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ORIGINAL ARTICLE

Median nerve injury does not contribute to early onset of decreased grip strength due to repetitive reaching and grasping tasks in rats

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Abstract

BACKGROUND: Workplace risk factors, such as repetitive tasks, can cause work-related musculoskeletal disorders. In a rat model, decreased grip strength and median nerve injury develop following repetitive reaching and grasping tasks, involving negligible force.
OBJECTIVE: We investigated whether median nerve injury is involved in the early onset of decreased grip strength due to such tasks
METHODS: Sprague-Dawley rats were divided into: non-task-performing (0-week) and task-performing (1-, 2-, and 3-week) groups. After an initial training period, the task-performing groups continued to perform the task for 2 h/day, 3 days/week, for 1–3 weeks. Grip strength and relative muscle weight of the flexor digitorum superficialis (FDS) muscle were measured. Median nerve injury was evaluated by histopathology and immunohistochemistry.
RESULTS: Grip strength of the reach limb (forelimb used in tasks) was significantly lower in the 3-week group compared with the other groups and was significantly lower than that of the non-reach limb in all groups. There were no significant differences in the relative FDS muscle weights of either limb among groups. No evidence of median nerve demyelination was observed and no cells expressed activating transcription factor-3, a specific marker of peripheral nerve injury, in the anterior horn of the spinal cord.
CONCLUSION: Median nerve injury does not contribute to the decreased grip

strength caused by 3 weeks of repetitive reaching and grasping tasks, involving negligible force, in rats.

Abbreviations:

WMSD	- work-related musculoskeletal disorder
ATF3	- activating transcription factor 3
DRG	- dorsal root ganglion
CFA	- complete Freund's adjuvant
Sp1	- specificity protein 1
FDS	- *flexor digitorum superficialis*
LFB	- Luxol fast blue

INTRODUCTION

Work-related musculoskeletal disorders (WMSDs) are defined as injuries or musculoskeletal effects caused by exposure to certain risk factors, such as excessive repetitive work, in the workplace (Waters *et al.* 2011). These disorders are collectively the most common form of occupational disease in Europe and in other parts of the industrialized world (Colombini & Occhipinti, 2006). Furthermore, the main WMSD symptoms, motor dysfunction and pain, contribute to high medical care costs. Thus, WMSDs are a serious problem in occupational health. Although excessive repetitive work has been established as a primary risk factor causing WMSD (Waters *et al.* 2011), this type of work is often necessary. However, because the mechanism of WMSD onset following repetitive work remains unclear, it has not been possible to develop effective treatments or prevention methods targeting this mechanism. Therefore, it is urgent to elucidate the pathological condition. Here, we studied the mechanisms involved in using a rat model of WMSD in which the rats perform repetitive reaching and grasping tasks, involving negligible force. Previous work by our group found that repetitive reaching and grasping tasks in this model caused a loss of grip strength and muscle mechanical hyperalgesia in the rats beginning after 3 consecutive weeks of task performance (Fujiwara *et al.* 2017). Previous studies using similar tasks in rats reported that continuing the reaching and grasping tasks for 6 weeks increased the number of macrophages that are ED1-immunoreactive (ED1 is a monoclonal antibody against CD68) in the median nerve of rats (Clark *et al.* 2003). Clark *et al.* (2003) also reported that an increase in connective tissue in the carpal tunnel compressed the median nerve, causing nerve injury. However, the cause of the dysfunction resulting from repetitive tasks, which begins after 2 or 3 consecutive weeks of task performance, is unclear. Furthermore, whether median nerve injury is involved in the onset of the dysfunction is also unknown.

Activating transcription factor 3 (ATF3) is a stress-inducible transcription factor that is not expressed in normal nerve cells. Notably, 96.4% of the dorsal root ganglion (DRG) labeled with a retrograde tracer from the cut end of the sciatic nerve express ATF3, suggesting that ATF3 is a highly sensitive marker for peripheral nerve injury. ATF3 was observed to be expressed in the DRG of the operative side and in the motor neuron cell body of the spinal anterior horn in rats with an injured sciatic nerve (Tsujino *et al.* 2000). In addition, when formalin was intraperitoneally administered to rats, tissue inflammation, but not peripheral nerve injury, occurred, and ATF3 expression was not observed in the neuronal cell body of the DRG or spinal cord anterior horn. Furthermore, ATF3 expression was not observed even when the sciatic nerve was given an electrical stimulation of an intensity that stimulates C fiber, which is a nociceptor responsive to noxious stimuli that transmits pain information, in conjunction with an injection of complete Freund's adjuvant (CFA) below the skin. Thus, ATF3 expression is not induced by tissue inflammation or other noxious stimuli but is specific to peripheral nerve injury.

Previous research in which the sciatic nerve of the rat was entrapped found that the myelin thickness of the operative sciatic nerve was significantly decreased and that the proportion of ATF3-positive cells was significantly higher in the operative DRG (Austin *et al.* 2015). Furthermore, Isacsson *et al.* (2005) reported that ATF3 expression was observed in the DRG on the operative side in rats with nerve entrapment due to the tube of the sciatic nerve and that contraction force from electrical stimulation in the triceps surae during surgery was significantly decreased compared with the opposite side. Together, these findings suggest that muscle weakness occurs in the skeletal muscle of the dominant nerve area at the time of peripheral nerve injury. Thus, we hypothesized that nerve injury is involved in the loss of grip strength that follows repetitive reaching and grasping tasks in rats.

