The Biology of Muscle Memory- Exploring Neural and Muscle-based Mechanisms

Olivia Hallman

Abstract

The phenomenon known as muscle memory is a result of a diverse number of highly complex biological systems. Two notable biological systems contributing to muscle memory are the muscular system and the neuronal system.

It is hypothesized that the myonuclei within muscle cells allow the muscle cell itself to retain a 'memory'. However, the concept of memory in the musculoskeletal system is divergent from the hypotheses of memory in the central nervous system. For example, an increase in nuclear content within a muscle cell (i.e. myonuclear content) results in the muscle cell getting stronger faster compared to a muscle cell with less myonuclei. Increased activity causes a muscle cell to produce more myonuclei, which even after detraining stays within the muscle cell for a period of time. Thus, if a person detrains after doing previous training, when they retrain again, their muscles will be more receptive to the training and become stronger at a quicker rate compared to before the initial training.

Neurons, specifically the amount of acetylcholine receptors (AChR) present at the neuromuscular junction (NMJ), is thought to play a role in the underlying mechanisms of developing muscle memory. This is because in rodent preclinical trials there is a positive correlation with the amount of AChR present in the neuromuscular junction and the amount that the muscle is exercised. However, it's important to note that divergent results have been seen in human clinical trials due to multiple factors that will be discussed in this paper. Understanding the conditional mechanisms responsible for muscle memory is beneficial to many groups of people, including the average person, athletes, and people with neuromuscular disorders.

Introduction

Exercise is defined as any planned physical activity that stimulates the body's muscles by making them extend and/or contract ("NHIS - Adult Physical Activity - Glossary"). Exercise can consist of, but not be limited to, running, lifting weights, swimming, etc. Exercise has positive effects on mental health, the cardiovascular system, bone integrity, risk of disease, and even fighting off different diseases such as cancer, and diabetes. The reason why exercise positively affects such a vast number of bodily functions is partially due to the multiorgan nature of exercise. For example, exercise engages multiple organ systems outside of the musculoskeletal system, including the cardiovascular, metabolic, immune, and central and peripheral nervous systems. For the purposes of this review, the literature search has been focused on the latter, the review of preclinical and clinical exercise-induced changes in the musculoskeletal brain connection. The preclinical trials include studies of rodents, and the clinical trials include studies of humans ("NHIS - Adult Physical Activity - Glossary").

Procedural memory, otherwise known as muscle memory, is the result of highly complex biological systems at the molecular level (Cleveland Clinic). Muscle memory is responsible for the phenomenon that results in reduced conscious mental effort to produce movements that are routinely executed (Cleveland Clinic). For example an infant initially has difficulty walking, since they need to learn how, but eventually they grow up and walking becomes a regular function in their everyday lives that takes little to no conscious thinking in order to complete. Muscle

memory also maintains bodily health as it can prevent muscle loss if movements are repeated progressively across time. Athletes can also find usefulness in this phenomenon as the muscle dynamics that allow the athlete to play their sport are repetitive, it reduces the cognitive load allocated to perform a particular movement (Posture Practice). The reduction in conscious cognitive energy allows for reallocation of this energy, thereby permitting the athlete to focus on other complex variable and non variable factors specific to the sport being played (Posture Practice).

Though there is a colloquial understanding of muscle memory, the underlying mechanisms of this phenomenon, especially at molecular and physiological levels, remain largely uncharacterized. This paper reviews two possible hypotheses for the underlying mechanisms of muscle-memory: 1) myonuclear content changes and 2) neurotransmitter receptor density changes. Here, both hypotheses are explored from the framework of results from preclinical translational research done on animals, and clinical data done on humans to better understand the strengths and gaps in the translational understanding of this topic.

The myonuclei hypothesis section of this review consists of multiple preclinical and clinical trials supporting the hypothesis, as a majority of the research found was in support of this hypothesis. As for the hypothesis relating to the receptors within the neuromuscular junction, preclinical trials on rodents support this hypothesis, while a majority of clinical trials on humans do not. The reason for this is likely because there are not as many clinical trials as there are preclinical trials. Clinical trial initiation requires significant modeling and stages as human safety is a prerequisite for these studies. Additionally, rodents were the primary test subjects in preclinical trials, and it has been proven that rodent neuromuscular junctions are unlike humans (Jones et al.). This would explain why the clinical trials and preclinical trials reviewed in this paper demonstrated different results.

Musculoskeletal system background

A key aspect of organismal physiology is movement. Movement is mediated through multiple cell and tissue types, the principle of which are myocytes or muscle fibers (Noto and Edens). Beyond movement, other organ systems rely on muscle cells to carry out key physiological processes. These organ systems include but are not limited to the heart, stomach, and intestines. Diverse functions are required for different essential body functions to employ properties of muscle cells and coregulate physiological function required for survival (Noto and Edens).

