associated with senescence including sarcopenia (age-related loss of muscle mass), osteoporosis, insulin resistance, incidence of accidental falls, bone fracture, and even cognitive decline (Anton et al., 2013; Cederholm et al., 2013; Engelke et al., 2006; Gregory et al., 2013; Liu-Ambrose and Donaldson, 2009; Visser, 2011; Westcott, 2012). Resistance training typically results in positive adaptations of the neuromuscular system including myofibers and neuromuscular junctions (NMJs) which link the motor nervous system with skeletal muscles that are activated by those motor neurons (Andersen and Aagaard, 2010; Deschenes et al., 2000; Folland and Williams, 2007). Clearly, there are distinct advantages to adding resistance training to the exercise regimens performed by the aged. Less clear, however, is whether the neuromuscular systems of older individuals respond to the stimulus of resistance training in a manner similar to the neurons and myofibers of younger individuals. This is a legitimate concern as it has recently been reported that adaptations of the NMJ to endurance training – treadmill running – were different among young and aged animals (Deschenes et al., 2011). The purpose of the present investigation was to determine whether aging affected adaptations of the NMJ and associated myofibers to resistance training.

MATERIALS AND METHODS

Subjects

Twenty young adult (9 months old) and 20 aged (20 months old) male Fisher 344 rats were purchased from the National Institute on Aging Colonies and randomly assigned to either resistance trained (RT) or control (CTL) treatment groups resulting in a total of four groups with N=10/group. The average life expectancy of male Fisher 344 rats is 25.5 months (Turturro et al., 1999) thus at 20 months of age the older rats used in this study had lived 78.5% of their lifespan. Relative to the average lifespan of men in the United States which currently is 76 years (United States Census Bureau, 2012) these older rats would be the equivalent of 60 years old or the age considered to be the onset of senescence by some health organizations and experts (Bloom DE, Boersch-Supan A, McGee P, Seike A, 2011; Forman et al., 1992).

Animals were provided standard rat chow and water ad libitum, and were housed in a constant temperature of 21–22° C with a 12-hour light/dark cycle. All procedures utilized were approved beforehand by the institutional animal care and use committee operating in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Resistance training

The resistance training protocol used consisted of three sessions per week for 6 weeks. Animals climbed a ladder that was 1 meter long and set at an 85° angle with additional weights attached to their tails using Velcro strips. Each training session featured 8 repetitions of ladder climbing and added resistance was initially set at 50% of body mass with 30 g increments added weekly. When necessary, rats were motivated to climb the ladder via a cool water spray. This animal model of resistance training has been shown to effectively recruit the hindlimb extensor muscles (i.e. Gastrocnemius, Plantaris, Soleus) as well as forelimb muscles (Kim et al., 2012; Linderman et al., 1994). Animals assigned to control conditions simply remained in their tubs throughout the 6 week intervention. At the end of the intervention period, all animals were euthanized, and hindlimb muscles were surgically removed, cleared of fat and connective tissue and quickly frozen at resting length in isopentane chilled with liquid nitrogen. Muscles were then stored at –80° C until analysis. The Soleus and Plantaris muscles were selected for analysis because although both are ankle extensors, they have vastly different myofiber type distributions and recruitment patterns. More specifically, the Soleus is mainly comprised of slow-twitch, or Type I fibers, and as a postural muscle displays a high duty cycle while the Plantaris principally expresses fast-twitch, or Type II myofibers, and is only extensively recruited during locomotor activity (Delp and Duan, 1996; Laughlin and Armstrong, 1982).

Cytofluorescent staining

To visualize NMJs, 50 µm thick longitudinal sections of the middle one-third of the muscle, and along its most superficial region, were obtained at –20°C on a cryostat (Cryocut 1800; Reichert-Jung, NuBloch, Germany). To prevent contraction of sections, microscope slides were pretreated in a 3% EDTA solution as previously described (Pearson and Sabarra, 1974). Sections were washed 4 x 15 min in phosphate buffered saline (PBS) containing 1% bovine serum albumin (BSA). Sections were then incubated in a humidified chamber overnight at 4°C in supernatant of the primary antibody RT97 (Developmental Studies Hybridoma Bank, University of Iowa), diluted 1:20 in PBS with 1% BSA. The RT97 antibody reacts with non-myelinated constituents of pre-synaptic nerve terminals (Anderton et al., 1982). The next day, sections were washed 4 x 15 min in PBS with 1% BSA before incubating for 2 h at room temperature in fluorescein isothiocyanate (FITC) conjugated secondary immunoglobulin (Sigma Chemical, St. Louis, MO) that was diluted 1:150 in PBS with 1% BSA. Sections were then washed 4 x 15 min in PBS with 1% BSA. Following this, sections were incubated in a humidified chamber overnight at 4°C in a solution containing rhodamine conjugated α -bungarotoxin (Invitrogen, Grand Island, NY) diluted 1:600 in PBS along with either anti-slow (Soleus) or anti-fast (Plantaris) myosin heavy chain ascites fluid (Sigma Chemical, St. Louis, MO) diluted 1:40. Bungarotoxin (BTX) recognizes post-synaptic acetylcholine (ACh) receptors, while the anti-slow or anti-fast immunogen enabled