Here, we measured ATF3 expression in the spinal cord and peripheral nerve tissue of a rat model of WMSD to clarify whether peripheral nerve injury is a cause of the loss of grip strength associated with repetitive reaching and grasping tasks that involve negligible force.

MATERIALS AND METHODS

Animals

A total of 51 female Sprague-Dawley rats, aged 10 weeks, were used for this study. Rats were purchased from Japan SLC and housed under controlled temperature (22–24°C) and lighting (08:00–20:00 light) conditions. Rats were divided randomly into food-restricted Control (n = 12) or Task (n = 39) groups (Figure 1A). All rats were restricted from food throughout the experiment. These rats were food deprived to maintain 80%–90% of full body weight, as defined by the weights of age-matched normally fed rats. At the beginning of the experiment, all 51 rats were trained for 2 weeks to perform repetitive reaching and grasping tasks, involving negligible force, as previously described (Barbe *et al.* 2003; Fujiwara *et al.* 2017). There was a 2-week training period, during which the rats were placed in an experi-

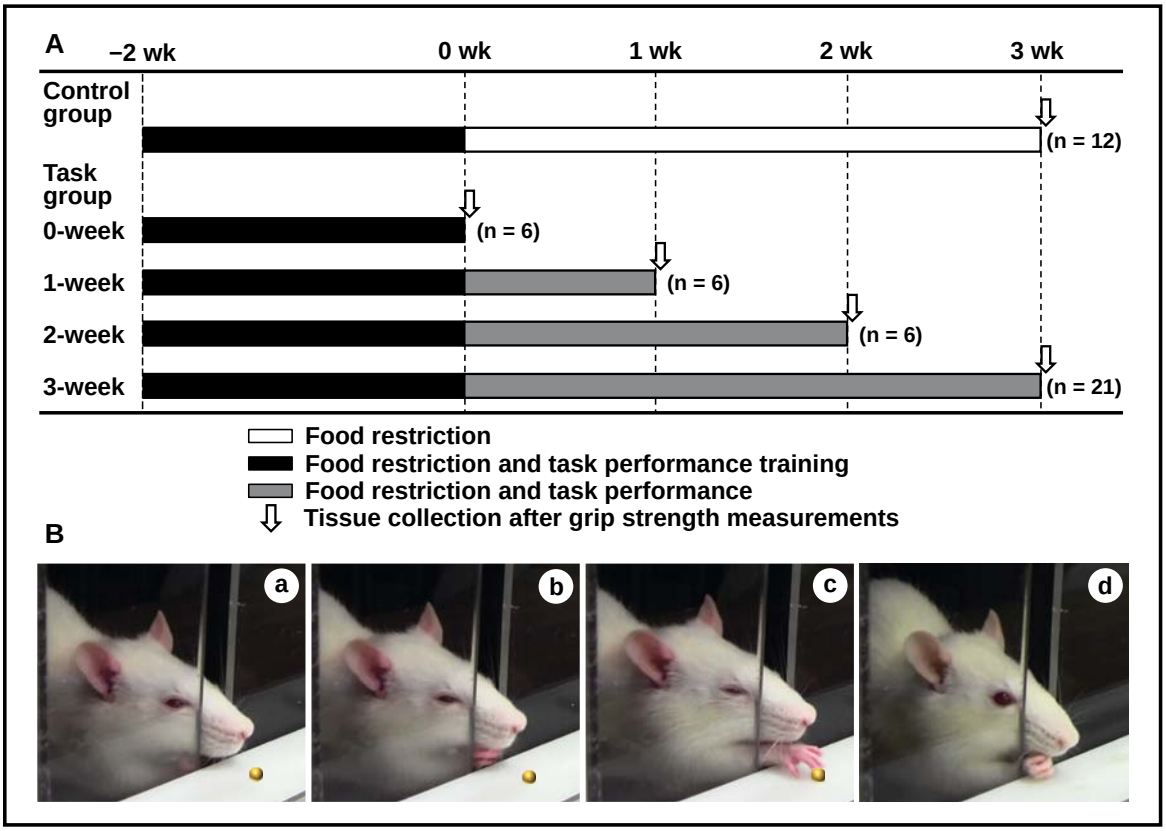

Fig. 1. Experimental setup.
(A) Schematic timeline for the repetitive reaching and grasping task, measurement of grip strength, and tissue collection (wk: weeks). (B) During the task, the rat repeatedly performs the action shown in the photographs once every 15 s (a–d). (a) The food pellet is dispensed on the shelf attached to the test box upon which the rat has been placed. (b and c) The rat reaches for and grasps the food pellet. (d) The rat eats the food pellet grasped in its paw.

mental box for 10 min daily, and food pellets were placed on the table by a slit in the box, so that the rats could learn to repeatedly reach and grasp the food pellets. Subsequently, the rats performed the reaching and grasping tasks at a controlled speed of 4 reaches/min for 2 h/day, 3 days/week, for 0, 1, 2, or 3 consecutive weeks (0-week, 1-week, 2-week, and 3-week groups, respectively, with six rats in each) (Figure 1B). We defined the forelimb used by the rat to perform reaching and grasping as the reach limb and the limb on the opposite side as the non-reach limb.