Muscle cells are composed of specialized organelles called myofibril (Noto and Edens). Myofibrils are longer, smaller tubes inside the muscle cell that all run parallel to each other. Inside the myofibrils lie the sarcomere, which is responsible for muscle extension and contraction. The sarcomere further contains actin and myosin. Myosin binds to the actin and “pulls” on the actin. The sarcomere is further subdivided into the thin filament (actin rich), and the thick filament (myosin rich). The thin filament “pulls” on the thick filament, causing the thick filament to contract. In a single muscle fiber, thousands of sarcomeres coordinate this process simultaneously, causing the muscle fiber to contract, and this allows the movement of the muscle (Noto and Edens).

Muscle cell function differs depending on where they are located in the body (Noto and Edens). Different muscle types include: cardiac muscles, skeletal muscles, and smooth muscles. Muscle cells are categorized based on striation, multinucleation, or uninucleation.

Under a microscope, striations are visible. Dark and light bands alternate along a muscle fiber. This is a result of the arrangement of sarcomeres within it (Noto and Edens).

Cardiac muscle cells are striated but cannot be consciously controlled due to their connection with the visceral nervous system (automatic nervous system). These cells are found in the walls of the heart and allow the heart to beat rhythmically in order to constantly push blood to the rest of the body (Noto and Edens).

Skeletal muscle cells are striated, but in contrast to the cardiac muscle cells, can be controlled consciously. This is due to their connection with the somatic nervous system, which allows for conscious control. Skeletal muscle types are found in muscle fibers attached to the skeleton (Noto and Edens).

Smooth muscle cells are nonstriated, unlike both other muscle types. This is due to the fact they contain no sarcomeres along with a different mechanism underlying expansion and contraction. The actin and myosin in these muscle cells are not organized in a way that creates sarcomeres, but instead in such a way that allows for the expansion and contraction in organs for flexibility in the system. Additionally, they are connected to the visceral nervous system, and therefore cannot be consciously controlled. Smooth muscle cells are found in the walls of hollow organs such as the liver, pancreas, and intestines and are in charge of expanding or contracting the walls of the organ as needed (Noto and Edens).

Musculoskeletal plasticity during exercise

While there are many different hypotheses related to what ‘muscle memory’ is, a well supported hypothesis is that the muscle cells contain growth stimuli, which correlate with how often and intensely the muscle is exercised (Murach et al.). Growth stimuli within muscle cells include both positive and negative stimuli. Positive growth stimuli can be triggered when a muscle is being exercised. It allows for the muscle to keep ‘memory’ of an exercise over a long period of time. This would allow for the muscle to be able to get stronger quicker after retraining than the first time it was exercised. Conversely, negative growth stimuli can be triggered when a muscle has not been exercised for a prolonged period of time. Instead of keeping ‘memory’ of an exercise done to a muscle, it keeps ‘memory’ of muscle detraining. In short, when the muscle that triggered negative growth stimuli during a period of detraining is retrained again, it may be increasingly difficult and require increased time for the muscle to function prior to the detraining (Murach et al.).

Preclinical studies of musculoskeletal plasticity

Multiple studies have explored the biological underpinnings of ‘muscle memory’. For example, researchers explored the effects of exogenous testosterone in the murine model of muscle growth (Egner et al.). In this study, female mice were given testosterone for 14 days, leading to a 66% increase in myonuclei count and a muscle fiber size increase of 77%. When exogenous testosterone treatment was halted, animals were subsequently monitored for 21 days. At day 21 it was noted that the mice which received testosterone had similar muscle fiber size to the control mice, but significantly increased myonuclear content retained when compared to control mice. Both groups of mice then were subjected to six days of overload exercise. Results showed that the control group only had a 6% increase within their fiber size, however,

the experimental group's fiber size had increased by 31% (Egner et al.). This increase indicates that increased myonuclei numbers increases the pace of making more muscle fiber.

Similarly, in another growth stimulus, Insulin Growth Factor (IGF-1), exerts a similar effect on the rodent myonuclear number and muscle fiber size. Specifically, it has been proven that IGF-1 induces an increase in muscle mass defined as hypertrophy (Jacquemin et al.). Hypertrophy has been shown to last throughout the life of the mouse and effectively prevent the decline of muscle mass with age in murine models (Jacquemin et al.).

