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Enhancement of neuromuscular dynamics and strength behavior using extremely low magnitude mechanical signals in mice

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ABSTRACT

Exercise in general, and mechanical signals in particular, help ameliorate the neuromuscular symptoms of aging and possibly other neurodegenerative disorders by enhancing muscle function. To better understand the salutary mechanisms of such physical stimuli, we evaluated the potential for low intensity mechanical signals to promote enhanced muscle dynamics. The effects of daily brief periods of low intensity vibration (LIV) on neuromuscular functions and behavioral correlates were assessed in mice. Physiological analysis revealed that LIV increased isometric force production in semitendinosus skeletal muscle. This effect was evident in both young and old mice. Isometric force recordings also showed that LIV reduced the fatiguing effects of intensive synaptic muscle stimulation. Furthermore, LIV increased evoked neurotransmitter release at neuromuscular synapses but had no effect on spontaneous end plate potential amplitude or frequency. In behavioral studies, LIV increased mouse grip strength and potentiated initial motor activity in a novel environment. These results provide evidence for the efficacy of LIV in producing changes in the neuromuscular system that translate into performance gains at a behavioral scale.

1. Introduction

Numerous studies using Whole Body Vibration (WBV) of varying frequencies and magnitudes, or limb specific vibration, including LIV, suggest it can improve skeletal and muscular function. Specifically, high frequency, low magnitude vibration stimulates bone formation, and may suppress adipose tissue generation (Gilsanz et al., 2006; Holguin et al., 2009; Reyes et al., 2011; Rubin et al., 2007; Slatkovska et al., 2010; Xie et al., 2006; Xie et al., 2008). Additionally, improvements in muscular function have also been demonstrated in humans (Gilsanz et al., 2006; Muir et al., 2011; Reyes et al., 2011; Russo et al., 2003) and animals (McKeehen et al., 2012; Xie et al., 2008). However, as opposed to human studies, there have been few if any reports of the efficacy of WBV or LIV at the behavioral level in animals.

Here we examine the influence of brief daily exposure to LIV on the musculature of both young and old mice as a means of understanding the mechanisms by which these mechanical signals modulate muscle function. We describe a number of potentially beneficial effects using both physiological and behavioral approaches. An addition, we propose a partial mechanism for one aspect of the observed changes, which may help to explain conflicting results from this new field.

2. Methods

2.1. Animals

Muscle electrophysiology, *ex vivo* isometric tension, and preceding LIV exposure were approved by the Institutional Animal Care and Use Committee and performed at the University of Missouri Dalton Cardiovascular Research Center. For *ex vivo* isometric tension and intracellular recording experiments, male C57BL/6 mice of 8 and 104 weeks of age, with $n=4$ and $n=3$, respectively, were subjected to LIV every day for 4 weeks and sacrificed 2–3 days after the last exposure. LIV exposure was performed for 20 min each day with mice in empty cages on a custom manufactured platform oscillating vertically at 30 Hz with 0.3 g peak acceleration (Marodyne Medical, Lakeland, FL). Control mice were set in identical housing near the platform during LIV exposure.

Behavioral experiments and preceding LIV exposure were approved by the Institutional Animal Care and Use Committee and performed at The University of Texas Southwestern Medical Center. Behavioral experiments were performed during the light cycle under dimmed lighting. Male C57BL/6J mice were purchased from Jackson Laboratory at 8 weeks of age and allowed to acclimate to housing for

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one week. Mice were group housed with 4 per cage. Half of the mice (two from each cage) were assigned as the LIV treatment group ($n=12$) with the other half being controls. Exposure was performed as above at 5 times per week for 3 weeks before behavioral testing and continued one more week, after daily testing. Behavioral testing, in order, consisted of Wire Grid Hang, Wire Hang, Grip Strength, and Open Field.

2.2. Ex vivo isometric tension and electromyogram measurements

These experiments were performed as previously described (Rafuse et al., 2000). Briefly, mice were sacrificed by CO_2 asphyxiation and semitendinosus muscle with intact nerve supply was dissected out and placed into well-oxygenated Tyrode's solution maintained at 27–29 °C. Fine-tipped polyethyl suction electrodes were used to stimulate individual nerves and measure electromyograms from the midbelly of the muscle. A linear force transducer was used to measure muscle contractions. Nerves were stimulated repeatedly 4–5 times at frequencies of 1, 10, 20, 50, 100, and 200 Hz. The average peak force from each stimulation set was used in statistical testing.

2.3. Intracellular recordings

Intracellular recordings were also performed as previously described (Rafuse et al., 2000). Semitendinosus muscles, with the nerve supply intact, were isolated as described above. Miniature end plate potentials (MEPPs) and evoked end plate potentials (EPPs) were recorded using sharp glass electrodes impaled near the motor end plate under standard conditions. The quantal content per cell was calculated by the mean amplitude of the first EPP from all the train stimuli (from 10 to 200 Hz) divided by the mean amplitude of the MEPPS from the same cell recording. Recording in which MEPPS were not observed at the same time that EPPS from the same recording cell were not used in the calculations. The mean quantal content was calculated as the mean of all the individual QCs from the control and treated animals.

2.4. Neuromuscular junction staining

Post electrical evaluation, the muscle preparations were incubated with an α-bungarotoxin-based stain (α-BTX-Alexa 555, Invitrogen, 100 nM) for 15 min in oxygenated Tyrode's solutions. After 15 min the preparation was washed 3 times with new fresh Tyrode's solution. The preparation was maintained under oxygenation and imaged in a fluorescence Olympus BX51WI microscope with a 40 × water immersion lens. Images were captured by a Retiga EXi-Fast (Qimaging) in binning mode at 8 bits.

