Adaptations of the neuromuscular junction to exercise training

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The neuromuscular junction (NMJ) is the synapse that enables proper function of the neuromuscular system. Like other synapses, the NMJ is sensitive to changes in its pattern of use and adapts accordingly. Exercise training stimulates the NMJ and elicits functional and morphological remodeling resulting in improved performance. Physiologically, exercise training increases quantal content, safety margin, alters spontaneous release of neurotransmitter, and improves resistance to fatigue during a train of stimuli. Structurally, exercise training leads to enhanced presynaptic nerve terminal branching, number of vesicles, along with a greater number of postsynaptic receptors. Generally, presynaptic to postsynaptic coupling is unaffected by exercise. Although the NMJ responds to both endurance training (e.g. running, swimming) and resistance training, it appears that the constant stimulus of endurance training imparts a more powerful stimulus to the NMJ. The plasticity of the NMJ is elegantly demonstrated with exercise training.

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Introduction

The neuromuscular system is one of the most essential and adaptable of the 11 major physiological systems of the human body. At its core is the neuromuscular junction (NMJ) which is the synapse that joins the motor nervous system with the skeletal muscle fibers that contract enabling important and life sustaining functions including breathing, digestion, and locomotion. Moreover, the NMJ plays an essential role in the activities of daily living, as well as recreational activities such as sports that add to the quality of life.

As with all synapses, the NMJ demonstrates an impressive degree of plasticity in response to alterations in the amount of activity that it experiences on a daily basis. For example, total (i.e. paralysis, denervation), and subtotal disuse (e.g. bed rest, limb immobilization) evoke detrimental adaptations such as alterations in the amount of the neurotransmitter acetylcholine (ACh) released both spontaneously, and when electrically stimulated [20,38]. Similarly, disuse results in significant presynaptic and postsynaptic morphological remodeling, including a greater number of nerve terminal branches, and increased dispersion of ACh receptors, respectively [2••,12,14]. Here, however, only adaptations to increased neuromuscular activity in the form of exercise training will be addressed.

Endurance exercise training

This form of increased neuromuscular activity is characterized by extended periods of continuous neuromuscular activity performed at a moderate, submaximal intensity. Commonly, this is presented in the form of running, swimming, or bicycling. Unfortunately, relative to inactivity, little research has been directly conducted into the effects of endurance training on the physiological function of the NMJ. However, in a particularly informative investigation, Fahim [17••] examined electrophysiological parameters of neuromuscular synapses of the gastrocnemius muscles of adult rats who had completed a 12 week treadmill running program. When compared to untrained sex and age matched controls, it was determined that quantal content, or the amount of neurotransmitter released onto the postsynaptic endplate's ACh receptors with a single stimulus, was significantly increased among the trained muscles. Moreover, the safety margin of stimulation, or the degree of postsynaptic depolarization in excess of that required to open voltage gated postsynaptic ion channels, also was enhanced among trained NMJs. Each of these adaptations would act to ensure effective presynaptic to postsynaptic communication during prolonged periods of neuromuscular activity. And since whole muscle function is linked to effective NMJ function, these adaptations also postpone, and mitigate the onset of muscular fatigue during prolonged activity [2••]. Similar training-induced improvements in NMJ transmitter release have been documented elsewhere [16].

One of the important enzymes that enables effective neuromuscular transmission is acetylcholinesterase (AChE). This enzyme is concentrated at the postsynaptic end plate in close proximity to ACh receptors [22,31,39]. AChE actually comprises several isoforms, each with its own specific function to play in neuromuscular transmission. While one specific isoform of AChE cleaves apart

the molecule of neurotransmitter bound to its receptor, another isoform is responsible for removing the bypro-ducts of that reaction. In doing so, AChE clears the way for binding of a new ACh molecule to that receptor, and continued ionic flux across the postsynaptic membrane (see Figure 1). Several studies have reported that the specific isoform of AChE that is responsible for inactivat-ing receptor-bound ACh, that is, G_4, markedly increases its expression in the synapses of muscles endurance trained either via running [28], or swimming [24]. These adaptations improve protracted neuromuscular transmis-sion, and by extension, resistance to muscle fatigue. Physiological adaptations of the NMJ to exercise can be found in Table 1.