A supporting case study tested how IGF-1 interacts with rats and whether exercise affects the rats muscle mass (Allen et al.). Control and experimental groups were designed to differentiate the effects of IGF-1, hindlimb suspension (restrains muscle movement), and occasional exercise on the body. It demonstrated that the only group which resulted in a higher muscle mass was the group which was hindlimb suspended, injected with IGF-1, and occasionally exercised (Allen et al.). These experimental results support the theory that IGF-1 maintains muscle mass, but does not increase muscle mass either, indicating that while IGF-1 does help maintain muscle mass over a long period of time, exercise is also needed in order to increase muscle mass.

A longitudinal study in mice through *in vivo* time-lapse microscopy explored how myonuclei react to muscles undergoing detraining (Bruusgaard and Gundersen). A plasmid containing the GFP gene was transferred into the nuclei to visualize and distinguish the resulting data. In mice subjected to detraining for 28 days, muscle fiber size decreased by greater than 50%, the myonuclear content stayed the same as in the initial muscle training (Bruusgaard and Gundersen).

Further, a separate report assessed the rate at which previously trained muscles grew in comparison to non previously trained muscles (Lee et al.). Researchers formed four cohorts of different groups of mice; untrained (control group), initial training, detraining, or retraining. Results demonstrated that the amount of myonuclei within the initial training, detraining, and retraining groups was elevated than in myonuclei within the untrained (control) group. This suggests that training significantly influences the number of myonuclei within the muscle and this influence for elevated levels of myonuclei remains during detraining periods. Additionally, it was found that the muscle fiber size within the retraining group was higher than the muscle fiber size seen within the initial training group (Lee et al.). Since the myonuclei from the initial training was still present at the start of the retraining but was not present before the initial training, this indicates that an increased number of myonuclei at the start of training accelerates the muscle fiber growth compared to having fewer myonuclei.

Clinical studies of musculoskeletal plasticity

Human-focused case studies supporting this hypothesis have also been published. One such trial evaluated the impact of strength training for 20 weeks, followed by 30-32 weeks in a cohort of six women previously untrained in strength training (Staron et al.). This cohort underwent strength training for 20 weeks, before participating in 30-32 weeks of detraining followed by six weeks of retraining. The researchers found that a majority of the women's dynamic strength and muscle fiber mass increased after the initial strength training slightly decreased again after undergoing detraining (though not to pre-strength training levels), and increased back to values seen after the initial training when they started training again (Staron

et al.). The women gained just as much muscle in the retraining as they did in the initial training, in roughly a ⅛ of the time.

Another study building upon this topic focused on a human trial centered around the amount of methylation in the muscles of both younger and older individuals as observed after initial muscle training, muscle detraining, and muscle retraining (Blocquiaux et al.). The results indicated that previously trained muscles are more receptive to muscular training compared to previously untrained muscle. Additionally, it was concluded that regular muscle resistance training can slightly repair age-related methylome transformations (Blocquiaux et al.).

An additional researcher studied a similar aspect to one of the previous studies mentioned (Staron et al.), focused on determining how the amount of myonuclei and the muscle fiber size is affected by initial muscle training, detraining, and retraining (Kristoffer Toldnes Cumming et al.). For this study, 12 men and 12 women were gathered, all with previously untrained arm muscles, and were directed to complete 10 weeks of unilateral elbow-flexor strength training, 16 weeks of detraining, and 10 weeks of retraining. The results showed that the number of myonuclei remained elevated during detraining, which in turn allowed the muscle to create more muscle fiber during retraining compared to the initial training (Kristoffer Toldnes Cumming et al.).

Another related study was concentrating on the correlation between the magnitude of muscle response and myonuclear content after stimulus presentation (Petrella et al.). 66 individuals were gathered that had previously untrained knee muscles, and put them through 16 weeks of knee extensor resistance training. At the end of the 16 weeks, they distributed the participants into 3 different groups; Extreme responders (those with lots of muscle fiber growth), moderate responders (those with moderate muscle fiber growth), and nonresponders (those with no muscle fiber growth). Each group reflected how responsive their muscles were to the training stimulus. At the end of the 16 weeks, it was concluded that extreme responders had a 26% increase in the number of myonuclei, whereas moderate responders had a 9% increase in the number of myonuclei, and non-responders had no change in the number of myonuclei (Petrella et al.). This does not correlate to the hypothesis that retraining a muscle after initial training increases the muscle memory function, however, it does support the notion that the amount of muscle fiber growth is affected by myonuclear content (i.e. a positive correlation between myonuclear content and muscle growth trajectory).