After 3 weeks of the task period, the Control and Task groups were further subdivided for certain analyses, so as to avoid artefacts being introduced into the muscle tissue analyses as a result of prior behavioral testing. Therefore, a subset of the Control (n = 5) and Task groups at 3 weeks (n = 6) were not subjected to behavioral analysis, but instead muscle samples were collected for histological analyses (measurements of relative muscle weight and the identification of fiber types I, IIA, and IIB by myosin ATPase staining). Behavioral analysis data (grip strength and pain) were collected for each group of rats, but each parameter was compared in individual rats on a weekly basis. For the '0-week' and '3-week' tissue samples, histopathological and immunohistochemical evaluations were performed in the spinal cord and median nerve from rats in which grip strength had been measured (six rats at each time point). In contrast, pain was assessed during the 3 consecutive weeks of the task period [i.e., in the '3-week group' (n = 9) and the age-matched Control group (n = 7) in which muscle pain was measured].

All procedures used in this study were approved by the Ethics Committee for Animal Experimentation at the Nagoya University School of Health Science (approval number: 026-024) and complied with the ARRIVE guidelines, the Ethical guidelines for the care and use of laboratory animals published by the International Association for the Study of Pain, and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Grip strength measurements

We performed grip strength measurements on the rats as previously described (Clark *et al.* 2004; Fujiwara *et al.* 2017). Briefly, rats were lifted by the tail and induced to grasp a rigid bar attached to a digital force gauge (Aikoh Engineering Corporation, Osaka, Japan). Each rat was gently pulled backward by the tail and the tension reading of the digital force gauge, just before the rat released the bar, was recorded. The test was per-

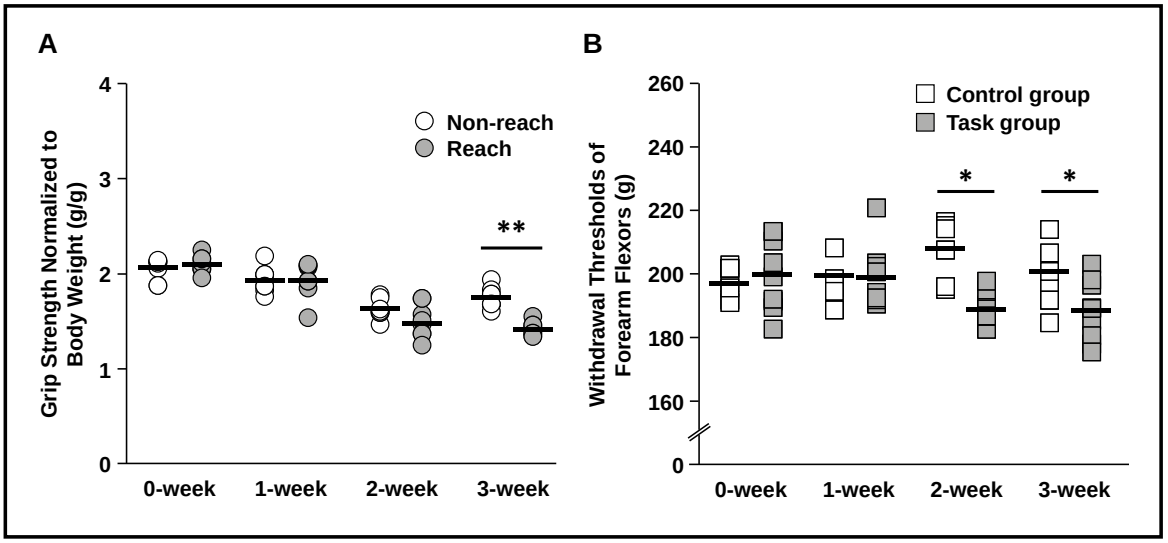

Fig. 2. Grip strength and forearm flexor withdrawal threshold following pressure stimulation.
(A) Forearm grip strength as normalized to body weight (in grams) of the non-reach limb and reach limb of rats in the 0-week, 1-week, 2-week, and 3-week groups. Horizontal bars indicate the mean values (n = 6 per group). $^{**}p < 0.01$, compared with the non-reach limb in the 3-week group, according to Student's *t*-tests. (B) The withdrawal thresholds of forearm flexors from rats after performing repetitive tasks for 0–3 weeks are shown. These data were obtained by measuring the same individual rats over time. Declines in the withdrawal thresholds of the forearm flexors following pressure stimulation are indicative of muscular mechanical hyperalgesia. Forearm withdrawal thresholds were measured in response to gradually increasing the mechanical forces applied to the forearm flexors, and the value of the force when the rat withdrew their forearm was recorded as the withdrawal threshold. The Control group (white) shown in the graph is composed of the non-reach limbs of rats that did not perform the repetitive reaching and grasping tasks, and the Task group (gray) is composed of the reach limbs of rats that performed the tasks. Horizontal bars indicate the mean values (n = 6–9/ per group). $^{*}p < 0.05$, compared with the age-matched Control group, according to Student's *t*-tests.

formed five times and the highest value was defined as the grip strength. Grip strength was measured in both the reach limb and non-reach limb on the day following the completion of the training period or the completion of the final task.

