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Plasmalemma Function is Rapidly Restored in *Mdx* Muscle after Eccentric Contractions

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Abstract

Purpose: Muscle that lacks dystrophin, as in the *mdx* mouse, has a heightened sensitivity to eccentric (ECC) contraction-induced strength loss, but an enhanced rate of recovery. However, the timeline and mechanisms underlying why *mdx* muscle recovers quicker has yet to be determined. We used an electromyographic (EMG) approach to analyze plasmalemma electrophysiological function during and following ECC contraction-induced injury to test the hypothesis that loss of plasmalemmal excitability is a transient event in *mdx* muscle.

Methods: Mice were implanted with stimulating electrodes on the common peroneal nerve and EMG electrodes on the tibialis anterior muscle. Anterior crural muscles of anesthetized mice performed 1 or 2 bouts of 50 injurious ECC contractions, and recovery of maximal isometric torque and M-wave root mean square (M-wave RMS) were assessed following each bout.

Results: Maximal isometric torque and M-wave RMS were equally reduced 62% ($P < 0.001$) in *mdx* mice immediately following the initial ECC injury. For these *mdx* mice, M-wave RMS was still reduced at 2 days post-injury ($P = 0.034$) but was not different from pre-injury values by 6 days ($P = 0.106$), while torque took up to 9 days to recover ($P = 0.333$). M-wave RMS did not change ($P = 0.390$) in wildtype mice in response to ECC injury while torque decreased 35% ($P < 0.001$) and recovered by day 2 ($P = 0.311$). Results from the second bout of ECC contractions were similar to those observed during and after the initial injury.

Conclusion: Functional dystrophin is necessary for excitation to occur at the plasmalemma during ECC contractions but is not essential for complete recovery of plasmalemma electrophysiological function or maximal isometric strength.

Keywords

damage; Duchenne muscular dystrophy; dystrophin; force; injury

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INTRODUCTION

Duchenne muscular dystrophy (DMD) is a devastating and fatal X-linked recessive disease that affects 1 in ~3500–5000 boys (1, 2). DMD is characterized by skeletal, respiratory and cardiac muscle deterioration due to the absence of dystrophin, a cytoplasmic protein associated with the plasmalemma (3). Dystrophin links the actin cytoskeleton to the transmembrane dystrophin-glycoprotein complex and is responsible for helping to maintain membrane integrity and cellular homeostasis (4–6). Skeletal muscle weakness, a clinical hallmark of DMD, develops in childhood and progressively worsens with age. Over time, patients with DMD lose ambulation and become wheelchair bound in their early teens, ultimately dying in their late twenties from respiratory and cardiac failure (3). Due to the nature of DMD, there is a great urgency to develop therapies that mitigate disease progression and increase the lifespan and overall health of these patients.

The *mdx*, dystrophin deficient, mouse is the most widely used animal model to study DMD (7). A key feature of the *mdx* mouse is dramatic reductions in muscle function due to exercise that accentuates eccentric (ECC) contractions. Indeed, strength loss immediately following isolated ECC contractions is 20–60% greater in *mdx* mice compared to wildtype mice, a finding that has been demonstrated in muscle *in situ* and *ex vivo* (8–11), as well as entire muscle groups *in vivo* (12–15). Susceptibility to ECC contraction-induced strength loss has therefore become a standard outcome measure in pre-clinical studies to assess disease severity and the efficacy of potential therapies for Duchenne muscular dystrophy. Despite the widespread use of ECC contraction-induced injury, the mechanisms underlying the initial loss and subsequent recovery of strength, and the impact eccentric exercise has on muscle function in *mdx* mice remains poorly understood. For the purpose of this manuscript, we define injury as the loss of muscle function (i.e., strength loss) due to any structural disruption or modification that occurs because of ECC contractions.