Comparing preclinical and clinical studies of musculoskeletal plasticity

Each case study presented in this paper cannot be directly compared due to experimental differences. However, one common conclusion, among research results related to the hypothesis that myonuclear content is partially responsible for muscle memory, is the consistent observation that the muscle fiber size decreases as muscles go into detraining, however the number of myonuclei remains increased (Allen et al.; Blocquiaux et al.; Bruusgaard and Gundersen; Egner et al.; Jacquemin et al.; Kennedy; Kristoffer Toldnes Cumming et al.; Lee et al.; Murach et al.; Petrella et al.; Staron et al.). This, in practice, would suggest that once a muscle is detrained, the mass of muscle fiber decreases, but the myonuclei content would remain as high as during the initial training, allowing for the muscle to create new muscle tissue at a faster rate during retraining compared to that seen during the initial training further supporting the results of additional research that demonstrate the number of myonuclei is the principal component driving the process of muscle fiber regrowth. Another conclusion that many

of the papers came to validate the hypothesis that the number of myonuclei truly is the main factor in the rate at which muscle fibers grow.

Neuro-muscular plasticity background

Another popular hypothesis is that the synaptic plasticity between skeletal muscles and neuromuscular junctions are partly responsible for 'muscle memory' (Kennedy). This hypothesis arises from a phenomenon known as Synaptic Plasticity, which describes the process in which neural pathways between the brain and neurons become stronger or weaker depending on how regularly the neuron is used. A multitude of different factors occurring at the molecular level contribute to how synaptic plasticity works, however, the most prominent rationale is that an increase in receptors being formed on the postsynaptic cleft as action potential signals become more frequent. An increase in the amount of existing receptors allows for action potential signals to move through it more quickly and efficiently due to availability increase (Kennedy).

Synaptic plasticity is found between practically every synapse in the human body. One such synapse, known as the neuromuscular junction, delivers the messages sent from the brain to a specific muscle to activate it. In turn, it allows for the muscle to move and perform the action signaled by the Central Nervous System (CNS) (Personius and Balice-Gordon). Since neuromuscular junctions between specific muscles are used more frequently, the receptors on the postsynaptic cleft increase in number, allowing for the neurotransmitters to be received by the receptors more efficiently because of the increased amount. This positive feedback loop effectively improves muscle movement and allows for faster muscle response times to external stimuli (Personius and Balice-Gordon).

Preclinical studies on exercise-induced neuromuscular plasticity

One study opted to analyze AChR located within the neuromuscular junction in response to increased exercise (Desaulniers et al.). To execute this, a group of rats were gathered and put through 16 weeks of endurance training. The results of this study showed an increase in the number of AChR within the neuromuscular junction (Desaulniers et al.). Indicating increased exercise results in more AChR.

A related study observed how AChR gene expression reacts to different endurance training regimens (Gorzi et al.). This study was accomplished through the random assignment of groups of Widstar rats; no training (control), high-intensity interval training (HIIT), endurance training (END), and mixed interval training (MIX). Trials lasted for 8 weeks, 5 days per week, training both the gastrocnemius muscle and the soleus muscle. Results indicated an increase in AChR in the HIIT group within both muscles, an increase in AChR in the END group within the soleus muscle, and no significant change in AChR in either muscle within the MIX group (Gorzi et al.). This shows that overall, exercise increased the amount of AChR expression within the neuromuscular junction.

Another related study centered on the behavior of the calcitonin gene-related peptide (CGRP) and AChR, in the neuromuscular junction related to increased resistance and endurance training (Parnow et al.). 25 male rats were assigned to 3 different groups; sedentary, endurance training, and resistance training. The configurations of rat groups completed 12 weeks of this experiment, 5 times a week, for 60 minutes per day. The results showed AChR

receptor numbers increased for both the resistance training group and the endurance training group (Parnow et al.). This suggests that increased exercise results in more AChR.

Researchers ran a trial on rats to investigate the structural effects of activity versus inactivity on the neuromuscular junction (Deschenes et al.). The rats were split into 3 groups; increased activity, decreased activity, and normal activity (control). 10 weeks after the trial was started, the rats were euthanized and their soleus muscles were removed and frozen. Soleus muscles were then examined by cytofluorescent and histochemical procedures. The results showed that nerve terminal branching was enhanced while the endplate size remained unchanged (Deschenes et al.). Though this does not support the idea that the amount of receptors within the neuromuscular junction increases with increased exercise, it indicates that increased exercise does have an effect on the overall health and structure of the neuromuscular junction. This suggests that there is a link between neurons and resistance training. More exercise equates with stronger, more efficient synapse links.

Clinical studies on exercise-induced neuromuscular plasticity

After synthesizing the information from these different medical trials, it can be inferred that this hypothesis may only be true for animal trials, or at least animal trials conducted on rodents (Boehm et al.; Soendenbroe et al., 2022; Desaulniers et al.; Deschenes et al.; Gorzi et al.; Khalil et al.; Parnow et al.; Sarto et al.) This is because there are many more articles that conclude there is a decrease in the amount of receptors within the neuromuscular junction as activity levels are increased within humans. This disproves the earlier hypothesis, that the amount of receptors within the neuromuscular junction would increase when activity levels increase. Additionally, it has been proven that the neuromuscular junction of rodents and humans is distinctly different in the way they work (Boehm et al.).