Presynaptic morphological adaptations

In addition to physiological adaptations of the NMJ to endurance training, numerous morphological adaptations also have been observed. These include an increase in the number of presynaptic nerve terminal branches, enhanced total nerve terminal branch length and branch-ing complexity, but a maintenance of average branch length of those same terminals [9,11,15,35,47,49•]. While the absence of adaptation in average branch length may be surprising, it actually makes sense given the nature of the conduction of action potentials toward the active zones where docked vesicles containing ACh are posi-tioned. The myelination along the axon that assists the movement of action potentials toward the nerve terminal
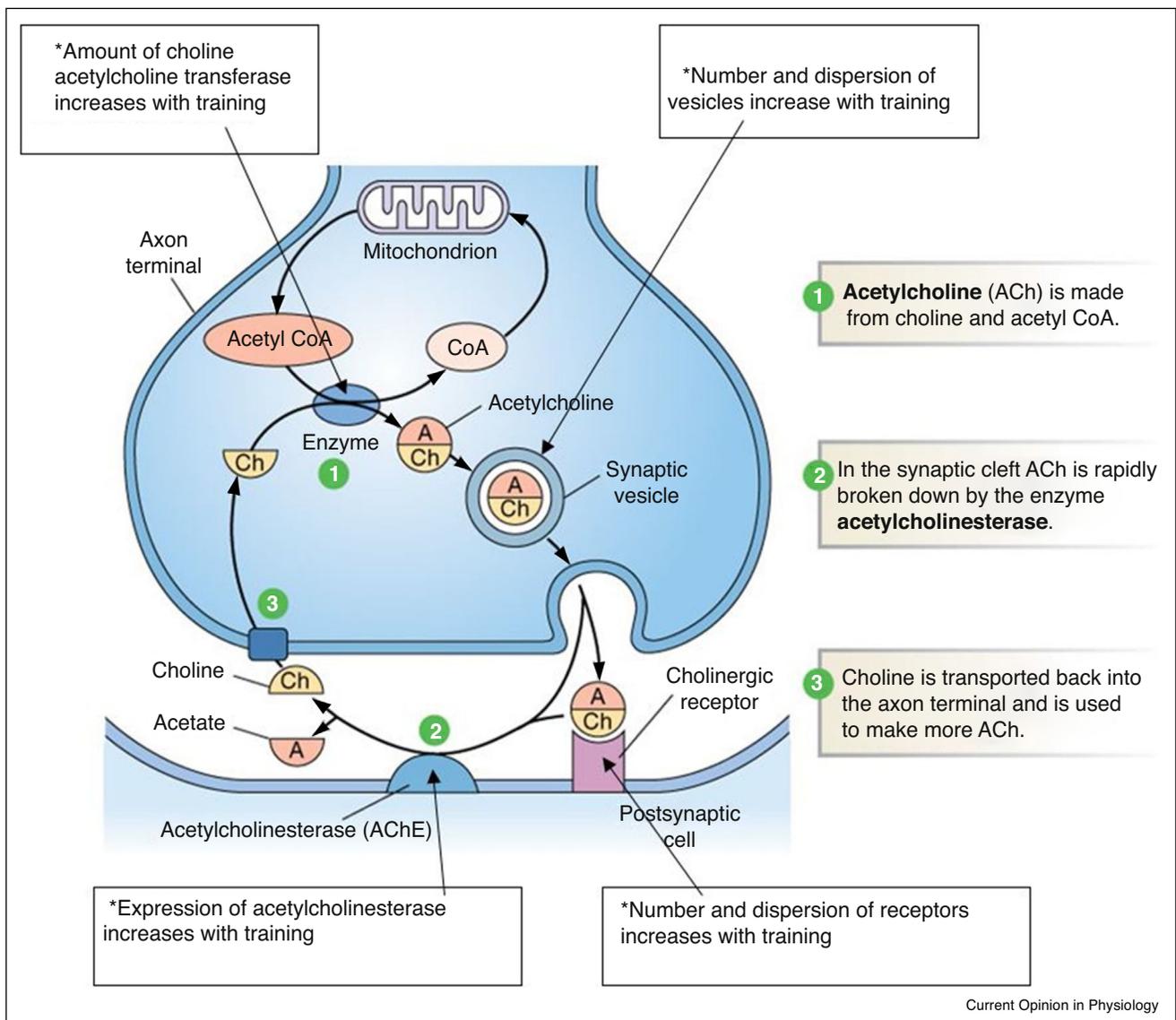
Figure 1

Illustration of how acetylcholinesterase (AChE) functions at the neuromuscular junction and examples of subsynaptic adaptations induced by exercise training.

