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Chronic repetitive reaching and grasping results in decreased motor performance and widespread tissue responses in a rat model of MSD

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Abstract

This study investigated changes in motor skills and tissues of the upper extremity (UE) with regard to injury and inflammatory reactions resulting from performance of a voluntary forelimb repetitive reaching and grasping task in rats. Rats reached for food at a rate of 4 reaches/min, 2 h/day, and 3 days/week for up to 8 weeks during which reach rate, task duration and movement strategies were observed. UE tissues were collected bilaterally at weekly time points of 3–8 weeks and examined for morphological changes. Serum was tested for levels of interleukin-1α (IL-1) protein. The macrophage-specific antibody, ED1, was used to identify infiltrating macrophages and the ED2 antibody was used to identify resident macrophages. Rats were unable to maintain baseline reach rate in weeks 5 and 6 of task performance. Alternative patterns of movement emerged. Fraying of tendon fibrils was observed after 6 weeks in the mid-forelimb. After 4 weeks, a general elevation of ED1-IR macrophages were seen in all tissues examined bilaterally including the contralateral, uninvolved forelimb and hindlimbs. Significantly more resident macrophages were seen at 6 and 8 weeks in the reach limb. At 8 weeks, serum levels of IL-1α increased significantly above week 0. Our results demonstrate that performance of repetitive tasks elicits motor decrements, signs of injury and a cellular and tissue responses associated with inflammation.

Introduction

The Occupational Safety and Health Administration [28] defines work-related musculoskeletal disorders (MSD) as disorders of the muscles, nerves, tendons, ligaments, joints, cartilage, blood vessels, or spinal discs associated with exposure to ergonomic risk factors. These factors include repetition, force and awkward or static postures [28]. In 2000, US private industry reported approximately 242,000 cases of occupational illness due to repeated trauma, which comprised 67% of all occupational illnesses that year [7]. At an average cost of $8000 per claim [24,31], workers' compensation costs were approximately $2 billion in 2000 for MSDs. There are additional costs associated with lost wages and productivity, particularly for cases associated with work absences or limited duty. Clearly, MSDs represent a serious occupational health issue.

MSDs of the distal upper extremity (UE) may result from increases in pressure in the carpal tunnel or loading of musculoskeletal tissues during repetitive, awkward or extreme positions and movements of the wrist and forearm [25,39]. This pressure and loading on the tissues can lead to mechanical injury of cellular membranes and intracellular structures [10,16,35]. Tissue injury typically leads to a localized release of soluble cytokines as well as collagen and fibronectin fragments which first attract neutrophils and then macrophages, hallmarks of an acute inflammatory response [19–21]. The release of pro-inflammatory cytokines into the extracellular matrix, whether during the acute or chronic phases of inflammation, can stimulate a systemic immune reaction [9,21]. Chronic inflammation, seen 2–4 days after the onset of

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the acute response, is primarily associated with the increased presence of infiltrating, immature macrophages [19,21]. Macrophages, a heterogeneous group in terms of location and function, secrete growth factors and cytokines that stimulate the proliferation of many cell types and induce additional influxes of inflammatory cells from the vasculature [23]. Activated macrophages (phagocytic) also release proteolytic enzymes and reactive oxygen species during phagocytosis, which can contribute directly to further tissue injury [11]. Infiltrating macrophages are the predominant cell type in the immune response in progressive fibrotic diseases [14,42], following thermal injury [27] and in tissues adjacent to implanted biomaterials [29]. Within 48 h of injury, macrophages are also the dominant immune cell in tendon [18], and muscle [3,9,20,21]. Repeated tissue injury leads to chronic inflammation [11], residual scarring [33,35,36] and, eventually, loss of muscular or neural function. This chronic injury scenario is consistent with the signs and symptoms reported in workers with MSD and illustrates the progression of MSD to chronic and disabling conditions. Effective management of MSD requires a thorough understanding of the response of tissues to repeated exposure to damaging stimuli.

Epidemiological and field studies have demonstrated an association between chronic performance of repetitive and/or forceful tasks and the development of localized musculotendinous injuries [2,3,16,35,36], but a clear relationship between exposure and tissue pathophysiology is still being established. This lack of information impedes the regulation of such disorders in the workplace (e.g., Congressional repeal of the OSHA ergonomics Program Rule in March 2001). Since it is methodologically difficult to control exposure and virtually impossible to examine biopsy specimens in human workers, the use of animal models is essential if investigators are to determine the effects of repetition on motor behavior and tissue pathology.

We have developed a rat model of MSD using voluntary movements in a repetitive hand and wrist-intensive task [4,5]. Rats were trained to perform a repetitive reaching and grasping task that shares certain key characteristics of movements performed by humans in some occupational settings. For example, investigations of industrial workers by Silverstein and colleagues [30, 31] have defined risk levels for repetitiveness to be high when reaching and grasping motions are performed faster than 30 s/cycle. Force is considered negligible to low if less than 15% of maximum voluntary contraction (MVC) is required and high if it is above 50% MVC. In our model, rats were trained to perform a paced reaching and grasping task that required a high reach rate (<15 s/cycle) and a negligible grip force (<1% of maximum grip force as measured in our laboratory according to Bertelli and Mira [6]). Whishaw has quantified the similarities between rats and humans in targeted reach submovements of the UE [40]. Although humans do not usually perform paced work on all fours, the UE movements of the reaching limb should not be appreciably altered in the bipedal position. Furthermore, in our model, the repetition and force parameters in the rat have been scaled to resemble occupational tasks in humans as further explained in Barr and Barbe [4]. Vikari-Juntura et al. [37,38] state that laboratory studies of animals examining the effect of repetitive loading on nerve, tendon, and muscle function may be extrapolated to human exposures and responses. Therefore, our rat model of a paced reaching and grasping task may be generalized to humans in terms of both behavioral and tissue responses for some types of physically constrained and paced occupational tasks. An example of such a paced task would be packing, in which a worker repeatedly places small objects presented on a conveyor belt into a package crate. Furthermore, this model has clear applicability for future intervention studies.