The evidence from this research (Boehm et al.; Soendenbroe et al., 2022; Desaulniers et al.; Deschenes et al.; Gorzi et al.; Khalil et al.; Parnow et al.; Sarto et al.) has indicated that the receptors within the neuromuscular junction in both animals and humans behave differently from each other. This may indicate that animal trials have insufficient homology with humans in the study of how the neuromuscular junction receptors work (Jones et al.). Importantly, there are limited studies completed with humans as clinical studies done on the synaptic plasticity within the neuromuscular junction are difficult to conduct and samples or perform in clinical trials as it could pose a health risk to patients.

Different procedures which can test for the number of receptors within the neuromuscular junction include serological tests, genetic testing, and muscle cell biopsies. Serological tests and genetic tests are both relatively harmless tests with minimal side effects (Khalil et al.). However, both of these tests are not very accurate in showing the number of receptors within a neuromuscular junction. The serological tests only test for the autoantibodies which are against AChR and genetic testing would only test for the mRNA expression levels of the AChR. This is not very accurate as AChR are built by a multitude of different factors, not just mRNA, so this method does not account for any other factors when testing, resulting in inaccurate results. The one test that offers a more robust assessment of the neuromuscular junction is muscle biopsy, however, the muscle biopsy of the neuromuscular junction is still in development and needs addition research to better understand potential adverse effects the procedure may have on humans (Mylène Aubertin-Leheudre et al.). Current traditional muscle biopsies of the skin cause minor adverse effects such as rash, bruising, and pain (Santos et al.) but since muscle biopsies

at the neuromuscular junction are not completely tested, they could prove to have worse effects. Therefore, muscle biopsies at the neuromuscular junction have not been used by many scientists yet as they require further testing.

Despite the evidence that this hypothesis is incorrect for humans, one research study did support this hypothesis in humans (Soendenbroe et al., 2020). Researchers elected to investigate how the neuromuscular junction in seniors reacts to increased exercise. With this aim, the researchers brought together both younger and older women to participate in resistance training specified to one leg, while the other leg was left unexercised. Their muscles were then biopsied at 4.5 hours up to 7 days post exercise for tissue analysis and cell culture. The results of this experiment showed that in both young and elderly women, the number of AChR within the exercised leg was significantly higher than in the unexercised leg (Soendenbroe et al., 2020). This suggests that increased exercise results in higher numbers of AChR within the neuromuscular junction. Though this study did seem to support this theory to be true for humans, results from other studies are contradictory, suggesting a requirement for further research.

A following paper authored by the same scientist as the previous paper (Soendenbroe et al., 2020) opted to find out if heavy resistance training could reverse the denervation in denervated muscle fiber cells (Soendenbroe et al., 2022). To address this, they established a control group and an experimental group, both compromising of elderly male individuals. The experimental group underwent 16 weeks of resistance training, while the control group refrained from exercise. The results from this trial showed a decrease in the AChR, mRNA messengers by the end of the 16 weeks within the experimental group, which indicates less AChR was being produced as exercise persisted (Soendenbroe et al., 2022).

Another study had a group of healthy men participate in 10 days of lower limb suspension followed by 21 days of resistance training (Sarto et al.). The goal of the study was to investigate how the neuromuscular junction reacts with increased exercise. At the end of the lower limb suspension part of the experiment, it was shown that the mRNA messenger was upregulated. This indicates that while the participants were undergoing lower limb suspension, AChR levels increased (Sarto et al.). This result contradicts the hypothesis stated beforehand as this data suggests the amount of AChR receptors increased instead of decreased as the neuromuscular junction was unused.

In conclusion, both human and rodent neuromuscular junctions behave differently in the aspect of how AChR acts. This is because there are many key differences in the structures and functions of human and rodent neuromuscular junctions. Rodent neuromuscular junctions are typically larger than seen in humans. This results in more neural branching, bigger nerve terminal size, more AChR receptors, larger endplate areas, and larger axonal diameters. On the other hand, humans have a smaller neuromuscular junction than rodents. This results in all of these key features to be smaller in humans. This would explain why the receptors within humans and rodents act differently, because they have many differences between their structures. Therefore, the differences between their structures could account for why the earlier stated hypothesis is not accurate for humans (Boehm et al.).