The excessive loss of strength in *mdx* mice following ECC contractions provokes the idea that the primary cause(s) of the injury may be different from that of wildtype mice (12, 13). In wildtype mice, ECC contraction-induced strength loss is largely due to excitation-contraction uncoupling occurring between the voltage-sensitive dihydropyridine receptors embedded in the T-tubule membrane and the sarcoplasmic reticulum (SR) Ca²⁺ release channel (i.e., ryanodine receptor) (16–19). Excitation-contraction uncoupling in skeletal muscle can broadly be described as any disruption between electrical events occurring at the plasmalemma and SR Ca²⁺ release. However, given the location and function of dystrophin in skeletal muscle, it is plausible that the site of disruption in *mdx* mice could be at the plasmalemma and impair the generation and/or conduction of an action potential across the plasmalemma. Electrophysiological dysfunction of the plasmalemma has historically been overlooked, likely because muscle of *mdx* mice typically have normal resting membrane potentials and excitability (13, 20–22). What has not been realized until very recently is that plasmalemma electrophysiological properties are drastically impaired during and immediately following ECC contractions in *mdx* mice (13, 14, 23), data that has largely gone unnoticed.

Interestingly, despite greater strength deficits observed during and immediately after ECC contractions in *mdx* mice, *in situ* and *in vivo*, strength recovers faster compared to that of wildtype mice (8, 12). A disconnect between strength loss and recovery further supports that sites of excitation-contraction uncoupling differ between wildtype and *mdx* mice. Given that the site causing strength loss is thought to be at the plasmalemma in *mdx* mice (13, 23), it seems logical that recovery of strength would therefore be dependent on restoring plasmalemma electrophysiological function. However, a detailed electromyographic (EMG) analysis of the time course of recovery in *mdx* mice has not yet been examined, as previous studies only measured one recovery time-point following the injury. Thus, the aim of *experiment #1* was to substantiate that plasmalemma electrophysiological function is impaired when *mdx* mice perform repetitive ECC contractions and determine its subsequent rate of recovery. We hypothesized that electrophysiological function of the plasmalemma would be compromised during and immediately after a bout of ECC contractions, and fully recover in the days following the injury.

Skeletal muscle possesses an intrinsic ability to adapt after ECC contraction-induced injury, in such a way that it is protected against injury from future bouts of ECC contractions (i.e., repeated-bout effect) (24). When compared to the initial injury, adaptations in normal muscle manifest as increased strength, attenuated ECC contraction-induced strength loss and an enhanced rate of strength recovery (12, 24, 25). Interestingly, when *mdx* mice perform several bouts of ECC contractions, the muscle eventually becomes stronger but never adapts to strength deficits during or immediately after injurious ECC contractions (12). It is plausible that the plasmalemma continues to be the main site of disruption in *mdx* mice regardless of prior ECC contraction bouts, and that recovery of plasmalemma electrophysiological function is paramount to the return of strength. Therefore, in *experiment #2* we aimed to measure plasmalemma electrophysiological function after a second bout of ECC contractions and determine the subsequent rate of recovery. We hypothesized that irrespective of whether the injury bout was novel or repeated, electrophysiological function of the plasmalemma would be impaired and recover in parallel with strength in *mdx* mice.

METHODS

Ethical approval and animal models

Male wildtype (C57Bl/10) and *mdx* (C57Bl/10ScSn-DMD^{*mdx*}) mice were obtained from Jackson Laboratory (Bar Harbor, ME, USA) or bred locally from these mice, and were 3–6 months old at the time of the experimental protocols. Mice were initially anesthetized in an induction chamber using isoflurane, and then maintained by inhalation of 1.5% isoflurane mixed with oxygen at a flow rate of 125 ml/min for all surgical procedures. This anesthetic regimen was also used when torque and EMG measurements were made. Following the final contraction protocol, mice were euthanized with an overdose of sodium pentobarbital (150 mg/kg body mass). All animal procedures were in accordance with the standards set by the Institutional Animal Care and Use Committees at the University of Minnesota.