The purpose of this study was to determine if chronic performance of a repetitive reaching and grasping task causes decrements in motor performance, tissue injury or tissue and cellular responses associated with an inflammatory reaction. We investigated changes in reach rate, duration of task performance and alterations in movement patterns used to perform the task. UE tissues were examined for histological changes. An immunomarker for type I collagen was used to examine the morphology of UE tendons. We used immunomarkers for both recruited, infiltrating macrophages (ED1; [12]) and resident, tissue macrophages (ED2; [12,15]) to examine the macrophage response to this task in tissues in the reach limb. In an effort to determine if the task had more widespread consequences than a localized infiltration or proliferation of macrophages, we examined the contralateral, non-reach limb and hindlimb for increased numbers of ED1-IR macrophages and serum for elevation of IL-1α, a pro-inflammatory cytokine.

Methods

Animals

Fifty-seven adult female Sprague-Dawley rats (age 12–14 weeks at onset of experiment) were used. Thirty-nine rats were trained to perform a highly repetitive forelimb reaching and grasping task with negligible force for up to 8 weeks. Four rats served as shaped-only rats and were sacrificed following an initial shaping period (see below); 14 animals served as age-matched, normal controls (no shaping). The normal and shaped-only rats did not participate in the performance of the task regimen. The experimental and shaped-only animals were food deprived so that they maintained 80–90% of full body weight as defined by weights of age-matched controls. Experiments were approved by the Temple University IACUC in compliance with NIH guidelines for the humane care and use of laboratory animals.

Behavioral task

Rats were placed in operant test chambers for rodents (Med. Associates, VT) with a portal located in one end. The portal was fitted

with a 1.5 cm wide tube that sloped downward 10° with respect to the chamber floor and was located at the animal's shoulder height. The tube was 2.5 cm in length so that the elbow had to be fully extended in order for the animal to reach pellets of food. Food pellets (45 mg; Biosource) were dispensed (Pellet dispenser, Med. Associates) every 15 s during the reach task. An auditory indicator (Stimulus clicker, Med. Associates) signaled that a pellet had been dispensed, thereby cueing the animal to attempt a reach.

Rats learned to reach for food during an initial 10–12 day shaping period. During this period, the rats were first encouraged to reach through open bars for food pellets placed on an elevated platform for 5 min/day. When they began to reach freely for the food, they were transferred to the test chamber until they could reach into the tube dispenser at a self-paced reach rate for 10–20 min/day. When they were able to perform the task consistently, they began the task regimen at the defined target rate of 4 reaches/min for 2 h/day, 3 days/week for 3–8 weeks. The daily task was divided into 4, 0.5 h training sessions separated by 1.5 h. Rats were allowed to use their preferred limb to reach, hereafter referred to as the reach limb.

An observer using a hand-operated counter and reach distance criteria logged the number of reaches. A reach was defined as an extension of the forepaw beyond a line drawn 0.5 cm within the tube. Gross movement patterns were also examined for deviations from the normal movements comprising reaching in rats [41]. The side used to reach was recorded in each session. There were 17 right-handed animals and 13 left-handed animals. Nine animals demonstrated ambidexterity either by using the non-preferred limb only occasionally (usually if a pellet was missed on the first reach attempt, $n = 5$) or by switching to more frequent use of the non-preferred limb after several weeks of task performance ($n = 4$). In these nine cases, reach rate and task duration data were used as a measure of overall task performance regardless of which limb the animals used to reach, however, the tissues of the non-preferred limbs were not included in the immunohistochemical analyses. The tissues of the preferred (i.e., originally used) limb of two ambidextrous animals were included as reach limbs in our experiments, one 6 week and one 8 week animal, both of whom continued to use the preferred limb throughout the final week of task performance.

Reach rate was defined as the average number of reaches performed per minute and was calculated on the last day of each week. Task duration was defined as the number of hours/day the rats participated in the task and was averaged over the 3 days within each week. Thirty-nine animals initiated the task regimen, but beyond 3 weeks, groups of animals were periodically euthanized for histological tissue analysis. Therefore, sample size of animals performing the behavioral task decreased over weeks of task performance. Based on previous studies [5], two distinct alternative reach movement patterns were defined. Scooping is a pattern in which the semi-open forepaw is placed over the food pellet and the pellet is dragged along the bottom or side surface of the tube and scooped into the mouth. Raking is an inefficient extreme of scooping in which repeated unsuccessful attempts to contact the food pellet with the semi-open forepaw result in repeated back and forth movements that resemble a raking motion. These behaviors were noted as present (>1/min) or absent on the last day of each week. They are expressed as the percentage of animals in which the behaviors were present.