Table 1

Exercise-induced physiological adaptations of the neuromuscular junction

• Amplitude of mini-endplate potentials	↑
• Frequency of mini-endplate potentials	↔
• Amplitude of stimulated quantal content	↑
• Depression of quantal content during train of stimuli	↓
• Safety margin of neuromuscular transmission	↑
• Acetylcholinesterase activity	↑
• Choline acetyltransferase	↑

is no longer present at the axon's terminal endings. This is to expose the voltage gated calcium channels that allow an influx of extracellular calcium which releases vesicles docked at active zones so they can, via exocytosis, release ACh into the synaptic cleft. It is important to recognize that because of the absence of myelination at the nerve terminal endings, action potentials must rely solely on passive diffusion on their way to the active sites. Thus, nerve terminal endings cannot be too long otherwise a diminished depolarization amplitude would be inadequate to open the voltage gated calcium channels co-localized with docked vesicles. In that case, there would be no exocytosis of neurotransmitter, and by extension, failure neuromuscular transmission; this does occur under conditions of neuromuscular fatigue.

To counter this, endurance training increases the total length of presynaptic nerve terminal branching by augmenting that synapse's branch number, not its average branch length. Mechanisms fully explaining how exercise elicits increased terminal branching have yet to be fully elucidated, but it has been shown that neurotrophin 4 (NT-4) which stimulates nerve terminal branching, positively responds to exercise [21,36] thus enabling the NMJ to store ~30% more vesicles [10**,16]. It appears that training does not do this by increasing the density of ACh vesicles per given branch length, which might interfere with optimal vesicular recycling. Rather, total branch length at trained NMJs is increased [9]. Similarly, when examining the stained clusters of vesicles at endurance trained NMJs, it is evident that their distribution is more dispersed than what is noted at the nerve terminals of untrained NMJs [9]. Because of the ongoing recycling of vesicles during continuous stimulation [37,48,50], it is understood that this dispersion enables more effective recycling and endocytotic uptake of membrane following the release of ACh into the synaptic cleft. In essence, increased trafficking of vesicular membrane during continuous use occurring during endurance training requires

more space to enable efficient ongoing neuromuscular transmission.

The importance of the presynaptic active zone docking of ACh filled vesicles pending their release upon the influx of calcium has previously been described in full [26•,33]. Recently, it has been found that subcellular modifications of the nerve terminal can be elicited by endurance training without accompanying cellular remodeling. More specifically, increases in the measured area of active zone bars, which anchor vesicles, have been detected as a result of endurance training without concomitant expansions of total nerve terminal length. Interestingly, it was noted in that same study that the ratio of vesicles to Bassoon – a major constituent protein of active zones – remains the same in trained and untrained NMJs suggesting that if more vesicles are to be located in trained NMJs, there needs to be a roughly equivalent increase in the number of docking sites at those synapses, as has been suggested elsewhere [32]. As another example of the sensitivity of NMJ remodeling, adaptations in presynaptic nerve terminals related to endurance training are confined to those myofibers, and NMJs, recruited during moderate intensity endurance training. Specifically, such remodeling is evident at low threshold, easily recruited slow-twitch myofibers, but not at higher threshold, less recruited fast-twitch myofibers and their NMJs [11,43]. Along with endurance training comes a greater rate of cellular protein turnover. In the motor neuron, newly synthesized proteins destined to function at the terminal endings must be transported from the cell body where protein synthesis occurs, to the nerve terminal arborizations via axoplasmic transport. A recent research project has confirmed that increased motor neuronal activity significantly enhances axonal transport capacity of that cell improving neuronal function of more active, that is, trained, NMJs [23].

Postsynaptic morphological adaptations
Along with presynaptic reconfiguration, postsynaptic remodeling of the NMJ occurs with endurance training. For example, increases in the number and dispersion of ACh receptors have been documented in endurance-trained NMJs [9,10**,15,25]. Interestingly, the training-induced expansion in the area occupied by ACh receptors matches that observed in the associated presynaptic area occupied by neurotransmitter filled vesicles (both ~30%). Indeed, it has been demonstrated that the presynaptic and postsynaptic features of the NMJ, particularly the ACh release and binding sites are tightly coupled, even in the face of the significant remodeling elicited by exercise training, or even aging [9]. In addition to the co-localization of presynaptic vesicles and postsynaptic receptors, presynaptic to postsynaptic coupling can also be determined by quantifying the area of postsynaptic binding sites relative to the length of presynaptic nerve terminal branching. When determined in this manner it is reaffirmed that synaptic coupling is maintained despite