Experimental Design

Experiment #1—To determine recovery of electrophysiological function of the plasmalemma in injured muscle, mice (wildtype, $n = 8$; *mdx*, $n = 14$) were chronically implanted with stimulating electrodes on the common peroneal nerve and EMG electrodes on the tibialis anterior (TA) muscle. Using these surgical techniques, we were able to utilize an *in vivo* approach to assess strength of the anterior crural muscles [TA, extensor digitorum longus, and extensor hallucis muscles] and plasmalemmal excitability of the TA muscle in a longitudinal study design. Specifically, anterior crural muscle isometric torque and TA muscle M-wave root mean square (RMS) were measured before, immediately after and at 1, 2, 6, 9 and 14 days following a single bout of 50 maximal ECC contractions (bout 1). Because the same mice were tested at every time-point, pre-injury values served as the control for all measurements.

Experiment #2—To determine if the plasmalemma continues to be the main site of disruption across repeated bouts of ECC contractions, a subset of mice from *experiment #1* (wildtype, $n = 6$; *mdx*, $n = 7$) were subjected to a second injury. Specifically, following the 14-day assessment, mice were injured for a second time with 50 maximal ECC contractions (bout 2) and tested immediately after and at 1, 2 and 14 days post-injury. All assessments in *experiment #2* were identical to those of *experiment #1*. When comparing changes across ECC contraction bouts or recovery time-points, each measurement was expressed relative to the initial ECC contraction or pre-injury isometric value for that given bout, respectively. Although this method of data interpretation may result in a less prominent repeated bout effect due to strength gains (12), the overall goal of the second injury in this study was to determine if M-wave RMS was reduced to the same degree between bouts 1 and 2 in *mdx* mice. Data pertaining to the repeated bout effect in muscle of wildtype mice when expressed as $\text{mN}\cdot\text{m}\cdot\text{kg}^{-1}$ of torque has been published by Ingalls and colleagues (19, 25).

Experimental Methodology

Surgical Procedures—An incision was made through the biceps femoris muscle in the left hindlimb and a nerve cuff made of platinum iridium wire (Medwire-Sigmund Chon 10Ir9/49T, Mt. Vernon, NY, USA) and silastic tubing was placed around the common peroneal nerve (13, 26, 27). The proximal end of the nerve cuff was run subcutaneously to the dorsal cervical region and connected to a stimulator and stimulus isolation unit (Models S48 and SIU5, respectively, Grass Technologies, West Warwick, RI, USA). No less than 21 days after implanting the stimulating nerve cuff, TA muscle EMG electrodes to record M-waves were then implanted in the anesthetized mouse (13, 26, 27). Briefly, deinsulated ends of two platinum iridium wires, offset by ~ 2 mm, were routed underneath the superficial fascial sheath of the TA muscle. The electrode wire spacing theoretically permitted sampling of EMG activity from the full thickness of the TA muscle beneath the electrodes (27, 28). The wires were secured to adjacent tissue and the proximal ends of the wires were run subcutaneously to the dorsal cervical region and connected to an EMG amplifier (Model P55, Grass Technologies). *In vivo* muscle testing with simultaneous M-wave measurements were initiated no less than 14 days after the EMG wire implantation.

Torque Measurements and Injury Protocol—*In vivo* maximal isometric torque of the anterior crural muscles was assessed as previously described (13, 18, 27, 29). The anaesthetized mouse (see above) was placed on a temperature-controlled platform to maintain rectal body temperature at 37°C, and the left knee was clamped and the left foot was secured to an aluminum footplate that is attached to the shaft of the servomotor system (Model 300B-LR; Aurora Scientific, Aurora, Ontario, Canada). Contractile function of the anterior crural muscles was assessed by measuring isometric torque as a function of stimulation frequency (20–300 Hz; 150-ms train with 0.1-ms pulses). The anterior crural muscles were then injured by performing 50 electrically stimulated maximal ECC contractions. During each ECC contraction the foot was passively moved from 0 deg (positioned perpendicular to tibia) to 19° of dorsiflexion where the anterior crural muscles performed a pre-lengthening 100 ms isometric contraction followed by an additional 20 ms of stimulation while the foot was moved from 19° of dorsiflexion to 19° of plantarflexion at 2000 deg s⁻¹. A 5-min rest following the ECC contraction protocol was given prior to reassessing contractile function via the aforementioned torque-frequency protocol.