Tissue collection and immunohistochemistry

Tissues from 23 rats were examined histologically: $n = 3$ each at weeks 3–6, $n = 4$ at week 8; normal ($n = 4$) and shaped-only control rats ($n = 3$) were considered as 0 weeks. Animals were euthanized by pentobarbital overdose (Nembutol; 120 mg/kg body weight) and perfused transcardially with 4% paraformaldehyde in PO_4 buffer (pH 7.4). Tissues were collected from all four limbs *en bloc*, equilibrated in 30% sucrose in PBS, and cut into longitudinal sections (16 μm) using a cryostat. For immunoperoxidase staining, sections were blocked with 3% H_2O_2 in methanol for 30 min, then with 4% goat serum in PBS. For immunofluorescence, the H_2O_2 step was omitted. The sections were incubated overnight at room temperature with primary antibodies diluted in 4% goat serum and PBS: anti-ED1 (1:250, Chemicon, CA), anti-ED2 (1:250, Serotec, NC), anti-IL-1α (1:250, Chemicon) and anti-type I collagen (1:100, Sigma). Sections incubated with ED1 were enzyme digested for 20 min using 0.5% pepsin in 0.01 N HCl prior to blocking with serum. Controls were performed without primary antibodies to verify specificity of the antibodies. Following incubation with primary antibodies, the sections were incubated for 2 h with appropriate secondary antibodies tagged with either peroxidase or fluorescent tags (diluted 1:100; Jackson Immuno). The peroxidase-conjugated secondary antibodies were visualized using DAB (Sigma) and the sections dehydrated and mounted with DPX media. Sections treated with fluorescent-tagged secondary antibodies were washed in PBS and mounted with 80% glycerol in PBS.

The numbers of infiltrating (ED1-IR) and resident (ED2-IR) macrophages were quantified bilaterally using a bioquantitation system (Bioquant TCW 98). Cells with a defined threshold of staining were counted in a 0.0768 mm^2 area using a 40× objective in tissues of the anterior palm and distal forelimb flexor mass proximal to the transverse carpal ligament. Three adjacent fields were measured for each tissue (loose areolar connective tissue (CT), tendon and muscle) per region and group means were calculated for each tissue per region. The mean number of ED1-IR macrophages was also quantified in 0, 5 and 6 week animals across tissue types in the forelimb extensor, brachial (biceps and triceps), shoulder (rotator cuff muscles), back (trapezius, rhomboids and erector spinae musles) and hindlimb (tibial) regions. Adjacent slides were counterstained with hematoxylin and eosin.

Protein isolation and cytokine analyses

Serum IL-1α levels were examined in rats that had performed the task for 0, 6 and 8 weeks ($n = 4$–8/group). Blood samples were collected from the heart, centrifuged, serum was aspirated and total protein was determined using BCA-200 protein assay kits (Pierce). Fifty μl aliquots were used for measuring IL-1α with enzyme-linked immmunosorbant assay (ELISA) kits (Biosource International, California) according to the manufacturer's protocol. ELISA data were normalized to μg protein. Each sample was run in triplicate.

Data analysis

A mixed model ANOVA for repeated measures was used to analyze differences in reach rate, task duration, and number of macrophages by week and by tissue. A p value of $\leqslant 0.05$ was considered significant for all analyses. Post hoc analyses were carried out using the Bonferroni method for multiple comparisons, and adjusted p values are reported. Reach rate and task duration were analyzed by week (8 levels). Post hoc analyses compared reach rate or task duration in week 1 (baseline) to the values at each subsequent weekly endpoint. Movement patterns over weeks of task performance are expressed as the percentage of animals observed to engage in either the scooping and/or raking patterns at each weekly endpoint.

For the distal forelimb and palm, differences in the number of ED1-IR macrophages were analyzed by week (6 levels), by tissue (CT, muscle, and tendon) and by limb (reach and non-reach). Microscopic field (3–5 fields/tissue) was used as a blocking factor in all of the histological analyses. Post hoc analyses compared the number of macrophages in control tissues (week 0) to subsequent weeks (3–6, and 8) in different tissue types and between reach and non-reach limbs. The number of ED2-IR macrophages in the reach limb of the distal forelimb tissues was compared by week (4 levels) and tissue (CT, musculotendinous junction, and tendon). Post hoc analyses compared the numbers of ED2-IR macrophages in control tissues (week 0) to those in subsequent weeks (5, 6, and 8). For both of the previous analyses, we chose not to analyze interactions between tissue type and the other main effects in order to increase statistical power and permit comparability with the other anatomical regions analyzed. For the forearm extensor, brachium, shoulder, back and hindlimb regions, the numbers of ED1-IR macrophages in all tissues combined were analyzed by week (0 and 5/6) and limb (reach and non-reach). Post hoc analyses compared the number of macrophages in control tissues (week 0) to those of animals exposed to the task for 5 or 6 weeks, and the reach to the non-reach limbs. Serum levels of IL-1α protein were analyzed by week (0, 6, and 8). Serum protein levels of control animals (week 0) were compared to those of animals exposed to the task for 6 and 8 weeks.