2.5. Behavioral experiments

2.5.1. Wire grid hang

Mice were placed first on a thin wire grid and then a wire rat cage grid as tests of muscle strength, endurance, and coordination (Hamann et al., 2003; Lee et al., 2009; Sango et al., 1996). The grid was shaken lightly to encourage mice to grip, then flipped upside down and the latency for mice to fall was recorded using a stopwatch. One trial was performed for each mouse with each grid.

2.5.2. Wire hang

This test was used as another measure of muscle dynamics (Allen et al., 2009; Baldo et al., 2012; Oddoux et al., 2009; Takahashi et al., 2009). Mice were lifted by the tail and allowed to grip onto a horizontal bar (diameter=3.175 mm) with their forepaws, then let go and allowed to hang until falling a short distance. All mice tested in this way were able to lift their lower bodies to grip the bar with all four limbs, as well as the tail. Two trials of wire hanging were performed at separate times on one testing day and averaged for statistical testing.

2.5.3. Grip strength

As a final measure of muscle strength (Derave et al., 2003; Mandillo et al., 2008; Whittemore et al., 2003), mice were placed on a horizontal wire grid attached to a spring loaded linear scale and allowed to grip it with all four paws, then were pulled by the tail until grip was released. The final weight pulled was then recorded. Three trials of this test were performed on each of 3 days. The average of each day's 3 trials was used for statistical testing.

2.5.4. Open field

This paradigm was used to test for effects of vibration on locomotion as well as general anxiety (Crawley, 1999). Mice were placed in a dimly lit square arena with walls 44 cm in length as a novel open field. Individual trials were 20 min in length, recorded using an overhead camera and analyzed using EthoVision v. 3.1.16. Measures in this study included total distance moved per arena region, distance moved per 4 min epoch, and total duration within specific regions of the testing arena.

2.6. Statistical testing

Two-way partial repeated measures ANOVA were performed on time series data from body mass and open field measures. Post-testing was performed using the Sidak method to correct for multiple comparisons. Two-way ANOVA was performed on *ex vivo* force production data. For other data, unpaired Student's *t*-tests were performed. Values of p less than 0.05 were considered to be significant.

3. Results

To understand how LIV could improve neuromuscular performance, we first used a physiological approach. Adult or aged mice were either left untreated (controls) or treated long term with LIV. From these subjects, *ex vivo* semitendinosus muscle preparations were then made for assessment of isometric force production in response to increasing synaptic stimulation rates (Fig. 1). In both young and old mice, LIV treatment resulted in overall higher isometric force (both $p<0.0001$). At higher stimulation rates, where untreated mouse muscle ceased to produce considerable force, LIV mice demonstrated continued, although reduced, force generation in young (Fig. 1A) and old (Fig. 1B) mice. In young mice there were significant increases at 20, 100, and 200 Hz stimulation rates. These were higher by 1.6-, 10-, and 29-fold.

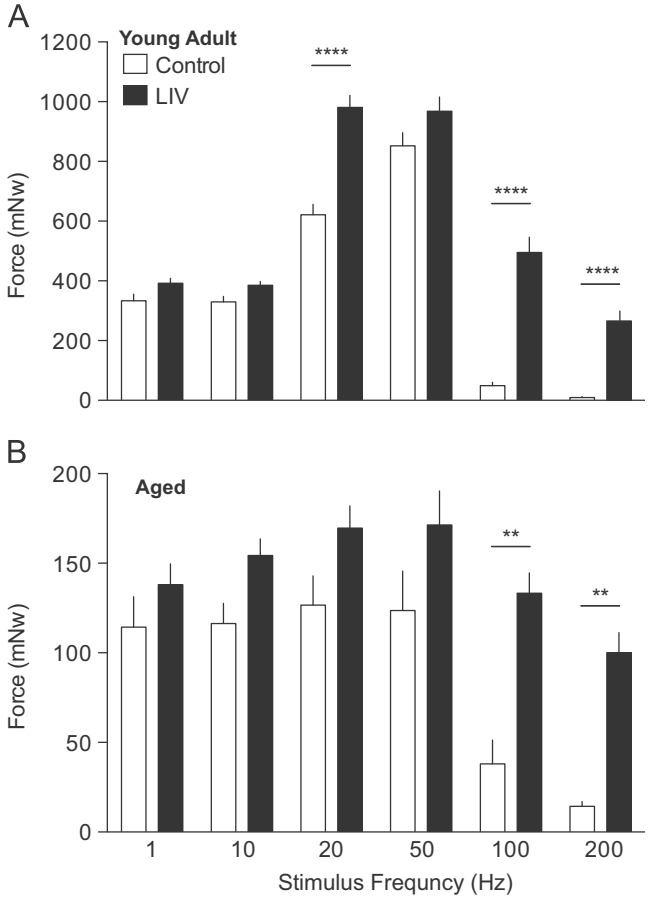

Fig. 1. LIV increases isometric force production and enhances neuromuscular synapse-driven responsiveness in mouse skeletal muscle. Effects of 4 weeks of LIV exposure on the isometric force generation of isolated mouse semitendinosus muscle at varying frequencies of intact direct nerve stimulation from young (12 week old, $n=4$, *A*) and aged (107 week old, $n=2$–3, *B*) mice are summarized. LIV increased force production overall in young and old mice ($p<0.0001$) (for both age groups). Data represent means with standard error. For per frequency comparisons **** is $p<0.0001$ and ** is $p<0.01$.