M-wave Assessment—Analysis of the electrically evoked myoelectric signal was done using the amplitude measure (i.e., M-wave root-mean-square [RMS]) as previously described (13, 26, 27). M-wave RMS was calculated for the full 150 ms of the isometric contractions during the torque-frequency protocols and across the first 100 ms (i.e., the isometric portion) of the ECC contraction protocol. Because the anterior crural muscles were maximally recruited via electrical stimulation of the common peroneal nerve, a decrease in M-wave RMS was interpreted as impairment of action potential generation and/or conduction (13, 26, 27). Fig. 1A & B depict representative isometric tetanic torque-time and M-wave-time tracings for wildtype and *mdx* mice.

Statistical Analyses

One-way repeated measures analysis of variance (ANOVA) were used to assess differences within strains across time, while two-way ANOVAs were utilized to probe for differences between strains across time or injury bout for torque and M-wave RMS. When significant interactions or main effects were calculated, differences were tested with Holm-Sidak post hoc tests. An α level of 0.05 was used for all analyses. Values are presented as means $\pm$ SE. All statistical testing was performed using SigmaPlot version 12.5 (Systat Software, San Jose, CA, USA).

RESULTS

Experiment #1

In both mouse strains, maximal ECC torque and M-wave RMS decreased over the injury protocol ($P < 0.001$; Fig. 2A & B), however deficits were far greater in *mdx* mice ($P < 0.001$). At the completion of the injury protocol, maximal ECC torque and M-wave RMS decreased $68 \pm 1\%$ ($P < 0.001$) and $78 \pm 3\%$ ($P < 0.001$), respectively, in *mdx* mice while only $28 \pm 2\%$ ($P < 0.001$) and $12 \pm 3\%$ ($P = 0.004$), respectively, in wildtype mice. Moreover, although maximal ECC torque and M-wave RMS both decreased over the

contraction protocol in wildtype mice, the reduction observed in M-wave RMS was greater than the deficit recorded in maximal ECC torque ($P = 0.003$).

As with maximal ECC torque and M-wave RMS during the injury protocol, reductions in maximal isometric torque and M-wave RMS assessed immediately post-injury were greater in *mdx* mice ($P < 0.001$). When compared to pre-injury values, maximal isometric torque and M-wave RMS decreased $62 \pm 3\%$ ($P < 0.001$) immediately post-injury in *mdx* mice (Fig 1B & 2D), whereas in wildtype mice maximal isometric torque only decreased $35 \pm 3\%$ ($P < 0.001$) and M-wave RMS did not change. ($P = 0.390$; Fig. 1A & 2C). By day 2, maximal isometric torque was not different from pre-injury torque in wildtype mice ($P = 0.311$). Maximal isometric torque and M-wave RMS took longer to return to pre-injury values in *mdx* mice compared to wildtype mice ($P < 0.001$). In *mdx* mice, M-wave RMS and maximal isometric torque were not different from pre-injury values by days 6 ($P = 0.106$) and 9 ($P = 0.333$), respectively (Fig. 1A & B, 2C & D).

Experiment #2

To characterize plasmalemma electrophysiological function during and after a repeated bout of ECC contractions, a subset of mice was subjected to a second bout of 50 maximal ECC contractions 14-days following the initial injury (Fig. 3A–D). In wildtype mice, maximal ECC torque at the 50th contraction was greater after bout 2 (105.94 ± 3.78 vs. 123.55 ± 4.63 $\text{mN}\cdot\text{m}\cdot\text{kg}^{-1}$; $P = 0.008$) and ECC torque deficits tended to be less (29 ± 1 vs. $24 \pm 1\%$; $P = 0.054$; Fig. 3A), while reductions in M-wave RMS did not differ between bouts (11 ± 4 vs. $8 \pm 2\%$; $P = 0.457$; Fig. 3C). Maximal ECC torque at the 50th contraction (52.55 ± 3.42 vs. 55.19 ± 2.64 $\text{mN}\cdot\text{m}\cdot\text{kg}^{-1}$; $P = 0.468$), and deficits in ECC torque (69 ± 2 vs. $67 \pm 2\%$; $P = 0.193$; Fig. 3B) and M-wave RMS (85 ± 2 vs. $81 \pm 3\%$; $P = 0.423$; Fig. 3D) were similar between bouts 1 and 2 in *mdx* mice.