Results

Behavioral degradation

Following the shaping period, all trained animals were able to perform the task at or above the target reach rate. Animals exceeded the target reach rate initially (mean repetition rate = 9.06 reaches/min ±4.10 SD on day 0). Reach rate declined by the end of the first week (mean = 8.27 reaches/min ±4.11 SD) and did not decrease further through week 4. There was a significant decrease in reach rate across weeks ($p = 0.0039$). Post hoc analysis revealed a significant decrease in reach rate at the end of week 5 ($p = 0.0028$, $n = 31$) and week 6 ($p = 0.0070$, $n = 26$) (Fig. 1A). This decrease in reach rate continued through week 7 (not significant, $p = 0.1645$; $n = 21$) but was followed by an increase toward baseline in week 8 with no significant difference from week 1 ($p = 0.4095$; $n = 19$). Post hoc analysis revealed that task duration was significantly different across weeks ($p = 0.0417$). Task duration decreased significantly by approximately 10 min/day in week 5 ($p = 0.05$, $n = 31$) (Fig. 1B). Although duration in weeks 3 ($p = 0.1253$; $n = 38$), 4 ($p = 0.5446$; $n = 34$), 6 ($p = 0.999$; $n = 26$) and 7 ($p = 0.1337$; $n = 21$) was not significantly lower than in week 1, there is a suggestion of a decrease of 10 min/day at these endpoints followed by an increase toward baseline in week 8 (Fig. 1B). The timing of these changes coincided with alterations in movement patterns of the upper limbs (Fig. 1C). We observed a gradual increase in scooping that peaked in week 5 in 47% of animals and then declined. A raking pattern continued to increase beyond week 5 and was observed in 100% of animals by weeks 7 and 8.

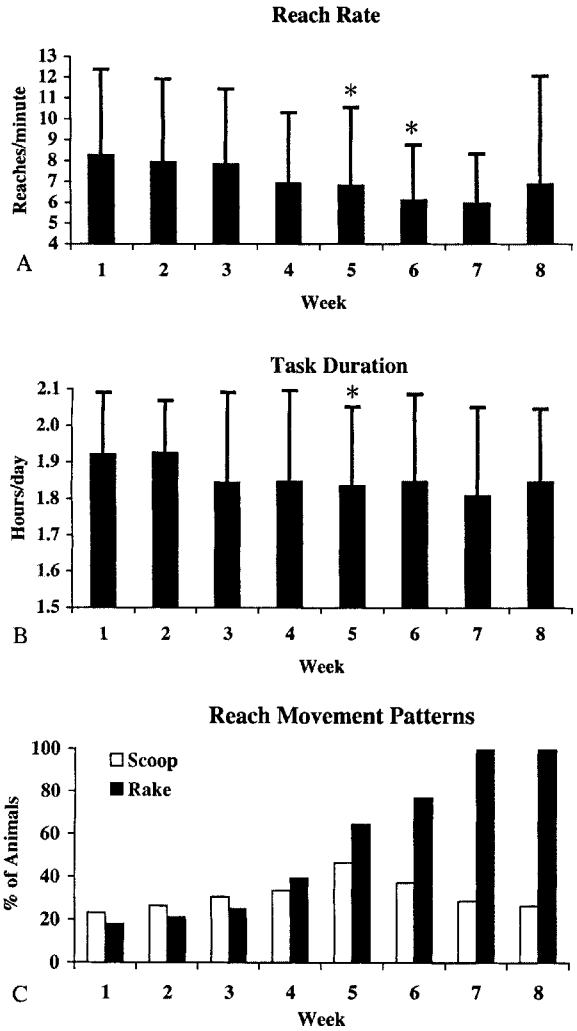

Fig. 1. Behavioral outcomes. (A) Note the significant decrease in weeks 5 and 6 for reach rate and (B) in week 5 for task duration (*: $p \leq 0.05$). These changes coincide with the emergence of raking (C), which is present in 100% of the animals by week 7.

Tissue injury

After 5 weeks, morphological alterations were seen in the anterior forelimb of the reach limb at the sites where myofibers merge into tendon fibrils. In all of the 6 and 8 week animals, many of the tendon fibrils appeared kinked at the muscle–tendon junctions (Fig. 2B), which typically reflects tendon fraying. No tendon fibril alterations were observed in any of the non-reach limb tissues.

Localized and widespread macrophage infiltration

A few to no ED1-IR macrophages were visible in tissues of the control rats (week 0) (Fig. 2C and E). Microscopic observation of sections reacted with anti-ED1 illustrates increases in this subpopulation of macrophages in tendon, muscle and loose CT with task performance (Fig. 2D and F). Bioquantification showed that, in the palm, the number of ED1-IR macrophages was significantly different across weeks ($p < 0.0001$), across tissues ($p = 0.0044$), and between limbs ($p = 0.0026$). The week × limb interaction was not significant for the palmar region ($p = 0.3339$). Post hoc analysis revealed that the number of ED1-IR macrophages rose significantly above control levels (week 0) by week 3 ($p = 0.005$; Fig. 3A and C) and remained elevated through week 8. The number of ED1-IR macrophages was significantly greater in CT when compared to both muscle ($p = 0.0057$) and tendon ($p = 0.0324$). Tendon and muscle were not significantly different from each other ($p > 0.999$) in the palmar region. The number of ED1-IR macrophages was greater in the reach limb than in the non-reach limb ($p = 0.036$).

In the distal forelimb, the number of ED1-IR macrophages was significantly different across weeks ($p < 0.0001$), across tissues ($p = 0.0018$), and between limbs ($p = 0.0119$). The week × limb interaction was significant for the distal forelimb ($p = 0.0052$). Post hoc analysis revealed that the number of ED1-IR macrophages