us to determine whether the endplate resided on a fast- or slow-twitch myofiber. The next day, sections were washed 4 x 15 min in PBS with 1% BSA before incubating them for 1 h at room temperature in AlexaFluor 647 (Invitrogen, Grand Island, NY) labeled secondary antibody to bind with the anti-slow or anti-fast primary antibody. Sections were given a final wash (4 x 15 min) before being lightly coated with Pro Long (Invitrogen, Grand Island, NY) and having cover slips applied. Slides were then coded with respect to treatment group to allow for blinded evaluation of NMJ morphology and then stored at -20°C , and in the dark, until analysis. An example of this cytofluorescent staining of pre- and post-synaptic components of the NMJ is displayed in Figure 1.

Pre-synaptic variables of NMJs assessed included: 1) number of branches identified at the nerve terminal, 2) the total length of those branches, 3) average length per branch, and 4) branching complexity which, as described previously (Tomas J, Fenoll R, Mayayo E, Santafe M., 1990) is derived by multiplying the number of branches by the total length of those branches and dividing that figure by 100. Post-synaptic variables of interest included: 1) total perimeter, or the length encompassing the entire endplate comprised of stained ACh receptor clusters and non-stained regions interspersed within those clusters, 2) stained perimeter, or the composite length of tracings around individual ACh receptor clusters, 3) total area, which includes stained receptors along with non-stained regions interspersed among receptor clusters, 4) stained area, or the cumulative areas occupied by ACh receptor clusters, and 5) dispersion of endplates, which was assessed by dividing the endplate's stained area by its total area and multiplying by 100. In this study, pre- to post-synaptic coupling was quantified by dividing the NMJ's post-synaptic stained area by its total length of pre-synaptic nerve terminal branching. Figure 2 displays how measurements were made of pre-synaptic branching, as well as an example of how line tracings were made either manually (total) or generated by software (stained) around post-synaptic ACh receptors.

Histochemical staining

To quantify myofiber profiles, 10 μm thick transverse sections were obtained from the midbelly of the muscle using a cryostat set at -20°C . Sections were stained for myofibrillar ATPase activity following pre-incubation at a pH of either 4.55 (Soleus) or 4.40 (Plantaris) according to Nemeth and Pette (1981). It should be noted that this staining technique allows identification of the three fiber types found in the Soleus (Types I, IIA, and IIX). However, the fourth fiber type included in the Plantaris (Type IIB) cannot be distinguished from Type IIX fibers. A representative sample of myofibers stained in this way can be seen in Figure 3. Slides were coded so that measurements could be conducted in a blinded fashion regarding treatment group.

Microscopy

An Olympus Fluoview FV 300 confocal system featuring three lasers and an Olympus BX60 fluorescent microscope (Olympus America, Melville, NY) was used to collect images of NMJs and to identify whether they were located on slow- or fast-twitch myofibers. Using a 100X oil immersion objective, it was initially established that the entire NMJ was within the longitudinal borders of the myofiber and that the area of interest was not damaged during sectioning. A detailed image of the entire NMJ was constructed from a z-series of scans

taken at 1 μm thick increments. To ascertain the myofiber type on which the NMJ resided, a single scan was collected of the fiber using the appropriate wavelength to detect AlexaFluor 647. Digitized, two-dimensional images of NMJs were stored on the system's hard drive and later quantified with the Image Pro-Plus software (Media Cybernetics, Silver Spring, MD). In the majority fiber type within each muscle, 10–12 NMJs were imaged and measurements averaged to represent NMJ structure, but due to paucity, a minimum of five NMJs on the minority myofiber type were used to determine average NMJ structural characteristics for that fiber type.

An Olympus BX41 phase contrast microscope was used to assess myofiber profiles with a 40X objective. Myofiber cross-sectional areas were quantified with the Image Pro-Plus software. A random sample of 125–150 myofibers from each muscle was analyzed to determine average myofiber size (i.e., cross-sectional area) and fiber type composition for that muscle.

Statistical Analysis

All data are reported as means $\pm$ S.E. For each variable of interest, a two-way analysis of variance (ANOVA) with main effects of age and treatment was conducted. In the event of a significant main or interactive effect, a Fisher PLSD post-hoc test was performed to identify significant pair-wise differences. In all analyses, statistical significance was established at $P = 0.05$.

RESULTS

Body mass

Prior to the start of the 6 week intervention program, Aged animals weighed significantly more than Young ones, but in neither age category were there differences in body mass between rats assigned to RT and CTL groups. When animals were weighed again at the conclusion of the experimental period it was once again noted that Aged ones weighed significantly more than Young ones. But unlike results at pre-intervention, the post-intervention ANOVA results indicated a significant main effect for treatment where the CTL animals weighed more than the RT group. But when post-hoc procedures were performed it was only among the Young rats that the effect of treatment category was found to be significant, i.e. trained rats weighed less than controls.