Withdrawal threshold measurements

Muscular mechanical hyperalgesia was assessed using the forelimb withdrawal threshold, which involved the application of gradually increasing mechanical forces to the forelimb flexors, including the *flexor digitorum superficialis* (FDS) muscle. The method used was a modification of that described by Nakano *et al.* (2012). Briefly, the head and trunk of each rat was wrapped with a cloth and the rat was suspended in a home-made hammock. We confirmed that the forelimb was freely movable under these conditions. The forelimb withdrawal threshold was quantified using a Pressure Application Measurement device (Ugo Basile, Comerio, Italy) equipped with a hand-made round-headed probe with a 5-mm tip diameter. It has been previously shown that a probe with this tip diameter is suitable for measurement of the muscle mechanical nociceptive threshold (Nasu *et al.* 2010). The pressure required to elicit forelimb withdrawal was determined five times at 1-min intervals, and the mean value was regarded as the forelimb flexor withdrawal threshold.

Tissue collection

After measuring the grip strength on the day after completion of the training period or completion of the final task, tissues were harvested from rats anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg Somnopentyl®; Kyoritsu Seiyaku Co., Tokyo, Japan). The spinal cord from C5 to Th2 was collected *en bloc*, and the collected spinal cords were immediately frozen with dry ice. The median nerve was then excised from the palm to the elbow joint, after which the whole length was divided into three segments and fixed in the extension position. The collected median nerve was fixed for 1 day with 4% paraformaldehyde diluted with 0.01 M phosphate buffer. After dehydration for 3 days with 30% sucrose diluted with 0.01 M PBS, each sample was frozen and embedded in optimal cutting temperature compound (Sakura Finetek, Torrance, CA, USA).

The FDSs were collected ~24 h after completion of the final task, to ensure that measurements reflected the effects of long-term exposure to repetitive motion, rather than immediate effects of the final exercise bout. Similarly, muscle tissue was collected 18–36 h after the final task in a previous study (Barbe *et al.* 2008). The FDS muscle was harvested from each reaching limb in rats anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg). The wet weights of the collected FDS muscles were measured immediately after collection, and the muscles were then were

quickly frozen by immersion in isopentane pre-cooled in liquid nitrogen. The tissues were then stored at −80°C until analysis.

Histopathological evaluation of peripheral nerves

For each median nerve collected, 10-μm-thick longitudinal sections were prepared using a cryostat (CM1510S, Leica, Wetzlar, Germany). The myelin sheath and axons in the longitudinal section were dyed via the Kluver-Barrera's Luxol fast blue (LFB) method (Geisler *et al.* 2002). The sections were first immersed in 95% ethanol for 2 min and then immersed in an LFB solution (Muto Pure Chemicals, Tokyo, Japan) at 56–58°C for 24 h. Thereafter, they were cooled at room temperature, washed with 95% ethanol in distilled water, then immersed for 5 s in a 0.05% aqueous solution of lithium carbonate (Muto Pure Chemicals), followed by fractionation with 70% ethanol. The sections were next thoroughly washed with distilled water for 5 min and immersed in a 1% aqueous solution of protein silver for 24 h at 37°C. The solution was then cooled to room temperature, reacted for 5 min with a reducing agent solution containing 1% hydroquinone and 4% anhydrous sodium sulfate, and then the sections were immersed in a 0.5% chloroauric acid aqueous solution for 50 min. The sections were further reacted with a 2% oxalic acid aqueous solution for 10 min, and finally with a 5% sodium thiosulfate aqueous solution for 5 min, before being washed with running water for 10 min, dehydrated, penetrated, and sealed. Using an optical microscope (BZ-9000, Keyence, Osaka, Japan), two stained sections per nerve were viewed at 40× magnification. Additional fields of view were examined until each entire section had been examined, allowing us to assess the extent of the LFB staining of the median nerve, which was used as an indication of the presence or absence of axonal demyelination.

Immunohistochemical evaluation of the spinal cord

For each collected spinal cord, five 20-μm-thick transverse sections were prepared from the C5–Th2 region, from which the median nerve arises, using a cryostat (CM1510S, Leica). After being fixed in 4% paraformaldehyde with 0.01 M PBS, each sample was subjected to immunohistochemical tissue staining as previously described (Kiryu-Seo *et al.* 2008). Initially, the samples were reacted for 30 min at room temperature with a blocking solution of 1% bovine serum albumin (Sigma-Aldrich) and 0.3% Triton X-100 (Sigma-Aldrich) in 0.01 M PBS. The samples were then reacted overnight at 4°C with anti-ATF3 rabbit IgG (1:1000; Santa Cruz Biotechnology), followed by a reaction for 2 h at room temperature with Alexa Fluor 594-conjugated goat anti-rabbit IgG (1:1000; Life Technologies) as a secondary antibody. Finally, the samples were mounted onto glass slides with fluorescence mounting medium (Dako, #S3023) and viewed using a fluorescence microscope (BZ-9000, Keyence) at a magnification of 20× until the entirety of each section had been assessed. The total number of ATF3-positive cells was counted.