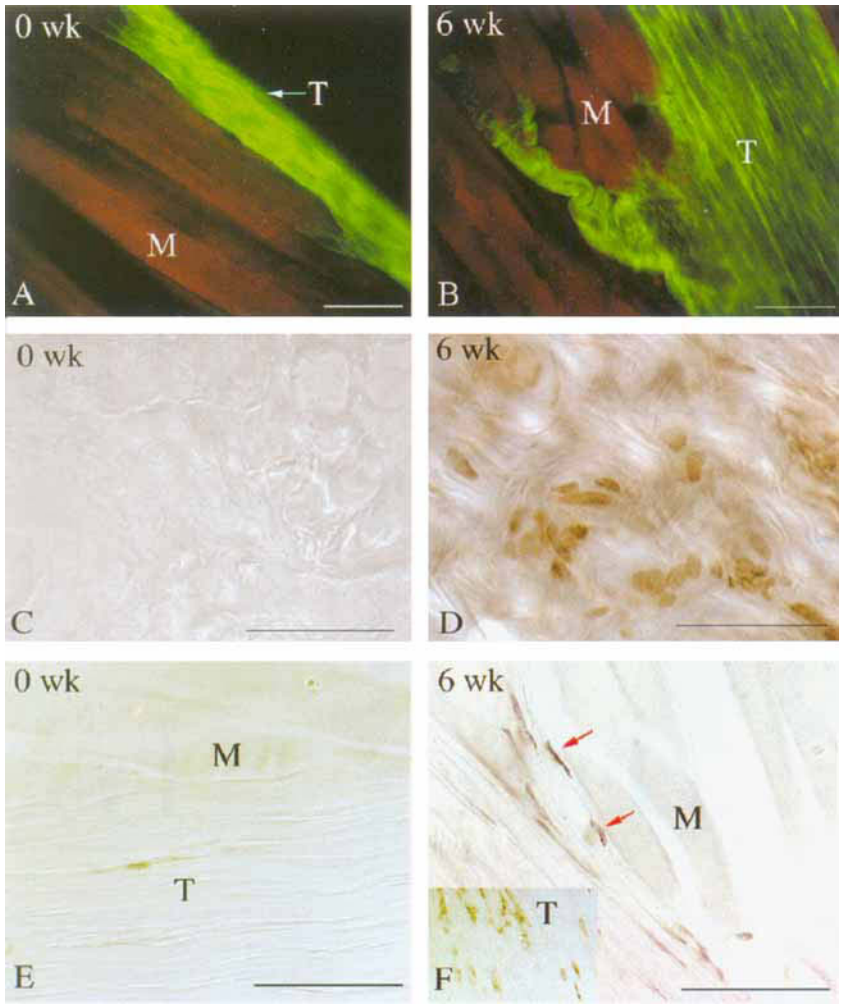

Fig. 2. Photomicrographs illustrating evidence of tendon microfray and localized infiltration of macrophages. (A,B) Type I collagen-IR (green) in the proximal tendon of flexor digitorum superficialis (M). A: At week 0, type I collagen-IR (green) in this tendon shows a neat transition between the myofibers (unstained) and tendon fibrils (T). B: A kink, or microfray, is visible in type I collagen-IR tendons of flexor digitorum superficialis of a 6 week rat. (C,D) ED1 immunoreactivity in loose CTs of the palm. C: No ED1-IR macrophages were visible in the loose CT of the palm in a control rat (week 0). D: By 6 weeks of task performance, many ED1-IR macrophages (brown staining cells) had infiltrated the palmar CT. (E,F) ED1 immunoreactivity in the flexor digitorum superficialis muscle at the junction of myofibers and tendon fibrils, located mid-forearm. E: No ED1-IR macrophages were present in the musculotendinous juncture site in a control (week 0) rat. F: By 6 weeks of task performance, ED1 immunoreactive macrophages (arrows) had infiltrated a comparable site in the flexor digitorum superficialis muscle of the reach limb. The inset illustrates a tendon from ipsilateral, proximal forearm and illustrates the presence of infiltrating ED1-IR macrophages in that tissue after 6 weeks of task performance. Bar = 50 μm.

in the distal forelimb region was significantly greater in CT when compared to both muscle ($p = 0.0021$) and tendon ($p = 0.0171$). Muscle and tendon were not significantly different from each other ($p > 0.999$). The number of ED1-IR macrophages in the reach limb was greater in weeks 4–6 ($p = 0.0016$) and week 8 ($p = 0.0416$) than in control tissues (Fig. 3C). In the non-reach limb (Fig. 3D), the number of ED1-IR macrophages increased above control levels in weeks 5, 6 and 8 ($p = 0.0016$). The number of ED1-IR macrophages was greater in the reach limb than in the non-reach limb at week 4 ($p = 0.0016$). The greatest peaks in the numbers of ED1-IR macrophages occurred in either week 5 or week 6 regardless of region (palm or distal forelimb), tissue type or limb (Fig. 3). The number of ED1-IR macrophages decreased toward baseline by 8 weeks in the palmar and distal forelimb regions in both limbs (Fig. 3).

In contrast to the infiltrating monocytes/macrophages (ED1-IR), the number of resident macrophages (ED2-IR) increased progressively above control levels through 8 weeks in loose CTs such as synovial sheaths, and in tendons and musculotendinous junctions of the anterior forelimb. There was a significant difference in the number of ED2-IR macrophages across weeks ($p < 0.0001$) and across tissues ($p < 0.0001$). Post hoc analysis revealed that the numbers of ED2-IR cells at 6 weeks ($p = 0.0288$) and 8 weeks ($p = 0.0003$) were significantly