Whole muscle wet weight

Upon euthanization at post-intervention, the Soleus and Plantaris muscles were surgically removed and weighed before they were frozen. In the highly recruited Soleus, a significant main effect of age was found (Aged > Young), while no effect for treatment was identified. Post-hoc results indicated that in both treatment groups, i.e. RT and CTL, aged Soleus muscles displayed greater mass than young ones. Conversely, data collected on the wet weight of Plantaris muscles demonstrated a significant main effect for treatment (RT > CTL) but not for age. Post-hoc analyses showed, however, that while resistance training resulted in heavier Plantaris muscles in aged rats, this was not apparent in young animals. Data regarding body mass and whole muscle wet weight can be found in Table 1.

NMJ morphology

Soleus (slow-twitch)—When examining the synapses found on the predominant myofiber type of the Soleus (slow-twitch) it was ascertained that neither resistance training, nor age impacted pre-synaptic structure. This was found to be true for each of the variables quantified regarding nerve terminal branching. But with respect to pre- to post-synaptic coupling, results indicate a significant effect of aging whereby aged NMJs showed a greater endplate area per total nerve terminal branch length. More specifically, this greater post- to pre-synaptic ratio was established in Aged, RT rats compared to Young, CTL and RT animals.

In examining post-synaptic endplate dimensions among slow-twitch NMJs, results confirmed that cumulative or total perimeter length of tracings surrounding stained clusters of ACh receptors in Aged, CTL exceeded those lengths in Young, RT rats. And when quantifying endplate area, both total area (which includes stained receptor clusters and unstained areas interspersed among clusters) and stained area (aggregate area of stained clusters only), it was revealed that aging had resulted in expanded areas under both control and trained conditions. When using data from total endplate area and stained endplate area to determine dispersion of ACh receptors, a treatment effect was identified indicating that RT resulted in a more compact – receptors occupying a greater proportion of total endplate area - or less dispersed, distribution of post-synaptic receptors among young, but not aged synapses.

Soleus (fast-twitch)—Similar to what was detected among NMJs of the predominant slow-twitch myofibers, synapses among the minority fast-twitch myofibers of the Soleus failed to display significant effects of age or treatment in nerve terminal branching parameters. And although pre- to post-synaptic coupling ratio was significantly influenced by age in slow-twitch NMJs, no such effect was discerned among NMJs residing on fast-twitch myofibers.

When quantifying perimeter lengths surrounding post-synaptic fast-twitch endplate regions, neither aging nor training modified either total or stained lengths. However, when quantifying total and stained endplate area, there was a significant effect of treatment (RT > CTL) in both young and aged animals. This differed from what was found in Solei slow-twitch NMJs where there was an effect of aging (Aged > Young) but no training effect. Finally, with respect to ACh receptor dispersion in fast-twitch NMJs, it was noted that resistance training resulted in more dispersed receptor clusters, but only among aged endplates. This, too, differed from what was observed in slow-twitch endplates where training was associated with more compact – i.e. higher percentage of total endplate area occupied by ACh receptors - or less dispersed NMJs, but only among young and not aged animals. All data on Soleus NMJ morphology are presented in Table 2.

Plantaris (fast-twitch)—In the Plantaris, it is fast-twitch myofibers that are predominantly expressed. In analyzing NMJs residing on those fast-twitch myofibers, a significant main effect for aging was detected for each pre-synaptic variable assessed. That is, aged synapses featured a greater number of nerve terminal branches, total branch length,

average branch length, and branching complexity relative to young NMJs. In contrast, results indicated that resistance training failed to alter pre-synaptic morphology. Moreover, neither age nor training altered pre- to post-synaptic coupling.

In examining post-synaptic endplates on these fast-twitch NMJs, statistical analysis revealed, again, a main effect of aging whereby aged endplates were found to have significantly longer perimeter lengths – both total and stained only measurements – as well as greater endplate areas – again both total and stained only measurements – compared to young animals. Yet no effect of training was identified in endplate morphology. Finally, dispersion of post-synaptic ACh receptors was impervious to both resistance training and aging.

Plantaris (slow-twitch)—In slow-twitch NMJs of the Plantaris, it was once again revealed that aging was coupled with significantly larger pre-synaptic nerve terminal branching patterns. This closely paralleled what was identified in the predominant fast-twitch NMJs. The sole exception being that average branch length in slow-twitch NMJs was unaffected by aging, whereas average branch length in aged fast-twitch NMJs was longer than in young fast-twitch synapses. And as was discovered among Plantaris fast-twitch NMJs, neither age nor resistance training altered pre- to post-synaptic coupling in slow-twitch NMJs of the Plantaris.