Maximal isometric torque and M-wave RMS in wildtype mice did not differ between bouts immediately after or at 1, 2 or 14 days following the injuries ($P = 0.132$; Fig. 4A & C). Maximal isometric torque in *mdx* mice was also similar between bouts immediately after, 1 and 14 days following the injuries ($P = 0.108$; Fig. 4B). Two days post-injury following the second bout of ECC contractions, *mdx* mice produced 32% more maximal isometric torque compared to the first bout ($P = 0.011$; Fig. 4B). No differences in M-wave RMS were measured between the bouts at any time-point during the maximal isometric contractions in *mdx* mice ($P = 0.266$; Fig. 4D).

DISCUSSION

We had four primary results from this study, all of which support our hypotheses. First, plasmalemma electrophysiological dysfunction is a major contributor to ECC contraction-induced strength loss in *mdx* mice. Second, loss of plasmalemma electrophysiological function is a transient event following ECC contraction-induced skeletal muscle injury in *mdx* mice. Third, excitation-contraction uncoupling at the plasmalemma is present across repeated bouts of ECC contractions. Lastly, a second ECC contraction bout does not exacerbate plasmalemma electrophysiological dysfunction beyond that of the initial injury or influence its subsequent rate of recovery. These results substantiate that a major

plasmalemma-based mechanism is one of the underlying causes for the loss and return of strength following ECC contractions in *mdx* mice, which is distinctly different from that of wildtype mice.

Loss of plasmalemma electrophysiological function due to a bout of ECC contractions was dramatic in *mdx* mice. M-wave RMS decreased 78% in *mdx* mice while only 12% in wildtype mice corresponding to maximal ECC torque deficits of 68 and 28%, respectively (Fig. 2A & B). In fact, it took less than four ECC contractions to achieve a ~12% reduction in M-wave RMS in *mdx* mice (vs. 50 ECC contractions in wildtype mice). As observed across the ECC contractions, maximal isometric strength and M-wave RMS in *mdx* mice were also drastically reduced immediately post-injury while maximal isometric torque deficits in wildtype mice did not parallel the loss in M-wave RMS (Fig. 1, 2C & D). These data corroborate two important physiological components of ECC contraction-induced strength loss previously reported by various research groups (14, 23), including our own (13, 27). First, functional dystrophin is required to maintain plasmalemmal excitability during maximal ECC contractions and second, ECC contraction-induced strength loss is mechanistically different between wildtype and *mdx* mice.

Transmission failure at the neuromuscular junction (NMJ) or an impaired ability to conduct an action potential along the plasmalemma, two proximal events during muscle excitation, would cause decrements in M-wave RMS in injured *mdx* mice. Evidence that NMJ transmission failure contributes to ECC contraction-induced strength loss is supported by histological data of altered NMJ morphology in injured muscle of *mdx* mice but not wildtype mice (14, 15). However, not all studies support neuromuscular transmission failure as a primary mechanism contributing to loss of M-wave RMS in *mdx* mice (13, 23). Roy et al. (23) reported that despite NMJ morphology being influenced by ECC contractions, bypassing the NMJ via direct stimulation of the injured muscle did not markedly improve strength in *mdx* mice. It should also be noted that the NMJ of *mdx* muscle is discontinuous and dispersed prior to ECC contraction-induced injury (14, 15, 23), yet *in vivo* maximal isometric torque is not different when compared to muscle with normal NMJ morphology (i.e., wildtype muscle) (14, 15, 30, 31). Thus, alterations in NMJ morphology may not always translate into NMJ dysfunction. Although we cannot fully rule out NMJ transmission failure in our study, these results suggest that plasmalemmal inexcitability in injured muscle of *mdx* mice does not originate at the NMJ, but rather occurs at a downstream site on the plasmalemma.