Myosin ATPase staining

FDL muscles were embedded in optimal cutting temperature compound (Sakura Finetek). Thereafter, 7-μm cross-sections were cut from the mid-portion of the muscles using a cryostat (CM1510S, Leica) and mounted on Superfrost Plus slides (Thermo Fisher Scientific, Tokyo, Japan).

Myosin ATPase staining was performed according to the protocol of Brooke and Kaiser (1970). Briefly, the sections were first pre-incubated in acidic buffer (0.1 M barbital acetate and 0.1 M hydrochloride, adjusted to pH 4.6) for 5 min, and then rinsed with a substrate solution (0.18 M calcium chloride and 0.1 M sodium barbital, adjusted to pH 9.4). Then, the sections were incubated in ATP staining buffer (0.18 M calcium chloride, 0.1 M sodium barbital, and 2.4 mM ATP disodium salt) at pH 9.4 for 45 min, washed three times in 1% calcium chloride solution for 3 min each, incubated with 2% cobalt chloride for 3 min, washed eight times with 0.01 M sodium barbital solution, and rinsed with distilled water for 2 min. Finally, the sections were incubated in 1% ammonium sulfide for 1 min and rinsed with distilled water five times. Following staining, each section was sealed with Canada balsam and topped with a coverslip. Dark-stained fibers (slow fibers) were classified as type I fibers and light fibers as fast fibers. Type IIA fibers appeared white, whereas type IIB fibers were stained gray (Lind & Kernell, 1991).

Images of the stained cross-sections were captured using an optical microscope (BZ-9000, Keyence) at 20× magnification. For microscopic analysis, we selected an area containing more than 50 fibers on a given section and determined the percentages of each fiber type using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Two areas per section and two serial sections per skeletal muscle were analyzed, and the means and error estimates for the percentages in the four areas were calculated.

Statistical analysis

Values are presented as the means ± standard error of the mean (SEM). Comparisons of grip strengths was performed using Student's *t*-tests for group-matched data from the non-reach limb and reach limb. Comparisons of forearm flexor withdrawal thresholds, relative muscle weight (ratio of muscle wet weight normalized to body weight) values, and fiber-type distribution were performed using Student's *t*-tests between the data from the reach limbs of rats in the Task group and the non-reach limbs of the Control group. A p value of < 0.05 was considered significant. Data were analyzed using SigmaPlot 13 statistical software (Systat Software Inc.).