Discussion

In review, “muscle memory” is a result of highly complex biological systems at both the neural level and the muscular level. As specific movements are completed more often, both neurons and muscles adapt in order to optimize efficiency and precision of said movement, and retain a molecular and physiological “memory” of that movement over a longer period of time. Muscles have the ability to retain the results of specific movements, even after a long time of de-training. This is enabled by the observation that when a certain muscle is exercised, both the muscle fiber size and number of myonuclei increase within the muscle fiber (Murach et al.). As detraining occurs, the muscle fiber size decreases while the myonuclear number remains stable. This results in the muscle fiber having a higher myonuclear content within the cell than before initial training, even after de-training. During re-training, when muscle fiber size returns to baseline measurements, it is the increased myonuclear content that enables the muscle fiber size to increase more quickly than seen in the initial training (Murach et al.).

Neurons become increasingly efficient with more frequent movements, based on observations in clinical and pre-clinical reports. Early work focused on the AChR in the neuromuscular junction, and their relative increase in density as certain movements became more repetitive. This results in more receptors available to receive neurotransmitters from the presynaptic cleft, increasing the overall signal's efficiency. However, further research suggested this is possibly only true in rodent pre-clinical trials, as most clinical trials suggested the number of AChR goes down with repeated movements. This discordance between preclinical and clinical findings may be due to species-specific mechanisms/physiology, and is an area of research that would benefit from additional investigation.

Human research conducted on the neuromuscular junctions AChR density as a function of frequent exercise differs from preclinical rodent studies to human studies (Boehm et al.; Soendenbroe et al., 2020; Soendenbroe et al., 2022, Cecilie J. L. Bechshøft, et al.; Desaulniers et al.; Deschenes et al.; Gorzi et al.; Khalil et al.; Parnow et al.; Sarto et al.). Importantly, it should be noted that both human studies reviewed in this paper did not report similar findings to the preclinical literature. It is critical to note differences in methodology as the primary research done was completed by measuring the mRNA levels of AChR, not testing through muscle biopsies. Muscle biopsies on the neuromuscular junction are not yet completely tested, which could result in severe adverse effects due to the procedure (Mylène Aubertin-Leheudre et al.; Santos et al.). This is likely why muscle biopsies on the neuromuscular junction were not used in these particular experiments. However, testing mRNA levels of AChR is not as precise as testing through muscle biopsy, because AChR density is influenced by a multitude of post-transcriptional and post-translational factors. The evidence supporting this hypothesis is described in one study (Soendenbroe et al., 2020) in which the researchers completed muscle biopsies of the patient's neuromuscular junction. This, in theory, would give these researchers more accurate results than those testing the mRNA levels, as they have access to many more factors contributing to the creation of AChR. The decrease in mRNA levels would, in theory, signify there is also a decrease in AChR, however it is limited to what can be made into a receptor, not necessarily what is developed into a functional protein.

Therefore, it should not automatically be assumed that the decrease in mRNA levels for AChR is directly associated with the number of AChR present. Additionally, this research did not encompass how different genders and ages of people are affected differently, as well as how the different muscle types are affected, so these factors should be further researched to discover how these affect muscle memory. Future research to further investigate this could include

discovering a safer and less difficult way to extract muscle biopsies from people's neuromuscular junction, allowing for scientists to safely conduct this experiment with more accurate results. Research should also be conducted to investigate the significance the amount of receptors on the postsynaptic cleft has on the overall effect on muscle movements and response. There has not been much research in this specific area, however, further research can allow us to understand how muscle memory works at a deeper level. Additional research could be conducted on another organism that has a more similar neuromuscular junction to humans than rodents do; for example, the neuromuscular junction of sheep and pigs (Boehm et al.).

In terms of societal impact, the potential of better characterizing of muscle memory has implications for human health and disease. For example, neuromuscular disorders represent a challenging class of conditions with high unmet medical need. For this population, it is possible that the molecular and cellular elucidation of muscle memory can unveil potential new, effective therapeutics. Hypothetically aiding muscle-memory through an intervention such as by modulation of myonuclei content within the muscle fibers could be a therapeutic intervention (Egner et al.). For instance, ALS (Amyotrophic Lateral Sclerosis), is a neuromuscular disorder in which nerves within an organism begin to slowly die over a period of time, resulting in the patient having less control over their voluntary muscles. Exercise can help this because even though it can't prevent or revert the nerve degeneration, it can keep the nerve stronger for longer, so it doesn't degrade as quickly (Kato et al.). Additionally, if we were able to characterize the musculoskeletal and neuromuscular mechanisms underlying muscle memory, perhaps a therapy could be generated to enhance neuromuscular function in these patients to alter disease trajectory.

Additionally, research and progress in this domain have implications beyond patients, including high-performance athletes and civilians alike. For the average person, an understanding of how to discern the impact of unconscious and conscious muscle movement, training, detraining, and memory may allow for optimization of their exercise routines and leveraging of these mechanisms for positive health outcomes.