Using the same *in vivo* injury model as described in the present study, Call and coworkers (13) demonstrated that a significant number of fibers were depolarized in *mdx* muscle. Immediately following a bout of ECC contractions, 50% of the *mdx* muscle fibers were more positive than -55 mV, compared to only 7% from that of wildtype muscle fibers (13). When skeletal muscle fibers are depolarized above -55 mV, they are rendered inexcitable and contribute minimally to force development (32–34). These data indicate that loss of M-wave RMS following the injury was due to sustained depolarization of a large number of *mdx* muscle fibers, which contributed to fewer action potentials being generated and/or conducted along the plasmalemma (13, 27). Theoretically, changes in transplasmalemmal K⁺ gradients, along with Na⁺, Cl⁻ and/or Ca²⁺ could explain muscle fiber depolarization and

thus reductions in M-wave RMS (23, 35). In support of this theory, a Cl^- channel inhibitor improved force ten minutes following a bout of ECC contractions in *mdx* mice, and authors attributed the improvement in force to an overall increase in muscle excitability (23). It is likely that multiple plasmalemma-associated proteins and channels are affected by ECC contractions (36, 37) and contribute to plasmalemma electrophysiological dysfunction in *mdx* mice.

It is also possible the ECC contractions additionally disrupted other sites distal to the plasmalemma, as *mdx* fibers that lost M-wave RMS were depolarized and inexcitable post-injury. For instance, ECC contractions have also been shown to impair force development at the myofilaments in *mdx* fibers. Blaauw et al. (38) demonstrated Ca^{2+} activated force by permeabilized *mdx* fibers, taken from muscle previously injured *in vivo*, generated 28% less force than uninjured fibers. By using this experimental approach, the researchers were able to bypass voltage-gated SR Ca^{2+} release and make conclusions about force development at the myofilaments (38). These data suggest ECC contractions may impair force development at the myofilaments in *mdx* mice, in addition to disrupting plasmalemma electrophysiological function.

In this experiment we also assessed recovery of maximal isometric torque and M-wave RMS at five time-points between 1 and 14 days following the ECC contractions. Plasmalemma electrophysiological function was only down 14% in *mdx* mice 2 days post-injury, despite a 62% deficit being recorded immediately after the ECC contractions (Fig. 2D). Maximal isometric torque also showed a remarkable rebound, although not quite as rapid as M-wave RMS. To put this rate of recovery into perspective, maximal isometric torque in wildtype muscle injured by 150 ECC contractions is still reduced by 25–30% after 7 days, even though the initial injury only causes a 45–55% deficit (39, 40). In the present study, maximal isometric torque was reduced 62% immediately after the ECC contractions, but only remained 18% lower 6 days into recovery (Fig. 2D). These data emphasize that because the underlying mechanisms of the injury are different in *mdx* and wildtype mice, the recovery processes will also be different. We suggest that full recovery of strength in *mdx* mice is primarily dependent on restoring plasmalemma electrophysiological function and any other damaged or modified sites downstream of the plasmalemma.

To further examine plasmalemma electrophysiological function in *mdx* mice, a subset of mice were injured for a second time. We show that reductions in maximal ECC torque and M-wave RMS did not differ between contraction bouts in *mdx* mice (Fig. 3B & D), while ECC torque deficits tended to be less in wildtype mice following the second injury (Fig. 3A). These results confirm previous reports that demonstrate *mdx* mice show no protection from ECC torque drop (12), which we contend is due to reductions in plasmalemma electrophysiological function that are present regardless of injury bout. As hypothesized, recovery of plasmalemma electrophysiological function was also similar across injury bouts in *mdx* mice (Fig 4B & D). These data confirm that repeated bouts of ECC contractions do not exacerbate muscle injury or recovery in *mdx* mice, which is partially facilitated by the loss and return of plasmalemma electrophysiological function.