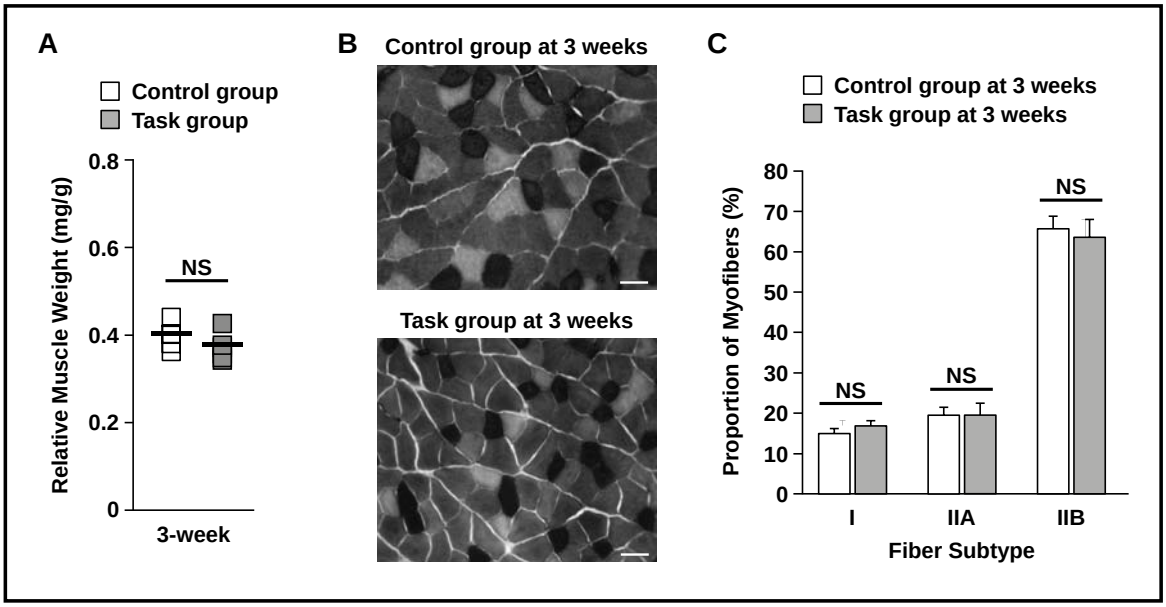

Fig. 3. Relative weight and fiber-type classification of the flexor digitorum superficialis (FDS) muscle of rats performing repetitive reaching and grasping tasks.
(A) Relative muscle weight (ratio of muscle wet weight normalized to body weight expressed in milligrams/grams) of the reach limb of the Task group, after 3 weeks (gray) and the non-reach limb of the Control group (white). Horizontal bars indicate the mean values (n = 5/Control group, n = 6/Task group). NS: not statistically significant ($p \geq 0.05$) compared with the age-matched Control group, according to Student's *t*-test. (B) Representative images of skeletal muscle histochemical staining at pH 4.6 for myosin ATPase in the FDS muscles of rats in the Control group (top) or Task group (bottom). Type I fibers appear dark, whereas type II fibers appear white (IIA) or gray (IIB). Scale bar = 50 μm. (C) Comparison of the average fiber-type distribution for all rats in the Control (white) and Task (gray) groups. Error bars indicate SEM (n = 5/Control group, n = 6/Task group). The Control group shown in the graph is composed of the non-reach limbs of rats that did not perform the repetitive reaching and grasping tasks, and the Task group is composed of the reach limbs of rats that performed the tasks. NS indicates not statistically significant ($p \geq 0.05$) compared with the fiber type-matched Control group, according to Student's *t*-tests.

RESULTS

Body weight, grip strength, and withdrawal threshold

To control for the body size of the rats, the rats in each group were weighed weekly as well as prior to sacrifice. Their average body weights of rats used in the behavioral analyses were 211.7 ± 1.41 g, 221.1 ± 1.7 g, 232.4 ± 2.1 g, and 234.0 ± 1.8 g in the 0-week, 1-week, 2-week, and 3-week groups, respectively. When the grip strengths were normalized to body weight, there were no differences in the grip strengths between the non-reach limb and reach limb of the rats in the 0-week, 1-week, or 2-week groups (Figure 2A). In contrast, the grip strength normalized to body weight of the reach limb was significantly lower than that of the non-reach limb in the 3-week group rats ($p < 0.01$); for these rats, the grip strength normalized to body weight of the reach limb was approximately 19.3% lower than that of the non-reach limb. These results indicate that the repetitive reaching and grasping tasks do not significantly lower grip strength until the tasks have been performed for 3 consecutive weeks.

The withdrawal thresholds of the forearm flexors in the same rats between weeks 0 and 3 of the study are shown in Figure 2B. A decline in the withdrawal threshold following pressure stimulation is indicative of mechanical hyperalgesia. The forearm withdrawal thresholds were measured in response to gradual increases in the mechanical force applied to the muscles, and the force when each rat withdrew its forelimb was recorded as the withdrawal threshold. The withdrawal thresholds were similar for the two groups at weeks 0 and 1, but significantly lower in the Task group at 2 and 3 weeks.

FDS muscle weight and fiber-type composition

Because the grip strength could be affected by the size of the FDS muscle, we also compared the relative muscle weights (ratios of muscle wet weight normalized to body weight) of the reach limbs of the Task group and the non-reach limbs of the Control group (Control group, n = 5; Task group at 3 weeks, n = 6). These rats weighed 236.6 ± 10.4 g and 239.2 ± 3.1 g, respectively. However, we found no significant differences in the relative FDS muscle weights between these limbs after 3 weeks of the intervention (Figure 3A).

Histological analyses were also conducted in cross-sections of the FDS muscles to determine whether performance of the task affected fiber-type distribution. Myosin ATPase staining showed that the FDS muscles of rats in the Control and Task groups had similar proportions of type I, IIA and IIB fibers after 3 weeks of the study (Figure 3B and C).

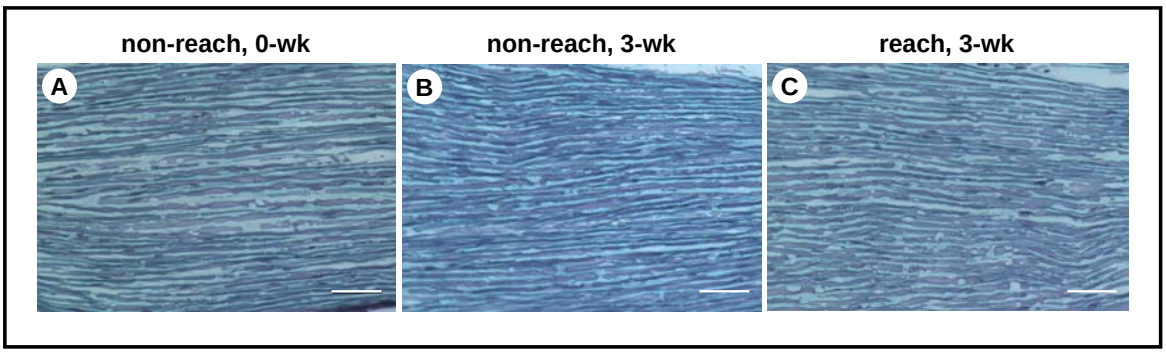

Fig. 4. Histopathology of median nerve sections stained with Luxol fast blue (LFB).
(A–C) Representative photomicrographs of LFB-stained median nerve sections from the non-reach limb in the 0-week group (non-reach, 0-wk) (A), the non-reach limb in the 3-week group (non-reach, 3-wk) (B), and the reach limb in the 3-week group (reach, 3-wk) (C). Scale bar = 50 µm.