Again, our analysis of post-synaptic slow-twitch Plantaris NMJs yielded results that mimicked what was found in endplates of the predominant fast-twitch NMJs of that muscle. More specifically, aging resulted in significant enhancement of total and stained perimeter lengths, along with significantly expanded total and stained endplate areas. Resistance training, on the other hand, had no effect on post-synaptic morphology. And as with fast-twitch endplates, neither age nor training altered the dispersion of ACh receptors in post-synaptic endplates of slow-twitch myofibers of the Plantaris. All data regarding NMJ morphology of Plantaris muscles can be examined in Table 3.

Myofiber morphology

Soleus—Statistical results from analysis of the heavily recruited, postural Soleus muscle inform that neither age nor treatment (i.e. resistance training) affected myofiber size when data from all fiber types were pooled together. This was also the case when examining predominant Type I (slow-twitch) fibers exclusively, as well as when Type IIA and IIX fibers were quantified by themselves. Similarly, neither age nor training significantly changed the fiber type composition of the Soleus.

Plantaris—In contrast to the Soleus, the Plantaris, while also an ankle flexor, is mainly comprised of Type II (fast-twitch) myofibers and is only sparsely recruited under resting conditions as its main function is to serve in locomotor activity. With data from fiber types collapsed together, Plantaris myofiber size was unaffected by age or resistance training. This same resilience to alterations in size was also observed when quantifying myofibers by individual fiber type (i.e. Type I, IIA, or IIX/B). However, when assessing fiber type composition of the Plantaris, it was found that although resistance training failed to alter fiber type distribution, aging did result in a significant modification. Specifically, it was

found that aging resulted in a significant decrease in the expression of Type II fibers that was accompanied by a similar increase in the percentage of Type I fibers comprising the Plantaris. This finding was mainly explained by a decline in the content of Type X/B fibers, as no appreciable variation in the percentage of Type IIA fibers was associated with aging. Myofiber profile results are presented in Table 4.

DISCUSSION

With the aging segment of populations in virtually all industrialized nations showing increasing growth, a major public health effort has been put forth to develop interventions to maintain good health so that health care costs associated with aging might be prevented from spiraling out of control (Charness, 2014; Pandya et al., 2013). Convincing evidence has been accrued demonstrating the success of exercise training as a cost-effective method of maintaining or improving health among senescent individuals (Allen and Morelli, 2011; Desveaux et al., 2014; Fleg, 2012). Included among exercise regimens prescribed for the aged is resistance training, or weight lifting. This mode of exercise may confer benefits such as greater strength and muscle mass, improved skeletal health, and better control of blood glucose levels (American College of Sports Medicine et al., 2009; Gasiorowski and Dutkiewicz, 2012). An important component of the neuromuscular system that is responsive to resistance exercise is the NMJ which functionally and anatomically connects the motor nervous system to skeletal muscle fibers (myofibers). It is known that aging results in remodeling of the NMJ (Andonian and Fahim, 1989; Anis and Robbins, 1987; Fahim et al., 1983; Jang and Van Remmen, 2011) and that it also influences NMJ adaptability to endurance training (Deschenes et al., 2011; Fahim, 1997). The main objective of the present investigation was to determine whether aging also impacted the ability of the NMJ to respond to resistance training. We examined this in two muscles with vastly different myofiber type compositions and principal functions. The Soleus is mainly composed of slow-twitch myofibers, and it is characterized by a high duty cycle meaning that it is regularly recruited due to its function as the main postural muscle. In turn, the Plantaris is primarily comprised of Type II (fast-twitch) myofibers and functions mainly as a locomotor muscle thus being recruited far less than the Soleus (Laughlin and Armstrong, 1982). One of the somewhat unexpected findings of this study was that among young rats, resistance trained soleus muscles weighed less than the soleus muscles of untrained Controls. This result mirrored what was found in body mass, that is, young trained rats weighed significantly less than young Controls. It can only be assumed that the training regimen sufficiently increased physical activity levels so that declines in body mass were observed. Alternatively, it is also possible that regular exercise curtailed the appetite of young rats so that they consumed fewer Calories during the intervention period, thus adding on less body mass than those subjected to control conditions.

More germane to the focus of the current investigation, our results showed that of the muscles examined it was the Soleus, and not the Plantaris, that displayed NMJ adaptations to resistance training. But those adaptations were evident only among NMJs that resided on the sparingly expressed fast-twitch myofibers and were assessed as increased post-synaptic endplate area noted among both young and aged rats, as well as greater ACh receptor dispersion among aged NMJs only. Also noted was a main effect of aging in pre- to post-

synaptic coupling of NMJs residing on slow-twitch muscle fibers. This could be explained by expansions of post-synaptic endplate size among the aged animals without a concomitant increase in nerve terminal branch length. Furthermore, no pre-synaptic training-induced adaptations were identified in either young or aged animals. This was true for nerve terminal branching quantified in both fast- and slow-twitch myofibers.