Athletes in particular can use this information to optimize their activity schedules in order to refine their schedules, allowing for peak performance and fluidity of muscle motions. If athletes take this research into consideration, it would allow them to understand that the more often they complete a specific motion, the stronger and more efficient that motion will become (Concordia St. Paul). Additionally, it remains to be seen how research in muscle memory can impact injury and recovery dynamics. This is an important facet of professional sports that further highlights the enormous potential molecular and cellular characterization of this phenomenon has for society at large.

Works Cited

Allen, David L., et al. "Growth Hormone/IGF-I And/or Resistive Exercise Maintains Myonuclear Number in Hindlimb Unweighted Muscles." *Journal of Applied Physiology*, vol. 83, no. 6, Dec. 1997, pp. 1857–61, https://doi.org/10.1152/jappl.1997.83.6.1857.

Blocquiaux, Sara, et al. "Recurrent Training Rejuvenates and Enhances Transcriptome and Methylome Responses in Young and Older Human Muscle." *JCSM Rapid Communications*, vol. 5, no. 1, Wiley, Oct. 2021, pp. 10–32, https://doi.org/10.1002/rco2.52.

Boehm, Ines, et al. “Comparative Anatomy of the Mammalian Neuromuscular Junction.” *Journal of Anatomy*, vol. 237, no. 5, June 2020, pp. 827–36, https://doi.org/10.1111/joa.13260.

Bruusgaard, Jo C., and Kristian Gundersen. “In Vivo Time-Lapse Microscopy Reveals No Loss of Murine Myonuclei during Weeks of Muscle Atrophy.” *Journal of Clinical Investigation*, vol. 118, no. 4, Apr. 2008, pp. 1450–57, https://doi.org/10.1172/jci34022.

Casper Soendenbroe, et al. “Human Skeletal Muscle Acetylcholine Receptor Gene Expression in Elderly Males Performing Heavy Resistance Exercise.” *AJP Cell Physiology*, vol. 323, no. 1, American Physical Society, June 2022, pp. C159–69, https://doi.org/10.1152/ajpcell.00365.2021.

Casper Soendenbroe et al. “Key Components of Human Myofibre Denervation and Neuromuscular Junction Stability Are Modulated by Age and Exercise.” *Cells*, vol. 9, no. 4, Multidisciplinary Digital Publishing Institute, Apr. 2020, pp. 893–93, https://doi.org/10.3390/cells9040893.

Cleveland Clinic. “Muscle Memory: What It Is & How It Works.” *Cleveland Clinic*, 19 Feb. 2025, my.clevelandclinic.org/health/articles/muscle-memory.

Concordia St. Paul. “The Science of Muscle Memory: Implications for Training & Rehab.” *Concordia St. Paul*, 29 July 2024, kinesiology.csp.edu/exercise-physiology-articles/science-of-muscle-memory/.

Desaulniers, Patrice, et al. “Endurance Training Increases Acetylcholine Receptor Quantity at Neuromuscular Junctions of Adult Rat Skeletal Muscle.” *NeuroReport*, vol. 9, no. 16, Nov. 1998, pp. 3549–52, https://doi.org/10.1097/00001756-199811160-00001.

Deschenes, M. R., et al. “Increased and Decreased Activity Elicits Specific Morphological Adaptations of the Neuromuscular Junction.” *Neuroscience*, vol. 137, no. 4, 2006, pp. 1277–83, https://doi.org/10.1016/j.neuroscience.2005.10.042.

Egner, Ingrid M., et al. “A Cellular Memory Mechanism Aids Overload Hypertrophy in Muscle Long after an Episodic Exposure to Anabolic Steroids.” *The Journal of Physiology*, vol. 591, no. 24, Nov. 2013, pp. 6221–30, https://doi.org/10.1113/jphysiol.2013.264457.

Gorzi, Ali, et al. “Muscle Gene Expression of CGRP-α, CGRP Receptor, NAchR-β, and GDNF in Response to Different Endurance Training Protocols of Wistar Rats.” *Molecular Biology Reports*, vol. 47, no. 7, July 2020, pp. 5305–14, https://doi.org/10.1007/s11033-020-05610-4.

“How Muscle Memory Affects Your Performance – Posture Practice.” *PosturePractice.com*, posturepractice.com/how-muscle-memory-affects-your-performance/.

Jacquemin, V., et al. “IGF-1 Induces Human Myotube Hypertrophy by Increasing Cell Recruitment.” *Experimental Cell Research*, vol. 299, no. 1, Sept. 2004, pp. 148–58, https://doi.org/10.1016/j.yexcr.2004.05.023.