Assessments of nerve injury in the median nerve and spinal cord

To determine if nerve injury is a cause of the lower grip strength observed after 3 weeks of performing repetitive reaching and grasping tasks, involving negligible force, we conducted histology and immunohistochemistry analyses of the median nerves and spinal cords, respectively, from the rats in each group. For the histology analysis, LFB staining, which identifies myelin, was used to identify demyelination. The results show that LFB staining was uninterrupted, indicating a lack of demyelination, in the median nerves of the non-reach limb in both the 0-week and 3-week group rats as well as in the median nerve of the reach limb in the 3-week group rats (Figure 4). Notably, we did not observe any demyelination in the median nerve, consistent with the findings of Okuwa *et al.* (2018).

We used immunohistochemistry to identify the expression of ATF3, which is a specific marker for peripheral nerve injury, in the anterior horn of the spinal cords. We confirmed an increase in ATF3 expression in the anterior horn following peripheral nerve injury, using the sciatic nerve injury model. In naive rats (the non-reach or reach sides in the 0-week group rats), ATF3 was not expressed in the spinal cord (Figure 5C and D). However, 3 days after injury of the right sciatic nerve in 10 sections prepared from a positive control rat, ATF3 was induced in virtually all motor neurons on the axotomized side (Figure 5B), but ATF3 was not expressed on the uninjured side (data not shown),

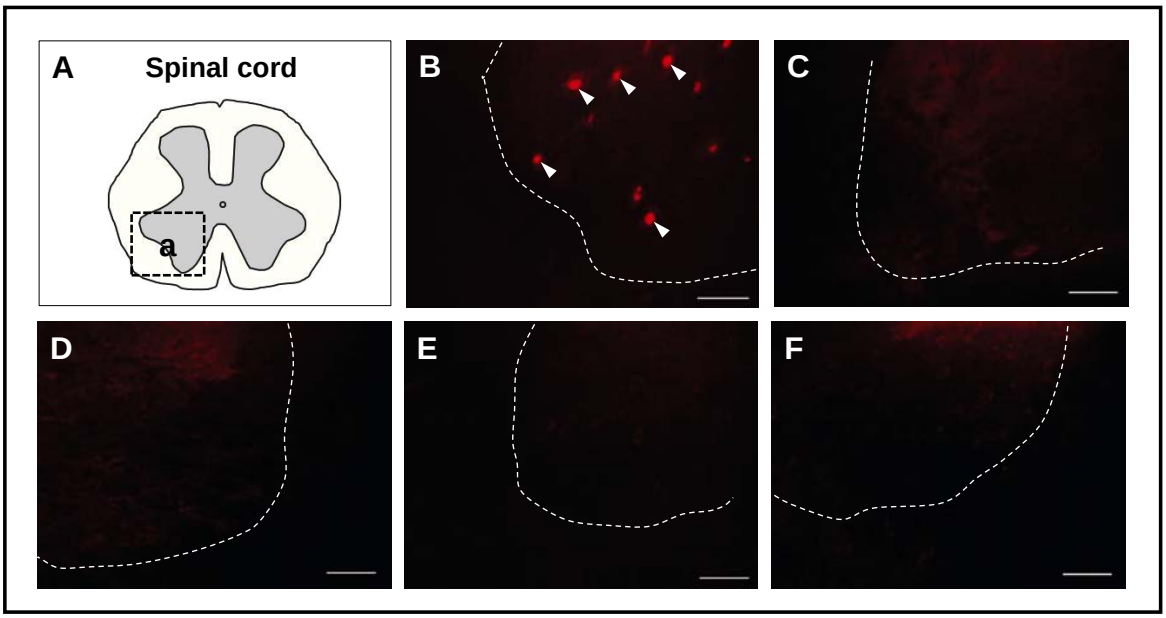

Fig. 5. Activating transcription factor 3 (ATF3) immunostaining of the spinal cord anterior horn.
(A) Schematic illustration of the spinal cord segments from C5 to Th2. The square area marked with an "a" indicates the anterior horn. (B–F) Representative fluorescence micrographs of ATF3 immunostaining in the spinal cord anterior horn from: a rat with sciatic nerve injury (positive control; arrowheads indicate ATF3-positive cells) (B), the non-reach limb side of a 0-week group rat (C), the reach limb side of a 0-week group rat (D), the non-reach limb side of a 3-week group rat (E), and the reach limb side of a 3-week group rat (F). Scale bar = 100 µm.

consistent with previous findings (Tsujino *et al.* 2000; Kataoka *et al.* 2007). In contrast, there were no ATF3-positive neurons in the anterior horn of the spinal cords from the non-reach or reach sides in the 3-week group rats (Figure 5E and F). Thus, we did not find any evidence of axonal injury, and specifically demyelination, in the median nerve due to repetitive reaching and grasping tasks, involving negligible force, over the 0–3-week timeframe.