In contrast to the effect of resistance training, which was apparent exclusively on post-synaptic measures of fast-twitch NMJs, the influence of aging was conferred solely upon the post-synaptic endplates of the predominant slow-twitch NMJs of the Soleus. As has been documented previously (Deschenes et al., 2011; Fahim, 1997), aging resulted in endplate expansion on slow-twitch myofibers of the Soleus.

Unlike post-synaptic parameters, not a single main effect of aging was detected for any pre-synaptic variable measured in either the predominant slow-twitch NMJs or the minority fast-twitch NMJs found in the Soleus. This absence of an aging effect may well be explained by the high recruitment patterns exhibited by that postural muscle.

Conversely, the Plantaris - composed mainly of fast-twitch myofibers and acting mainly as a locomotor muscle – almost uniformly evinced a main effect of aging. This effect was observed as expansions of all features of pre-synaptic nerve terminal branching, as well as post-synaptic endplate regions. Importantly, such age-related increases in NMJ parameters were observed in both fast-twitch and slow-twitch synapses of the Plantaris. Due to the pre- and post-synaptic uniformity of this age-related morphological growth, pre- to post-synaptic coupling was maintained in the face of aging, as was the dispersion of ACh receptors within the endplate region. Somewhat surprisingly, given its role as an ambulatory muscle, the NMJs of the Plantaris remained wholly devoid of training-induced adaptations. This may be related to the well-known difficulty in emulating the intensity and volume of resistance training typified in human weight lifters when using an animal model (Cholewa et al., 2014; Timson, 1990). Indeed, myofiber profiles of both the Soleus and Plantaris did not reveal any of the hypertrophy that would be expected with an effective resistance training program. This lack of myofiber hypertrophy is not supported by whole muscle wet weight data showing that Plantaris muscles of trained aged rats were significantly heavier than those of young animals. We believe that this result was likely mainly due to inconsistencies during the surgical removal of those muscles. Unlike the Soleus which has easily identified points of origin and insertion that are easily accessible when extracting the muscle, the Plantaris does not so readily present obvious cutting sites during surgical removal; it is also embedded in the Gastrocnemius muscle. As a result, sometimes smaller, or larger sections of what are intended to be whole muscles are removed that are then later weighed.

In contrast to myofiber cross-sectional area, myofiber type composition was affected by aging, at least in the Plantaris, in that Aged rats displayed a higher percentage of Type I (slow-twitch) myofibers that was concomitant to a decreased percentage of Type II, or fast-twitch myofibers. This pattern of fiber type conversion (II → I) is a cardinal feature of the sarcopenia observed in aged muscle. Interestingly, these data suggest that the first sign of sarcopenia may be fiber type conversion rather than fiber atrophy, and that sparsely recruited muscles (Plantaris) may be more vulnerable to the effects of sarcopenia than highly

recruited ones (Soleus). A caveat may be in order here, however. It has recently been reported that various staining procedures used to determine myofiber type composition in muscles may yield inaccurate results due to misclassification, especially among aged muscles (Purves-Smith et al., 2014). And of the numerous staining procedures used to assess fiber type composition the one employed here, i.e. ATPase histochemistry, is particularly vulnerable to such error.

The combination of using different age groups (Young vs. Aged) to study the effects of resistance training on disparate muscles (Soleus vs. Plantaris) along with different NMJs (fast- vs. slow-twitch) provided important new findings regarding the inherent synaptic plasticity of the peripheral nervous system. A vital finding was that it was among the NMJs of the highly recruited Soleus, but not the lightly recruited Plantaris, where morphological adaptations to the resistance training stimulus were apparent. Again, the resistance training paradigm used may have lacked adequate rigor to recruit the Type II predominant – thus high threshold – Plantaris.

It is important to note that these training-induced NMJ adaptations observed in the Soleus were detected among both young and older rats suggesting that aging, at least early onset aging, did not impact the sensitivity of synapses to an exercise stimulus. This bodes well for older people trying to derive the many health benefits associated with weight training. However, the training-provoked enhancement of post-synaptic endplate dimensions, i.e. perimeter length and area, of the Soleus were noted in fast-, but not slow-twitch NMJs. This can likely be attributed to the fact that under normal conditions fast-twitch myofibers of the Soleus are infrequently recruited, but during the weight training sessions they would be expected to be activated. In contrast, significant interactive effects among aging and training were observed in both fast-twitch and slow-twitch NMJs of the Soleus. More specifically, it was determined that resistance training increased dispersion of ACh receptors of aged NMJs, while that same stimulus brought about a more compact receptor distribution within the endplates of young NMJs. Clearly there is a nuanced, complex relationship between the influences of exercise training and aging with respect to NMJ remodeling.