Jones, Ross A., et al. “Cellular and Molecular Anatomy of the Human Neuromuscular Junction.” *Cell Reports*, vol. 21, no. 9, Nov. 2017, pp. 2348–56, https://doi.org/10.1016/j.celrep.2017.11.008.

Kato, Naoki, et al. “Effect of Muscle Strengthening Exercise and Time since Onset in Patients with Amyotrophic Lateral Sclerosis.” *Medicine*, vol. 97, no. 25, June 2018, p. e11145, https://doi.org/10.1097/md.0000000000011145.

Kennedy, Mary B. “Synaptic Signaling in Learning and Memory.” *Cold Spring Harbor Perspectives in Biology*, vol. 8, no. 2, Feb. 2016, https://doi.org/10.1101/cshperspect.a016824.

Khalil, Bassem, et al. “Physiology, Neuromuscular Junction.” *Nih.gov*, StatPearls Publishing, 1 May 2023, www.ncbi.nlm.nih.gov/books/NBK470413/.

Kristoffer Toldnes Cumming, et al. “Muscle Memory in Humans: Evidence for Myonuclear Permanence and Long‐Term Transcriptional Regulation after Strength Training.” *The Journal of Physiology*, vol. 602, no. 17, Wiley, Aug. 2024, https://doi.org/10.1113/jp285675.

Lee, Hojun, et al. “A Cellular Mechanism of Muscle Memory Facilitates Mitochondrial Remodelling Following Resistance Training.” *The Journal of Physiology*, vol. 596, no. 18, Aug. 2018, pp. 4413–26, https://doi.org/10.1113/jp275308.

Murach, Kevin A., et al. “Muscle Memory: Myonuclear Accretion, Maintenance, Morphology, and MiRNA Levels with Training and Detraining in Adult Mice.” *Journal of Cachexia, Sarcopenia and Muscle*, vol. 11, no. 6, Sept. 2020, pp. 1705–22, https://doi.org/10.1002/jcsm.12617.

Mylène Aubertin-Leheudre, et al. “Improved Human Muscle Biopsy Method to Study Neuromuscular Junction Structure and Functions with Aging.” *The Journals of Gerontology*, vol. 75, no. 11, Oxford University Press, Dec. 2019, pp. 2098–102, https://doi.org/10.1093/gerona/glz292.

“NHIS - Adult Physical Activity - Glossary.” *Cdc.gov*, 6 Nov. 2024, archive.cdc.gov/www_cdc_gov/nchs/nhis/physical_activity/pa_glossary.htm.

Noto, Rachel E., and Mary Ann Edens. “Physiology, Muscle.” *PubMed*, StatPearls Publishing, 1 May 2023, www.ncbi.nlm.nih.gov/books/NBK532258/.

Parnow, Abdolhossein, et al. “Effects of Endurance and Resistance Training on Calcitonin Gene-Related Peptide and Acetylcholine Receptor at Slow and Fast Twitch Skeletal Muscles and Sciatic Nerve in Male Wistar Rats.” *International Journal of Peptides*, vol. 2012, no. 1, Hindawi Publishing Corporation, June 2012, pp. 1–8, https://doi.org/10.1155/2012/962651.

Personius, Kirkwood E., and Rita J. Balice-Gordon. “Book Review: Activity-Dependent Synaptic Plasticity: Insights from Neuromuscular Junctions.” *The Neuroscientist*, vol. 8, no. 5, SAGE Publishing, Oct. 2002, pp. 414–22, https://doi.org/10.1177/107385802236970.

Petrella, John K., et al. “Potent Myofiber Hypertrophy during Resistance Training in Humans Is Associated with Satellite Cell-Mediated Myonuclear Addition: A Cluster Analysis.” *Journal of Applied Physiology*, vol. 104, no. 6, June 2008, pp. 1736–42, https://doi.org/10.1152/japplphysiol.01215.2007.

Santos, Jhonnatan Vasconcelos Pereira, et al. “Safety of Percutaneous Muscle Biopsy: An Update Based on over 2400 Procedures.” *Scandinavian Journal of Medicine & Science in Sports*, vol. 35, no. 11, Wiley, Nov. 2025, https://doi.org/10.1111/sms.70160.

Sarto, Fabio, et al. “Effects of Short‐Term Unloading and Active Recovery on Human Motor Unit Properties, Neuromuscular Junction Transmission and Transcriptomic Profile.” *The Journal of Physiology*, vol. 600, no. 21, Sept. 2022, pp. 4731–51, https://doi.org/10.1113/jp283381.

Staron, R. S., et al. “Strength and Skeletal Muscle Adaptations in Heavy-Resistance-Trained Women after Detraining and Retraining.” *Journal of Applied Physiology*, vol. 70, no. 2, Feb. 1991, pp. 631–40, https://doi.org/10.1152/jappl.1991.70.2.631.