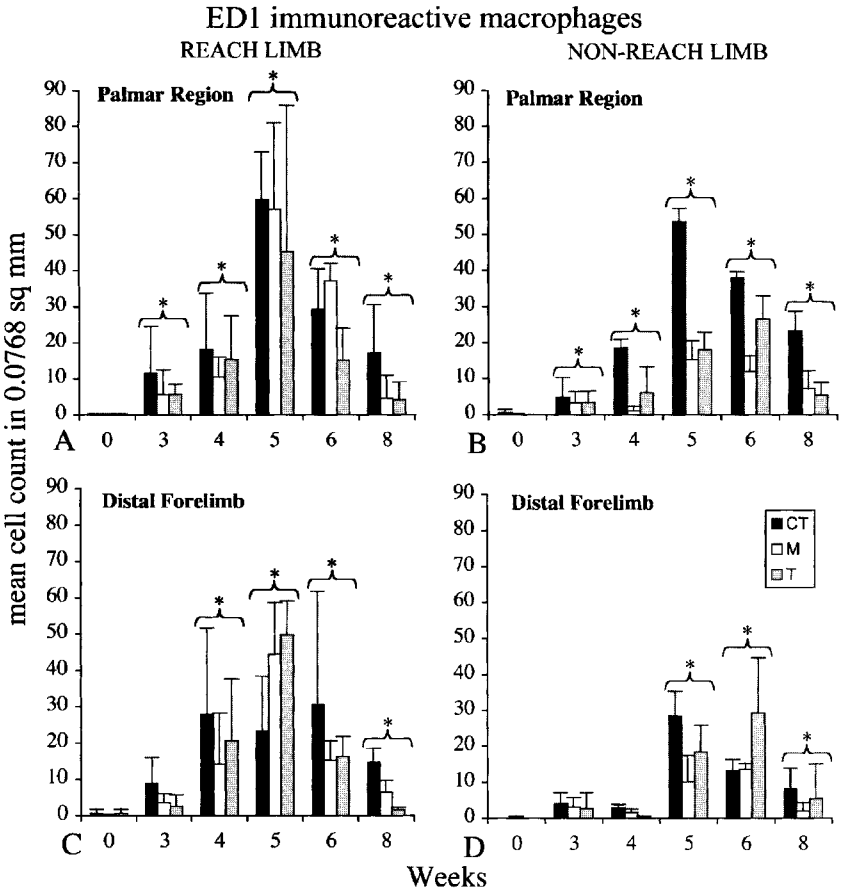

Fig. 3. Quantification of immature, infiltrating macrophages (ED1-IR) in tissues from palmar (A,B) and forelimb (C,D) regions in the reach and non-reach limbs. The mean number of ED1-IR cells was quantified in loose CT, tendons (T), and muscles (M) in the anterior palms and flexors of distal forelimbs. There were significantly more ED1-IR macrophages in all of the tissues examined in the palm after week 3, and in the distal forelimb after week 4 (reach limb) and week 5 (non-reach limb) compared to controls (*: $p < 0.05$).

higher compared to controls (Fig. 4). No significant increase in ED2-IR cells was observed within the muscle bellies of the anterior forelimb region (data not shown).

There was a significant increase in the number of infiltrating monocytes/macrophages (ED1-IR) above control levels within the musculotendinous and associated CTs of both the reach and non-reach sides by 5–6 weeks in the forearm extensor, brachium, shoulder, back and hindlimb regions ($p < 0.0001$; Fig. 5). Data for the back region is not shown in Fig. 5 (week 0: 0.9142 cells/0.0768 sq mm ± 1.8047 SD, weeks 5 and 6: 4.5312 cells/0.0768 sq mm ± 7.5817 SD, $p < 0.0001$). There was no significant difference between limbs in any of these regions.

Elevation of IL-1α in serum

A significant increase in the serum level of IL-1α was observed in week 8 ($p = 0.0188$) (Fig. 6A). Although IL-1α was elevated at week 6, the increase was not significant ($p = 0.0762$). One source of the IL-1α in serum is the infiltrating macrophages (ED1-IR) in the peripheral tissues (Fig. 6B). ED1-IR macrophages that co-

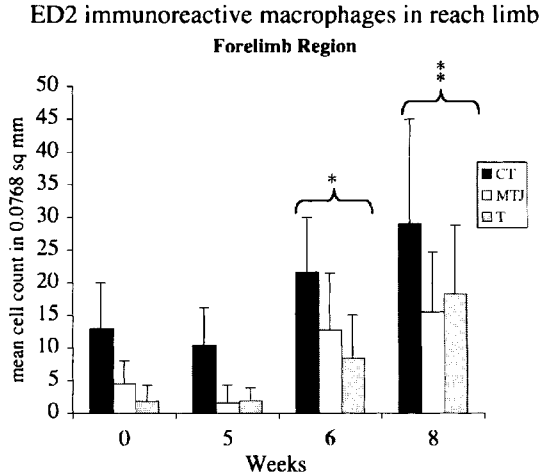

Fig. 4. Quantification of resident, tissue macrophages (ED2-IR) in tissues from the distal forelimbs of reach limbs. The mean number of ED2-IR cells increased significantly above controls levels at weeks 6 and 8 (*: $p < 0.05$; **: $p < 0.001$). MTJ = myotendinous junction.

expressed IL-1α immunohistochemically were visible in tissues of all four limbs by week 6 (data not shown).