Unlike the effects of resistance training which triggered NMJ adaptations only among the small number (~15%) of fast-twitch myofibers that comprise the Soleus, the effects of aging were identified in the more commonly expressed slow-twitch myofibers of that muscle. It was interesting to note that adaptations to both aging and training occurred only at post-synaptic regions of NMJs identified in the Soleus. But perhaps the most noteworthy outcome of our investigation was the fact that age-related synapse reconfiguration was uniformly evident in the Plantaris. That is, the effects of aging were manifested in both the predominant fast-twitch myofibers, as well as the sparingly expressed slow-twitch myofibers of the Plantaris. Moreover, this remodeling was revealed both in pre-synaptic nerve terminal branching, and the post-synaptic endplate region of the myofiber's sarcolemma. It appears then, that a very pervasive series of NMJ adaptations occurs during even the earliest stages of aging - recall that the aged rats used here are the equivalent of 60 year old humans, with that age serving as the threshold for the “youngest” of the aged population (Forman et al., 1992) - at least among lightly recruited muscles such as the Plantaris.

Overall, the data gathered in the present investigation indicate that lightly recruited muscles are more sensitive to aging, even at the very earliest stages of aging, than those muscles which demonstrate a higher duty cycle. There is also evidence that the effects of aging first appear in the post-synaptic region suggesting that this process may begin with the myofiber before progressing in a retrograde fashion up to pre-synaptic nerve terminals. Additionally, among muscles such as the Plantaris which are particularly sensitive to the effects of aging due to their modest habitual activity levels, aging-induced synaptic remodeling is apparent in the NMJs of both slow- and fast-twitch myofibers. In all cases, aging resulted in an expansion of pre- and post-synaptic features even without changes in the size of the underlying myofibers which are coupled with NMJ size increments noted during natural growth and development (Balice-Gordon and Lichtman, 1990).

Although the current study mainly focused on NMJs, an interesting finding gathered here is that sarcopenia may begin not with myofiber atrophy, but rather with myofiber conversion (Type II $\rightarrow$ I). Finally, although the resistance training protocol employed here was not sufficient to produce myofiber hypertrophy, the NMJs affected by that training regimen displayed expansions that have also been exhibited by endurance trained rats (Deschenes et al., 1993; Deschenes et al., 2011; Fahim, 1997). Apparently then, physical training of any kind elicits a sequence of molecular responses that results in larger pre- and post-synaptic components of the NMJ. Presumably, these exercise-induced adaptations result in improved physiological functioning of the neuromuscular system as previous reports (Fahim, 1997) indicate that morphological changes of the NMJ are linked with positive physiological adaptations, while age-related NMJ structural remodeling is associated with declines in neuromuscular function (Banker et al., 1983).

Acknowledgments

Grant support: This work was supported by National Institutes of Health and The Foundation for Aging Studies and Exercise Science Research to MRD.

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Figure 1.
Representative image of fluorescently stained neuromuscular junction. Presynaptic nerve terminal branches are stained in green, and post-synaptic ACh receptors are stained in red. Original magnification of X1000.

Figure 2.

Representative image of tracings used to quantify morphological aspects of the neuromuscular junction. Panel A depicts tracings used to quantify presynaptic nerve terminal branches, while panel B illustrates tracings (both manually drawn and generated by software) used to quantify postsynaptic ACh receptors.

Figure 3.

Sample of myofibers stained for myosin ATPase activity following acidic pre-incubation. Darkly stained myofibers are Type I, lightly stained fibers are Type IIA, and intermediately stained fibers are Type IIX/B. Original magnification of X100.

Table 1

Effects of resistance training (RT) and aging on body mass and whole muscle wet weight.

	Young control	Young RT	Aged control	Aged RT
Body mass (g)	352.7 ± 11.1 *	315.4 ± 6.7 *	450.9 ± 11.9	442.5 ± 8.1
Soleus wet weight (mg)	120.6 ± 5.7 ‡	111.2 ± 4.4 *	133.9 ± 6.7	136.8 ± 3.7
Plantaris wet weight (mg)	286.0 ± 18.0	299.0 ± 21.1	284.1 ± 6.9	336.8 ± 15.2 ‡

Values are means ± S.E.

* indicates significant (P = 0.05) difference from all other groups.

‡ indicates significant (P = 0.05) difference from Aged RT.

‡ indicates significant (P = 0.05) difference from Young control and Aged control.

Table 2

Effects of resistance training (RT) and aging on slow- and fast-twitch NMJs of the Soleus muscle.