Table 2

Exercise-induced morphological adaptations of the neuromuscular junction

Presynaptic	
● Number of nerve terminal branches	↑
● Total length of nerve terminal branching	↑
● Average length of nerve terminal branches	↔
● Complexity of nerve terminal branching	↑
● Quantity of acetylcholine vesicles	↑
● Quantity of acetylcholine receptors/branch length	↔
● Active zone size	↑
● Number of vesicles/active zone length	↔
● Presynaptic to postsynaptic coupling	↔
Postsynaptic	
● Number of acetylcholine receptors	↑
● Dispersion of acetylcholine receptors	↑
● Total area occupied by acetylcholine receptors	↑
● Perimeter length encompassing acetylcholine receptors	↑

significant structural remodeling [12,13•]. This stability of presynaptic to postsynaptic coupling is to be expected since unlike a complete, untraversed gap representing the synaptic cleft portrayed in many textbook images, presynaptic and postsynaptic factions are physically bound together by durable filamentous proteins such as fibronectin, laminin, and even calcium channels [34,40]. In short, despite considerable architectural alterations evoked by changes in neuromuscular activity, coupling between presynaptic release sites and postsynaptic binding sites are remarkably well maintained so that effective neurotransmission is assured. An excellent review of the importance of extracellular proteins, including fibronectins and laminins, in maintaining synaptic function despite overall remodeling has recently been published [18]. Morphological adaptations of the NMJ to endurance exercise are summarized in Table 2. Images of untrained (top) and trained NMJs (bottom) are presented in Figure 2.

Resistance exercise training

Another popular mode of exercise is resistance training, perhaps better known as weight lifting. While it is well known that resistance training elicits gains in muscle mass and strength [42], it is also a valued health maintenance intervention as it has been shown to improve the

symptoms of Type 2 diabetes, bone fragility, osteoporosis, and even hypertension [4,6,19]. Studying the effects of resistance training is difficult as small animals, with accompanying smaller muscles, are routinely used to examine the structural and functional characteristics of NMJs as they are located somewhere in the middle third of the whole muscle [41••,44]. Accordingly, the challenge is to establish a suitable model for weight lifting in rats, or mice [5,46]. Two commonly used models of resistance training for small animals is weighted ladder climbing, and synergist ablation to present constant overload to the unaffected synergistic muscle. While both of these methods have proven somewhat effective in stimulating muscle hypertrophy [1,7,27,30,45], neither faithfully mimics what is performed by a highly motivated human during resistance exercise trials. As a result, using such animal-based models to investigate the effects of resistance training must be approached with some degree of caution.

Nonetheless, there are some valuable insights suggesting that although weight lifting does result in some changes at the NMJ, those structural expansions are only about one half of what is reported as a result of endurance training, that is, ∼15% versus ∼30%, respectively. In addition, it appears that such NMJ adaptations to weighted ladder climbing are both muscle and myofiber type specific. Indeed, one study discovered that resistance training affected fast-twitch, but not slow-twitch myofibers by expanding the postsynaptic area occupied by ACh receptors, and even this was detected in soleus, but not plantaris muscles [13•]. In short, resistance training was found to elicit postsynaptic NMJ enlargement, but only among the high threshold, lightly recruited fast-twitch myofibers that typically are only infrequently recruited and among the postural soleus, but not the non-postural plantaris muscle. Presumably, the exercise stimulus was intense enough to recruit those higher threshold motor units that typically are only rarely activated, thus eliciting NMJ remodeling. Yet in another investigation employing weighted ladder climbing as the exercise stimulus, the NMJs of the soleus muscle displayed significant size increments irrespective of myofiber type [8]. It is likely that the different results regarding the NMJs of the soleus shown by those studies [13•,8] are due to the longer training duration, and greater weight lifted while ladder climbing used in the study revealing significant NMJ adaptations to resistance training [8].

When evaluating the effects of chronic overload, imparted via synergist ablation, on NMJ structure, it was revealed that although this mode of resistance exercise was successful in producing significant (18%) hypertrophy of the plantaris muscle fibers, no accompanying restructuring of the NMJs in those muscles could be found [14]. In seeking answers to these disparate results among the two modes of resistance training, it is plausible that the constant contractile state of synergist ablation