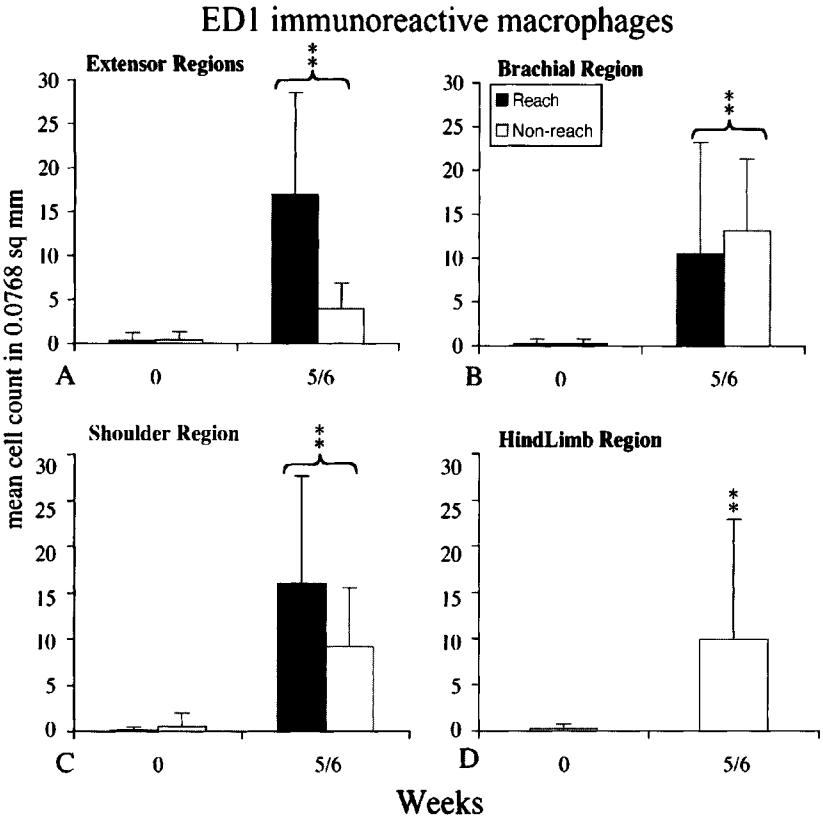

Fig. 5. Inflammatory response in regions distant from anterior forelimb and palm of reach limb. The number of ED1-IR macrophages was quantified in tissues (musculotendinous and associated CTs) of the (A) forearm extensor, (B) brachial, (C) rotator cuff, and (D) tibial regions. Weeks 5 and 6 were combined and compared to week 0. The number of ED1-IR macrophages was significantly higher in weeks 5 and 6 than week 0 (**: $p \leq 0.001$).

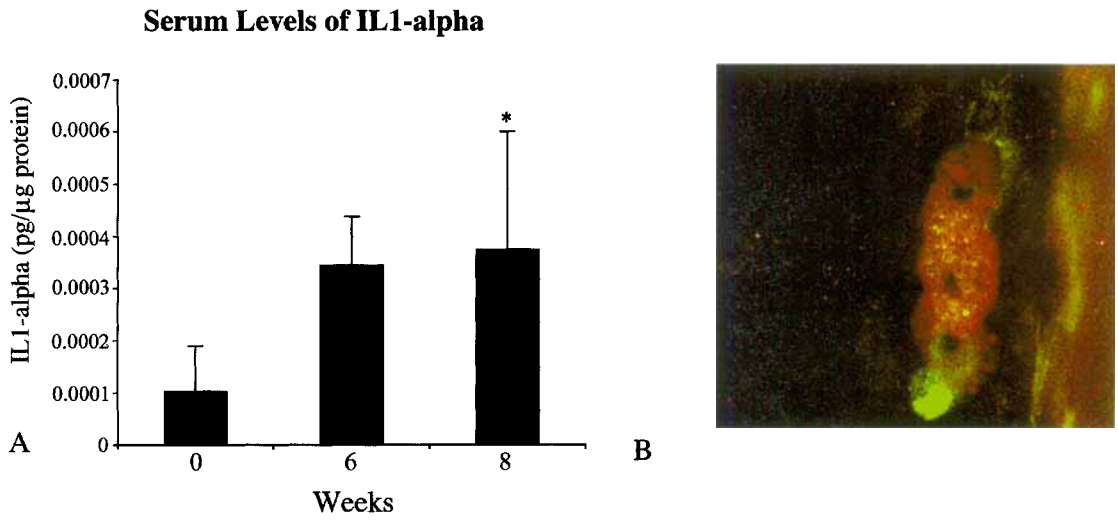

Fig. 6. Serum levels of IL-1α. (A) Protein levels of IL-1α increased significantly in the serum of 8 week rats compared to controls (*: $p < 0.05$). (B) Photomicrograph illustrating ED1-IR macrophages (green) that are co-expressing IL-1α (red).

Discussion

A highly repetitive task with negligible force in which food was retrieved using a constrained forelimb reach and grasp was found to decrease motor performance, induce histological changes associated with tissue injury and give rise to cellular and tissue responses associated with inflammation. There was a reduction in reach rate beginning in week 5 after an early learning effect between day 0 and the end of week 1. This reduction

coincided with a refusal to participate in the task for the full session time. These results taken together indicate that animals were injured and are unable to maintain their initial pace over time. The coincidental increase in the abnormal scooping and raking patterns further showed degeneration in motor behavior over time. The sequential emergence of scooping and raking indicated loss of motor control. During scooping, the animals ceased to fully close the digits and to lift the food pellet. In raking, open digit movement was repeated clumsily within a single reach and was associated with repeated loss of control of the food pellet. Performance of the raking movement pattern in particular may paradoxically increase risk exposure by increasing the repetitiveness of reach submovements. Since MSDs often involve inflammation, loss of motor performance and appearance of gross movement disorders [3,8,13], we hypothesize that tissue injury, inflammation and/or the resulting pain caused this motor degradation.