Pre-synaptic (slow-twitch)	Young control	Young RT	Aged control	Aged RT
Branch number	6.6 ± 0.6	6.4 ± 0.4	5.9 ± 0.6	5.8 ± 0.4
Total branch length (μm)	99.3 ± 3.1	109.6 ± 6.5	94.4 ± 13.8	104.5 ± 7.8
Average branch length (μm)	16.7 ± 1.0	15.4 ± 1.5	19.5 ± 0.9	16.5 ± 1.8
Branching complexity	7.7 ± 0.9	7.9 ± 0.9	7.8 ± 1.6	7.2 ± 1.0
Pre- to post-synaptic coupling	2.5 ± 0.3 [‡]	2.1 ± 0.2 [*]	2.9 ± 0.2	3.4 ± 0.4
Post-synaptic (slow-twitch)				
Total endplate perimeter (μm)	135.9 ± 10.7	120.1 ± 6.0	137.4 ± 9.3	132.3 ± 6.4
Stained endplate perimeter (μm)	223.3 ± 14.0	213.6 ± 15.8 [‡]	264.9 ± 19.0	240.8 ± 23.2
Total endplate area (μm ²)	410.2 ± 16.0 [*]	383.1 ± 40.5 [*]	564.3 ± 53.7	546.7 ± 52.8
Stained endplate area (μm ²)	222.6 ± 15.8 [*]	230.0 ± 27.6 [*]	323.4 ± 32.9	356.0 ± 38.3
Endplate dispersion (%)	50.0 ± 2.6 [¶]	65.6 ± 3.4	61.0 ± 1.0	62.0 ± 2.5
Pre-synaptic (fast-twitch)				
Branch number	6.2 ± 0.7	6.8 ± 0.8	5.8 ± 0.9	7.1 ± 0.5
Total branch length (μm)	106.9 ± 10.9	109.0 ± 13.6	101.4 ± 20.9	132.7 ± 12.4
Average branch length (μm)	16.5 ± 0.5	17.2 ± 1.2	17.4 ± 1.4	19.5 ± 1.5
Branching complexity	10.7 ± 1.4	8.4 ± 1.7	7.3 ± 2.8	12.4 ± 3.4
Pre- to post-synaptic coupling	2.4 ± 0.1	3.1 ± 0.8	2.3 ± 0.6	2.7 ± 0.5
Post-synaptic (fast-twitch)				
Total endplate perimeter (μm)	119.4 ± 8.2	125.6 ± 10.3	123.9 ± 28.0	131.2 ± 10.8
Stained endplate perimeter (μm)	243.0 ± 23.9	246.4 ± 9.7	186.2 ± 61.5	286.9 ± 38.9
Total endplate area (μm ²)	315.0 ± 48.8 [#]	504.2 ± 69.7	386.5 ± 166.9 [#]	557.5 ± 74.0
Stained endplate area (μm ²)	240.5 ± 23.0 [#]	326.1 ± 82.7	248.8 ± 110.6 [#]	352.4 ± 58.7
Endplate dispersion (%)	61.3 ± 1.5	62.5 ± 7.5	68.0 ± 4.9	58.8 ± 4.8 [‡]

Values are means ± S.E., Branching complexity = branch number x total branch length/100. Pre- to post-synaptic coupling = endplate stained area/total terminal branch length. Dispersion = stained endplate area/total endplate area x 100.

* indicates significant (P = 0.05) difference from Aged control and Aged RT.

[‡] indicates significant (P = 0.05) difference from Aged RT.

[‡] indicates significant (P = 0.05) difference from Aged control.

[¶] indicates significant (P = 0.05) difference from all other groups.

[#] indicates significant (P = 0.05) difference from Young RT and Aged RT.

Table 3

Effects of resistance training (RT) and aging on slow- and fast-twitch NMJs of the Plantaris muscle.

Pre-synaptic (fast-twitch)	Young control	Young RT	Aged control	Aged RT
Branch number	5.8 ± 0.4	5.3 ± 0.3*	6.7 ± 0.3	6.6 ± 0.4
Total branch length (µm)	130.0 ± 8.0*	128.7 ± 5.3*	172.5 ± 7.4	168.3 ± 8.8
Average branch length (µm)	23.1 ± 0.7*	24.2 ± 0.7*	26.4 ± 0.6	26.8 ± 1.0
Branching complexity	7.5 ± 1.0*	6.5 ± 0.6*	11.9 ± 1.1	11.5 ± 1.4
Pre- to post-synaptic coupling	2.9 ± 0.1	3.3 ± 0.2	3.3 ± 0.1	3.2 ± 0.2
Post-synaptic (fast-twitch)				
Total endplate perimeter (µm)	156.1 ± 5.5*	158.8 ± 9.5*	200.4 ± 7.8	191.4 ± 7.6
Stained endplate perimeter (µm)	292.4 ± 22.7*	287.1 ± 29.9*	434.4 ± 28.9	391.0 ± 29.4
Total endplate area (µm ²)	452.4 ± 35.1*	457.7 ± 48.3*	666.2 ± 50.7	615.5 ± 51.6
Stained endplate area (µm ²)	385.3 ± 26.0*	398.7 ± 38.7*	559.5 ± 42.9	525.1 ± 49.8
Endplate dispersion (%)	71.0 ± 2.4	73.3 ± 1.7	70.2 ± 1.7	70.0 ± 2.0
Pre-synaptic (slow-twitch)				
Branch number	5.5 ± 0.5	5.1 ± 0.6*	6.9 ± 0.3	6.8 ± 0.4
Total branch length (µm)	129.1 ± 10.1*	111.4 ± 11.4*	167.7 ± 7.4	169.5 ± 11.6
Average branch length (µm)	25.4 ± 4.1	23.3 ± 1.2	25.2 ± 1.2	25.5 ± 1.3
Branching complexity	7.5 ± 1.3*	6.5 ± 1.7*	12.3 ± 1.0	12.6 ± 1.6
Pre- to post-synaptic coupling	3.8 ± 0.3	3.1 ± 0.4	2.6 ± 0.4	2.5 ± 0.4
Post-synaptic (slow-twitch)				
Total endplate perimeter (µm)	163.2 ± 12.8 [†]	144.0 ± 15.0*	203.0 ± 9.2	191.1 ± 10.1
Stained endplate perimeter (µm)	327.5 ± 42.7 [†]	290.1 ± 28.4*	440.2 ± 37.2	377.2 ± 29.5
Total endplate area (µm ²)	492.2 ± 65.8	399.3 ± 35.0 [†]	629.8 ± 55.2	525.4 ± 53.1
Stained endplate area (µm ²)	413.9 ± 48.4	341.6 ± 29.1 [†]	533.4 ± 56.6	429.6 ± 46.8
Endplate dispersion (%)	68.5 ± 2.7	70.6 ± 2.3	68.0 ± 2.0	66.2 ± 3.6