Figure 2
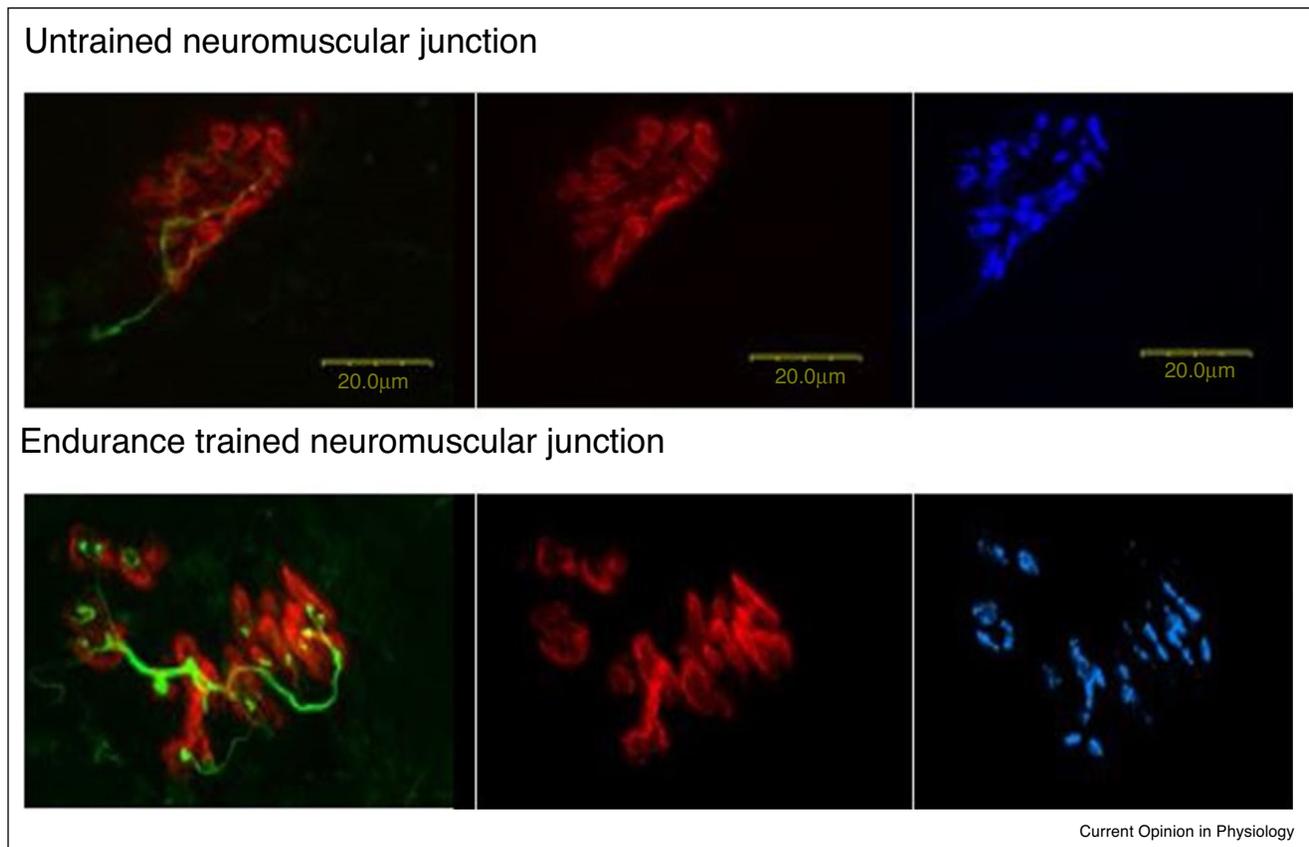
Untrained (top) and endurance trained (bottom) neuromuscular junctions. In both panels, left image is of nerve terminal branching (green) with postsynaptic acetylcholine receptors (red), while the middle image is of postsynaptic receptors only (red), and the right image is of presynaptic acetylcholine vesicles (blue). Note that the trained synapse is larger with greater branching and more dispersed vesicles and receptors (scale bar = 20 μm).

may trigger responses in the contractile, but not the excitatory proteins comprising the NMJ.

Natural postnatal development versus exercise

It has been previously shown that increases in the size of myofibers that naturally occur during postnatal development are associated with similar growth in NMJ dimensions [3]. But this relationship does not hold with modifications due to exercise training. For example, the myofiber hypertrophy resulting from chronic overload is not accompanied by similar NMJ growth [14]. Moreover, the moderate (10–15%) myofiber atrophy observed in endurance trained muscles is actually linked to significant enhancement of NMJ size [11,12]. These examples are clear evidence that when elicited by exercise, rather than progressive development, alterations in the morphology of NMJs do not necessarily emulate those of the myofibers on which they reside.