DISCUSSION

Our current study aimed to investigate a possible cause of the loss of grip strength in rats that occurs following repetitive reaching and grasping motions, which imitate high frequency repetitive work. We found that the grip strength of the non-reach limb was not different between any of the rat groups, whereas the grip strength of the reach limb was significantly lower than that of the non-reach limb in the 3-week group rats. This result is similar to the result of our previous study (Fujiwara *et al.* 2017). The finding that the grip strength in the reach limb was lower than that in the non-reach limb within individual subjects indicates that the repetitive reaching and grasping motions, despite involving negligible force, caused the grip strength to decrease. Although muscle strength and muscle size generally show a strong correlation with one another (Bamman *et al.* 2000; Fukunaga *et al.* 2001; Blazevich *et al.* 2009), the relative weights of the FDS muscles did not differ between the reach limbs of rats in the Task group after 3 weeks of performing the task and the non-reach limbs of the Control group, despite the difference in reach limb grip strength between these groups. Other experiments revealed there was also no change in the proportion of the muscle fiber type, i.e., the muscle fiber composition of the FDS muscle, following the performance of repetitive reaching and grasping tasks for 3 consecutive weeks (Figure 3B and C). Together, these findings suggest that although the grip strength of the reach limb decreased significantly following 3 weeks of repetitive reaching and grasping tasks as compared with the non-reach limb, histological changes, such as muscle mass and muscle fiber composition, in the FDS muscle were not involved in this grip strength reduction.

We hypothesized that median nerve injury might be responsible for the observed loss of grip strength described above, so we looked for evidence of demyelination in the median nerve of the rats. However, histopathological examination of the medullary sheath and axon of the median nerve revealed no evidence of demyelination in the median nerves of either the reach or non-reach limbs of any rats. Furthermore, we used immunohistochemical staining to examine the expression of ATF3, which is an index of peripheral nerve injury, in the anterior horn of a C5-to-Th2 section of spinal cord. However, no ATF3 expression was observed in the anterior horn of the spinal cord on either the non-reach side or the reach side in any of the rats, in contrast to the substantial expression identified in a positive control rat subjected to sciatic nerve injury. A previous study in which ATF3 expression was examined reported that ATF3 was not expressed in the anterior horn or DRG when CFA was injected into the plantar aspect of rat paws (Tsujino *et al.* 2000). In contrast, other work in which the sciatic nerve of the rat was entrapped reported that the proportion of ATF3-positive cells was significantly higher in the operative DRG (Austin *et al.* 2015). Together, these studies indicate that ATF3 expression in the anterior horn and DRG is likely due to axonal injury of peripheral nerves, not inflammation.

Our results from the histopathology of the median nerve and ATF3 immunohistochemistry of the anterior horn suggest that the continuation of reaching and grasping tasks, involving negligible force, for 3 consecutive weeks did not damage the median nerve. Clark *et al.* (2003) reported that when rats performed such tasks more than 9 consecutive weeks, the conduction velocity of the median nerve decreased. Thus, although this study found that peripheral nerve injury is not involved in the occurrence of reduced grip strength due to conducting repetitive reaching and grasping tasks for 3 weeks, peripheral nerve injury may occur when such tasks are continued for a longer period. However, in our previous study, we showed that the repetition of these tasks for 2 or more consecutive weeks causes muscle mechanical hyperalgesia in the forearm flexor, as measured by forearm withdrawal thresholds (Figure 2B), and this muscle mechanical hyperalgesia and the loss of grip strength both occurred around the same time (Fujiwara *et al.* 2017). Previous studies have reported potential factors of repetitive task-induced loss of grip strength, such as an increased number of ED1-immunoreactive macrophages in the median nerve as well as median nerve injury and/or skeletal muscle atrophy of the FDS muscle when rats performed such tasks for more than 6 consecutive weeks (Clark *et al.* 2003; Fujiwara *et al.* 2017). The mechanism underlying the initial loss of grip strength that occurs after only 3 consecutive weeks of performing repetitive reaching and grasping tasks is not understood.

While the present study found evidence that peripheral nerve demyelination is not the cause of the observed decrease in grip strength occurring after 3 consecutive weeks of reaching and grasping tasks, further work is needed to identify the factor(s) that are responsible for this phenomenon. We speculate that muscle fatigue could contribute to the decrease of muscle force. The forearm flexor, which is the agonist muscle of the grasping motion, is fast-twitch muscle (Delp & Duan, 1996), and Ursu *et al.* (2001) reported that fast-twitch muscle is less resistant to fatigue caused by repetitive muscle contraction compared with slow-twitch muscle. To test this possibility, future studies could use electrical stimulation measure the tension (contractile force) of ex

vivo FDS muscles from rats that have been subjected to repetitive tasks. Alternately, to determine if pain is a main cause of the decreased grip strength, measurements of the grip strength of similarly conditioned rats that were treated with an analgesic could be collected.

In conclusion, this study shows that the performance by rats of repetitive reaching and grasping tasks, involving negligible force, for 3 consecutive weeks induced a loss of grip strength. However, no changes in the muscle mass or detectable median nerve demyelination resulting from the tasks were observed. Thus, neither skeletal muscle atrophy nor median nerve injury contribute to the early onset of decreased grip strength in rats due to repetitive reaching and grasping tasks.

DISCLOSURES

The authors have no conflicts of interests to declare.

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DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on request.

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