Values are means ± S.E., Branching complexity = branch number x total branch length/100. Pre- to post-synaptic coupling = endplate stained area/total nerve terminal branch length. Endplate dispersion = stained endplate area/total endplate area x 100.

* indicates significant (P < 0.05) difference from Aged control and Aged RT.

[†] indicates significant (P < 0.05) difference from Aged control.

Table 4

Effects of resistance training and aging on myofiber profiles of Soleus and Plantaris muscles.

Soleus (cross-sectional area, μm^2)	Young control	Young RT	Aged control	Aged RT
Types combined	2159 $\pm$ 101	2051 $\pm$ 107	2243 $\pm$ 126	2226 $\pm$ 69
Type I	2217 $\pm$ 110	2086 $\pm$ 109	2285 $\pm$ 126	2242 $\pm$ 75
Type II (A&X)	1879 $\pm$ 102	1815 $\pm$ 116	2022 $\pm$ 180	2152 $\pm$ 84
Type IIA	1939 $\pm$ 111	1908 $\pm$ 118	2164 $\pm$ 193	2189 $\pm$ 105
Type IIX	1667 $\pm$ 135	1497 $\pm$ 77	1796 $\pm$ 195	1832 $\pm$ 167
<i>Soleus (fiber type composition, %)</i>				
Type I	85.2 $\pm$ 2.1	88.2 $\pm$ 1.2	86.0 $\pm$ 1.1	84.3 $\pm$ 1.1
Type II (A&X)	14.8 $\pm$ 7.5	11.8 $\pm$ 1.2	14.0 $\pm$ 1.1	15.7 $\pm$ 1.1
Type IIA	11.6 $\pm$ 2.2	8.0 $\pm$ 1.0	9.0 $\pm$ 1.2	11.7 $\pm$ 1.0
Type IIX	3.2 $\pm$ 0.7	3.8 $\pm$ 1.1	5.0 $\pm$ 0.8	4.0 $\pm$ 0.7
<i>Plantaris (cross-sectional area, μm^2)</i>				
Types combined	2218 $\pm$ 119	2100 $\pm$ 114	2017 $\pm$ 93	2106 $\pm$ 110
Type I	1359 $\pm$ 117	1467 $\pm$ 202	1292 $\pm$ 46	1427 $\pm$ 80
Type II (A&X)	2394 $\pm$ 119	2256 $\pm$ 111	2209 $\pm$ 109	2312 $\pm$ 120
Type IIA	2632 $\pm$ 173	2543 $\pm$ 157	2369 $\pm$ 112	2588 $\pm$ 117
Type IIX/B	1303 $\pm$ 159	1513 $\pm$ 188	1249 $\pm$ 86	1417 $\pm$ 99
<i>Plantaris (fiber type composition, %)</i>				
Type I	16.5 $\pm$ 1.3	20.1 $\pm$ 3.0	22.3 $\pm$ 1.8 [#]	25.7 $\pm$ 3.2 [*]
Type II (A&X/B)	83.5 $\pm$ 1.3	79.9 $\pm$ 3.0	77.7 $\pm$ 1.8 [#]	74.3 $\pm$ 3.2 [*]
Type IIA	67.8 $\pm$ 6.1	65.9 $\pm$ 3.7	66.6 $\pm$ 2.3	64.5 $\pm$ 6.3
Type IIX/B	15.7 $\pm$ 5.4	14.0 $\pm$ 3.7	11.1 $\pm$ 1.5 [#]	9.8 $\pm$ 1.8 [*]

* indicates significant (P 0.05) difference from Young, CTL.

[#] indicates trend (0.10<P<0.05) for difference from Young, CTL.