Conclusion

The NMJ is a unique synapse in that it is easily accessible, is simple in its connectivities, that is, a single presynaptic and a single postsynaptic cell, and displaying a normal postsynaptic depolarization from only a single pulse that exceeds the safety factor needed to generate a postsynaptic excitatory response by several-fold. None of these characteristics are exhibited by synapses of the central nervous system [29,41••,44]. Yet at the same time, the NMJ is similar to other synapses in that it demonstrates a high degree of plasticity, both physiological and morphological, in response to changes in its pattern of habitual use. Accordingly, this synapse demonstrates robust adaptability to both endurance, and resistance types of exercise training.

Future directions

Although much has been learned regarding the adaptations of the NMJ to exercise training over the last 30–40 years, this topic remains ripe with opportunity for

additional research endeavor. As previously mentioned, there is still much to be learned about the effects of resistance training for want of an adequate small animal model of weight lifting. Importantly, the focus of investigation of the NMJ and exercise science research should now turn to the subcellular, rather than the cellular level. Such subcellular topics might include adaptations of active zones, enzymes producing vital components such as choline acetyltransferase, internal vesicles, and proteins that properly dock those vesicles at active zones, among others. Information resulting from these studies would obviously result in a more complete understanding of how exercise training remodels the NMJ both physiologically and morphologically.

Conflict of interest statement
Nothing declared.

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Ahtiainen JP, Lensu S, Ruotsalainen I, Schumann M, Ihalainen JK, Fachada V, Mendias CL, Brook MS, Smith K, Atherton PJ *et al.*: **Physiological adaptations to resistance training in rats selectively bred for low and high response to aerobic exercise training**. *Exp Physiol* 2018, **103**:1513-1523.

2. Baehr LM, West DW, Marcotte G, Marshall AG, De Sousa LG,
•• Baar K, Bodine SC: **Age-related deficits in skeletal muscle recovery following disuse are associated with neuromuscular junction instability and ER stress, not impaired protein synthesis**. *Aging (Albany NY)* 2016, **8**:127-146.
This study demonstrates the essential role that the NMJ plays in the aging of the neuromuscular system, and how it determines the rate of recovery following disuse.

3. Balice-Gordon RJ, Lichtman JW: **In vivo visualization of growth of presynaptic and postsynaptic elements of neuromuscular junctions in the mouse**. *J Neurosci* 1990, **10**:894-908.

4. Bird SR, Hawley JA: **Update on the effects of physical activity on insulin sensitivity in humans**. *BMJ Open Sport Exerc Med* 2017, **2**:e000143.

5. Cholewa J, Guimaraes-Ferreira L, da Silva Teixeira T, Naimo MA, Zhi X, de Sa RB, Lodetti A, Cardozo MQ, Zanchi NE: **Basic models modeling resistance training: an update for basic scientists interested in study skeletal muscle hypertrophy**. *J Cell Physiol* 2014, **229**:1148-1156.

6. Coelho-Junior HJ, Goncalves IO, Camara NOS, Cenedeze MA, Bacurau RF, Asano RY, Santana J, Caperuto E, Uchida MC, Rodrigues B: **Non-periodized and daily undulating periodized resistance training on blood pressure of older women**. *Front Physiol* 2018, **9**:1525.

7. Degens H, Meessen NE, Wirtz P, Binkhorst RA: **The development of compensatory hypertrophy in the plantaris muscle of the rat**. *Ann Anat* 1995, **177**:285-289.

8. Deschenes MR, Judelson DA, Kraemer WJ, Meskaitis VJ, Volek JS, Nindl BC, Harman FS, Deaver DR: **Effects of resistance training on neuromuscular junction morphology**. *Muscle Nerve* 2000, **23**:1576-1581.

9. Deschenes MR, Kressin KA, Garratt RN, Leathrum CM, Shaffrey EC: **Effects of exercise training on neuromuscular junction morphology and pre- to post-synaptic coupling in young and aged rats**. *Neuroscience* 2016, **316**:167-177.

10. Deschenes MR, Maresh CM, Crivello JF, Armstrong LE,
•• Kraemer WJ, Covault J: **The effects of exercise training of different intensities on neuromuscular junction morphology**. *J Neurocytol* 1993, **22**:603-615.
This report elucidates the morphological adaptations of the NMJ to endurance training, and how these alterations are affected by training intensity.

11. Deschenes MR, Roby MA, Glass EK: **Aging influences adaptations of the neuromuscular junction to endurance training**. *Neuroscience* 2011, **190**:56-66.

12. Deschenes MR, Sherman EG, Glass EK: **The effects of pre-habilitative conditioning on unloading-induced adaptations in young and aged neuromuscular systems**. *Exp Gerontol* 2012, **47**:687-694.