Excitation-contraction uncoupling at the plasmalemma through repeated bouts of ECC contractions suggests that loss of excitability may be an adaptive mechanism in muscle of *mdx* mice. Recently, ECC contraction-induced strength loss in *mdx* muscle was theorized to function as a circuit breaker that potentially protects the muscle from cell-lethal structural damage (10). Our data support these concepts for several reasons. First, plasmalemma electrophysiological function was drastically impaired by a limited number of ECC contractions, regardless of the contraction bout (Fig. 2B & 3D). Second, loss of plasmalemmal excitability was a transient event, recovering soon after the injury (Fig. 2D). Lastly, muscle of *mdx* mice did not adapt to ECC contraction-induced loss of plasmalemma electrophysiological function (Fig. 3D), yet were still able to recover from repeated injuries (Fig. 4B & D). Additional studies are needed to determine if sites downstream of the plasmalemma are also disrupted by ECC contraction-induced injury and thus, if loss of plasmalemmal excitability is simply the most proximal circuit breaker in *mdx* muscle. Elucidating the roles of other proteins associated with the dystrophin-glycoprotein complex (e.g., dystroglycans, utrophin) that might contribute to ECC contraction-induced electrophysiological dysfunction of the plasmalemma is also important future work.

In conclusion, exercise that accentuates ECC contractions induces significant strength loss in *mdx* mice due to plasmalemma electrophysiological dysfunction, likely mediated by a failure to conduct action potentials in depolarized muscle fibers. Despite the rather dramatic perturbation observed in plasmalemma electrophysiological function during and immediately after the injury, *mdx* mice possess a remarkable ability to recover plasmalemmal excitability. Furthermore, a second injury does not appear to exacerbate the *mdx* phenotype, as plasmalemma electrophysiological function is lost and restored at a similar rate as the first injury. These results highlight that ECC contraction-induced strength loss is mechanistically different between wildtype and *mdx* mice. The data also indicate that functional dystrophin is necessary for excitation to occur at the plasmalemma during a series of ECC contractions, but not essential for complete recovery of plasmalemma electrophysiological function or peak isometric strength.

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Fig. 1. Isometric torque and EMG tracings acquired during a 150 ms, 200 Hz isometric stimulation of the anterior crural muscles. Tracings from a wildtype (A) and *mdx* (B) mouse acquired immediately pre- and post-ECC contraction-induced injury, and 1 and 9 days into recovery.

Fig. 2.

Effects of ECC contractions on maximal torque and M-wave RMS. Maximal ECC torque and M-wave RMS across 50 ECC contractions in wildtype (A) and *mdx* (B) mice. Maximal ECC torque and M-wave RMS were expressed as a fraction of initial (i.e., the 1st contraction). Recovery of maximal isometric torque and M-wave RMS after the ECC contraction-induced injury in wildtype (C) and *mdx* (D) mice. Maximal isometric torque and M-wave RMS were expressed as a fraction of pre-injury. Sample size per group: wildtype, $n = 8$; *mdx*, $n = 14$. The dashed line indicates the uninjured baseline level. Values are presented as means $\pm$ SE. *Significantly different from the uninjured baseline ($P < 0.05$), #Significantly different from wildtype ($P < 0.05$).

Fig. 3.

Effects of repeat bouts of ECC contractions on maximal ECC torque and M-wave RMS.

Loss of maximal ECC torque and M-wave RMS across 50 ECC contractions for bouts 1 and 2 in wildtype (A, C) and *mdx* (B, D) mice. Maximal ECC torque and M-wave RMS were expressed as a fraction of initial (i.e., the 1st contraction) for that given bout. Each bout was separated by 14 days. Sample size per group: wildtype, $n = 6$; *mdx*, $n = 7$. The dashed line indicates the uninjured baseline level. Values are presented as means $\pm$ SE.

Fig. 4.

Recovery of maximal isometric torque and M-wave RMS following repeated bouts of ECC contractions. Maximal isometric torque and M-wave RMS for bouts 1 and 2 in wildtype (A, C) and *mdx* (B, D) mice. Maximal isometric torque and M-wave RMS were expressed as a fraction of pre-injury for that given bout. Each bout was separated by 14 days. Sample size per group: wildtype, $n = 6$; *mdx*, $n = 7$. The dashed line indicates the uninjured baseline level. Values are presented as means $\pm$ SE. ^{\$}Significantly different from bout 1 ($P < 0.05$).