We observed discrete sites of disruption of the tendon fibers in rats that had performed the task for several weeks. Chronic inflammation of tendons has been shown to result from repeated tissue damage [2,16], perhaps due to increased pressure or friction on the tendons within the carpal tunnel or increased loads on these tissues during task performance [25,38,39]. Tendon injury has been shown to result in a localized increase in macrophages [18]. This migration of macrophages may be stimulated by several factors including the presence of fibronectin fragments and collagenase digests of type I collagen [22,26].

Our ED1 results reveal that continued performance of a highly repetitive task elicited an infiltration of phagocytic macrophages in regions of the forelimb used to perform the task after only a few weeks. The timing and magnitude of the appearance of the ED1-IR cells corresponds to and may precede the observed changes in motor performance. The reduction in reach rate and duration at 5 and 6 weeks may allow the onset of regeneration, which may, in turn, contribute to the decreased numbers of ED1-IR cells in the tissues at 8 weeks with the possible reduction of symptoms. The observation of a rebound in reach rate and duration toward baseline in week 8 further supports this hypothesis. Alternatively, the rebound in reach rate may indicate that the animals have learned to cope with the limitations imposed by the tissue damage. Further studies examining the presence or absence of pain at this stage should help us to clarify this question. We also observed an increase in the number of resident macrophages (ED2-IR) by 8 weeks. The chronological increase of these macrophage subtypes is consistent with the role of ED1 macrophages in muscle necrosis followed by that of ED2 macrophages in muscle regeneration [32]. Alternatively, the continued high levels of ED2 macrophages could suggest a chronic inflammatory response [14,29]. Jarvinen and colleagues [16] have speculated that mechanisms leading to tissue repair are prevented by the repeated cycle of tissue trauma.

A systemic inflammatory response is suggested by a general elevation of ED1-IR macrophages in all four limbs, including limbs not involved in performing the task, and the increase in serum levels of IL-1α. Pro-inflammatory cytokines such as IL-1α and IL-6 are released by macrophages in injury states following exertion-induced muscle cell injury [9] and repetitive motion-induced injury of tendon fibroblasts [1]. Pro-inflammatory cytokines induce the infiltration of macrophages into injured tissues as well as mediate the proliferation of a variety of cell types, including macrophages, both locally [9] and in distant hematopoeitic tissues [17]. These macrophages secrete pro-inflammatory cytokines, which, through autocrine regulation, further stimulate secretion of more cytokines [9,11]. Examination of rats that perform this task for longer than 10 weeks may prove interesting in light of this continued increase of IL-1α in the circulating blood.

Our results are supported by other studies using animal models to observe the effects of repetitive motion on tissues. Archambault et al. [2] showed that a repetitive movement injury model of a reflexive movement of the rabbit hindlimb resulted in histological changes consistent with localized inflammation of the Achilles tendon after 6–8 weeks. Two load-repetition protocols were used: 20 repetitions/min at 50% of isometric maximum and 75 repetitions/min at 30% of isometric maximum for 2 h/day, 3 days/week. Their results reveal cellular evidence of inflammation following repetitive loading. Stauber et al. [33,34] studied the effects of repeated forced-lengthening, at slow (10 mm/s) and fast (25 mm/s) velocities on soleus muscles of rats. The soleus was electrically stimulated while repeated stretching was carried out for brief periods (~65 and ~25 s for slow and fast velocities, respectively) 3 days/week for 4 weeks. Slow stretching resulted in muscle hypertrophy with an increase in muscle mass and cross-sectional area of myofibers. Fast stretching also induced an increase in muscle mass, but it resulted in two populations of muscle fiber sizes and an increase in non-contractile tissues without muscle fiber hypertrophy. Increasing the exposure and duration of the repeated forced-lengthening to 6 weeks of daily strain led to significant decreases in muscle mass, decreases in myofiber area and further increases in non-contractile tissues [36]. Although these models of passive motion, reflexive exertion and forced-lengthening of muscle do not incorporate the voluntary, submaximal exertions of the repetitive task in our study, they do illustrate the effect of both load and exertion of repetitive movements on the magnitude and morphology of tissue responses.

Future research might include the analysis of human serum in response to continued performance of

repetitive tasks. Such investigations could clarify the risk exposure–tissue pathophysiology relationship in humans. In addition, research is needed to determine if the deterioration in speed and quality of reaching and grasping is due to localized injury to peripheral tissues or cortical reorganization. For example, Byl et al. [8] have shown evidence for dedifferentiation of the somatosensory cortex in an owl monkey model of repetitive grasping.

In conclusion, our rat model of work-related MSD shows that cumulative exposure to a highly repetitive task with negligible force causes both tissue and behavioral responses. The behavioral responses include avoidance as well as reduced fine motor performance. The primary tissue response is a local as well as a general elevation of macrophages, which have infiltrated widespread sites of muscles, tendons and associated CTs. This response may be mediated systemically by pro-inflammatory cytokines within the first 3–8 weeks. We are as yet uncertain that the number of infiltrating macrophages will return to baseline with continued task performance or remain elevated after 8 weeks. The increase of inflammatory mediators in serum at 8 weeks above control levels indicates that a systemic response has been stimulated. Our observations are consistent with complaints of humans reporting MSDs. The evidence for a systemic response may help to explain the puzzling and unstable early symptoms among affected workers, which are often disregarded by health care providers because they defy diagnostic classification. We hypothesize that early intervention at this stage might include control of inflammation systemically along with reduction in risk exposure.

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