13. Deschenes MR, Sherman EG, Roby MA, Glass EK, Harris MB:
• **Effect of resistance training on neuromuscular junctions of young and aged muscles featuring different recruitment patterns**. *J Neurosci Res* 2015, **93**:504-513.
This is one of the first studies on the impact of resistance training on the NMJ. It was revealed that resistance trained adaptations of the NMJ were myofiber-type specific and were evident only at the postsynaptic endplate.

14. Deschenes MR, Tenny K, Eason MK, Gordon SE: **Moderate aging does not modulate morphological responsiveness of the neuromuscular system to chronic overload in Fischer 344 rats**. *Neuroscience* 2007, **148**:970-977.

15. Deschenes MR, Tenny KA, Wilson MH: **Increased and decreased activity elicits specific morphological adaptations of the neuromuscular junction**. *Neuroscience* 2006, **137**:1277-1283.

16. Dorlochter M, Irintchev A, Brinkers M, Wernig A: **Effects of enhanced activity on synaptic transmission in mouse extensor digitorum longus muscle**. *J Physiol* 1991, **436**:283-292.

17. Fahim MA: **Endurance exercise modulates neuromuscular
•• junction of C57BL/6NNia aging mice**. *J Appl Physiol (1985)* 1997, **83**:59-66.
This is a comprehensive examination of the impact of endurance training on the electrophysiological properties of the NMJ during continuous electrical stimulation.

18. Ferrer-Ferrer M, Dityatev A: **Shaping synapses by the neural extracellular matrix**. *Front Neuroanat* 2018, **12**:40.

19. Fiatarone MA, O'Neill EF, Ryan ND, Clements KM, Solares GR, Nelson ME, Roberts SB, Kehayias JJ, Lipsitz LA, Evans WJ: **Exercise training and nutritional supplementation for physical frailty in very elderly people**. *N Engl J Med* 1994, **330**:1769-1775.

20. Fischbach GD, Robbins N: **Effect of chronic disuse of rat soleus neuromuscular junctions on postsynaptic membrane**. *J Neurophysiol* 1971, **34**:562-569.

21. Funakoshi H, Belluardo N, Arenas E, Yamamoto Y, Casabona A, Persson H, Ibanez CF: **Muscle-derived neurotrophin-4 as an activity-dependent trophic signal for adult motor neurons**. *Science* 1995, **268**:1495-1499.

22. Gaspersic R, Koritnik B, Crne-Finderle N, Sketelj J: **Acetylcholinesterase in the neuromuscular junction**. *Chem Biol Interact* 1999, **119-120**:301-308.

23. Gillon A, Nielsen K, Steel C, Cornwall J, Sheard P: **Exercise attenuates age-associated changes in motoneuron number, nucleocytoplasmic transport proteins and neuromuscular health**. *GeroScience* 2018, **40**:177-192.

24. Gisiger V, Sherker S, Gardiner PF: **Swimming training increases the G4 acetylcholinesterase content of both fast ankle extensors and flexors**. *FEBS Lett* 1991, **278**:271-273.

25. Gyorkos AM, McCullough MJ, Spitsbergen JM: **Glial cell line-derived neurotrophic factor (GDNF) expression and NMJ plasticity in skeletal muscle following endurance exercise**. *Neuroscience* 2014, **257**:111-118.

26. Homan AE, Meriney SD: **Active zone structure-function**
• **relationships at the neuromuscular junction**. *Synapse* 2018, **72**: e22057.
An enlightening, and current review of how the structure of the presynaptic active zone, anchoring neurotransmitter containing vesicles, regulates neuromuscular function.

27. Hornberger TA Jr, Farrar RP: **Physiological hypertrophy of the FHL muscle following 8 weeks of progressive resistance exercise in the rat**. *Can J Appl Physiol* 2004, **29**:16-31.

28. Jasmin BJ, Gisiger V: **Regulation by exercise of the pool of G4 acetylcholinesterase characterizing fast muscles: opposite effect of running training in antagonist muscles**. *J Neurosci* 1990, **10**:1444-1454.

29. Jones RA, Harrison C, Eaton SL, Llavero Hurtado M, Graham LC, Alkhammash L, Oladiran OA, Gale A, Lamont DJ, Simpson H *et al.*: **Cellular and molecular anatomy of the human neuromuscular junction**. *Cell Rep* 2017, **21**:2348-2356.

30. Jung S, Ahn N, Kim S, Byun J, Joo Y, Kim S, Jung Y, Park S, Hwang I, Kim K: **The effect of ladder-climbing exercise on atrophy/hypertrophy-related myokine expression in middle-aged male Wistar rats**. *J Physiol Sci* 2015, **65**:515-521.

31. Mis K, Grubic Z, Lorenzon P, Sciancalepore M, Mars T, Pirkmajer S: **In vitro innervation as an experimental model to study the expression and functions of acetylcholinesterase and agrin in human skeletal muscle**. *Molecules* 2017, **22** http://dx.doi.org/10.3390/molecules22091418.

32. Neher E: **What is rate-limiting during sustained synaptic activity: vesicle supply or the availability of release sites**. *Front Synaptic Neurosci* 2010, **2**:144.

33. Nishimune H: **Active zones of mammalian neuromuscular junctions: formation, density, and aging**. *Ann N Y Acad Sci* 2012, **1274**:24-32.

34. Nishimune H, Sanes JR, Carlson SS: **A synaptic laminin-calcium channel interaction organizes active zones in motor nerve terminals**. *Nature* 2004, **432**:580-587.

35. Nishimune H, Stanford JA, Mori Y: **Role of exercise in maintaining the integrity of the neuromuscular junction**. *Muscle Nerve* 2014, **49**:315-324.

36. Ogborn DI, Gardiner PF: **Effects of exercise and muscle type on BDNF, NT-4/5, and TrKB expression in skeletal muscle**. *Muscle Nerve* 2010, **41**:385-391.

37. Rizzoli SO, Betz WJ: **Synaptic vesicle pools**. *Nat Rev Neurosci* 2005, **6**:57-69.

38. Robbins N, Fischbach GD: **Effect of chronic disuse of rat soleus neuromuscular junctions on presynaptic function**. *J Neurophysiol* 1971, **34**:570-578.

39. Rotundo RL, Rossi SG, Kimbell LM, Ruiz C, Marrero E: **Targeting acetylcholinesterase to the neuromuscular synapse**. *Chem Biol Interact* 2005, **157-158**:15-21.

40. Sanes JR: **Laminin, fibronectin, and collagen in synaptic and extrasynaptic portions of muscle fiber basement membrane**. *J Cell Biol* 1982, **93**:442-451.

41. Sanes JR, Lichtman JW: **Development of the vertebrate**
•• **neuromuscular junction**. *Annu Rev Neurosci* 1999, **22**:389-442.
This is an excellent overview of how the NMJ develops providing insight into how that synapse functions in adult vertebrates.

42. Schoenfeld BJ, Grgic J, Krieger J: **How many times per week should a muscle be trained to maximize muscle hypertrophy? A systematic review and meta-analysis of studies examining the effects of resistance training frequency**. *J Sports Sci* 2018:1-10.

43. Sieck GC, Prakash YS: **Morphological adaptations of neuromuscular junctions depend on fiber type**. *Can J Appl Physiol* 1997, **22**:197-230.

44. Slater CR: **The structure of human neuromuscular junctions: some unanswered molecular Questions**. *Int J Mol Sci* 2017, **18** http://dx.doi.org/10.3390/ijms18102183.

45. Terena SM, Fernandes KP, Bussadori SK, Deana AM, Mesquita-Ferrari RA: **Systematic review of the synergist muscle ablation model for compensatory hypertrophy**. *Rev Assoc Med Bras (1992)* 2017, **63**:164-172.

46. Timson BF: **Evaluation of animal models for the study of exercise-induced muscle enlargement**. *J Appl Physiol (1985)* 1990, **69**:1935-1945.

47. Valdez G, Tapia JC, Kang H, Clemenson GD Jr, Gage FH, Lichtman JW, Sanes JR: **Attenuation of age-related changes in mouse neuromuscular synapses by caloric restriction and exercise**. *Proc Natl Acad Sci U S A* 2010, **107**:14863-14868.

48. Van der Kloot W: **Loading and recycling of synaptic vesicles in the torpedo electric organ and the vertebrate neuromuscular junction**. *Prog Neurobiol* 2003, **71**:269-303.

49. Waerhaug O, Dahl HA, Kardel K: **Different effects of physical**
• **training on the morphology of motor nerve terminals in the rat extensor digitorum longus and soleus muscles**. *Anat Embryol* 1992, **186**:125-128.
This is an informative and pioneering investigation revealing that the structure endurance trained adaptations of presynaptic motor nerve terminals are myofiber-type specific.

50. Zefirov AL, Zakharov AV, Mukhametzyanov RD, Petrov AM, Sitdikova GF: **The vesicle cycle in motor nerve endings of the mouse diaphragm**. *Neurosci Behav Physiol* 2009, **39**:245-252.