Dose-Response Relationship between Reach Repetition and Indicators of Inflammation and Movement Dysfunction in a Rat Model of Work-Related Musculoskeletal Disorder

Ann E. Barr, Marata Amin and Mary F. Barbe

Proceedings of the Human Factors and Ergonomics Society Annual Meeting 2002 46: 1486
DOI: 10.1177/154193120204601617

DOSE-RESPONSE RELATIONSHIP BETWEEN REACH REPETITION AND INDICATORS OF INFLAMMATION AND MOVEMENT DYSFUNCTION IN A RAT MODEL OF WORK-RELATED MUSCULOSKELETAL DISORDER

Ann E. Barr*, Mamta Amin*, Mary F. Barbe*†
*Department of Physical Therapy, College of Allied Health Professions,
Temple University, Philadelphia, PA, USA
†Department of Anatomy and Cell Biology, Temple University
School of Medicine, Philadelphia, PA, USA

The purpose of this study was to compare the effects of a high or low repetition reaching and grasping task on serum levels of interleukin-1α (IL-1α) and IL-1β and on reach performance in a rat model of work-related musculoskeletal disorder (MSD). Forty-seven rats reached repetitively for 2 hours/day, 3 days/week for 6-8 weeks at a high or low rate. Reach rate and abnormal movement patterns were recorded. Serum was collected at 6 and 8 weeks for ELISA assay of IL–1α and IL-1β. High repetition animals experienced a 2-fold decline in reach rate, strong emergence of a raking movement, and increased IL-1α. IL-1α decreased in the low repetition group. IL-1β decreased in both groups. This model provides evidence that inflammation increases with repetition. At the higher reach rate, persistent IL-1α serum levels indicate chronic inflammation that is attenuated at the lower reach rate. These findings support the use of risk reduction in MSD prevention.

INTRODUCTION

Work-related musculoskeletal disorders (MSDs) accounted for 67% of all occupational illnesses reported by US private industry in the year 2000 (BLS, 2001). Never the less, the nature of tissue pathophysiology in the development of MSDs remains elusive. Animal models of MSD are an ideal means to study the link between exposure to physical risk factors and resulting physiological and behavioral responses.

We have developed a rat model of MSD using voluntary movements in a repetitive hand and wrist-intensive task (Barr et al, 2000; Barr and Barbe, 2002; Barbe et al, In Press). Rats were trained to perform a repetitive reaching and grasping task that shares key characteristics of movements performed by humans in some occupational settings. For example, repetitiveness is considered high when reaching and grasping motions are performed faster than 30 sec/cycle, and force is considered negligible to low if less than 15% of maximum voluntary grip force is required to perform a grasping task (Silverstein et al, 1986). In our model, rats performed a paced reaching and grasping task that required a high reach rate (<15 sec/cycle) and a negligible grip force (<1% of maximum grip force). Our rat model may be generalized to humans in terms of both behavioral and tissue responses for some types occupational tasks, such as packing, in which a worker repeatedly places small objects presented on a conveyor belt into a package crate.

The purpose of this project was to compare the effects of high and low repetition exposures over an 8-week period on serum levels of the proinflammatory cytokines interleukin-1α (IL-1α) and interleukin-1β (IL-1β) and on reach performance in a rat model of a repetitive reaching and grasping task with negligible force.

METHODS

Subjects

Fifty-seven adult Sprague-Dawley rats (age 12-14 weeks at onset of experiment; Ace) were used. Forty-seven rats were trained to perform a repetitive forelimb reaching and grasping task with negligible force for up to 8 weeks. Ten rats served as age-matched controls and did not perform the task regimen. The experimental 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

The 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 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 seconds (high repetition group, n=39) or every 30 seconds (low repetition group, n=8) during the reach task. An auditory indicator (Stimulus clicker, Med. Associates) coincided with pellet dispensation, thereby cueing the animal to attempt a reach. Animals performed the task for 2 hours/day, 3 days/week for 6-8 weeks. The daily task was divided into 4, 0.5-hour training sessions separated by 1.5 hours. An observer logged the number of reaches in a session using a hand-operated counter and reach distance criteria. A reach was defined as any time a rat reached beyond and then withdrew the forepaw behind a line drawn 0.5 cm within the tube.

Reach Performance Analysis

Reach rate was defined as the average number of reaches performed per minute and was analyzed by repeated measures analysis of variance (ANOVA) across weeks of task performance for the high and low repetition groups. Gross movement patterns were examined for deviations from normal movements comprising reaching in rats (Whishaw and Pellis, 1990). From previous studies (Barr et al, 2000; Barbe et al, In press), 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. Raking is typically associated with repeated loss of control of the food pellet due to uncoordinated movements of the digits. These behaviors were noted as present (>1/minute) or absent (<1/minute) at the end of each task week. They are expressed as the percentage of animals in which the behaviors were present.

Protein Isolation and Cytokine Analysis

Groups of animals were euthanized at weekly endpoints using Nembutol (120 mg/kg body weight) Serum IL-1α and IL-1β levels were examined in rats that had performed the task for 0, 6 and 8 weeks (n=3-8/group). Blood samples were collected from the heart, centrifuged, serum aspirated and total protein determined using BCA-200 protein assay kits (Pierce). Fifty µl aliquots were utilized for measuring IL-1α and IL-1β using enzyme-linked immmunosorbant assay (ELISA) kits (Biosource International, Camarillo, California) according to the manufacturer's protocol. ELISA data were normalized to µg protein. Each sample was run in triplicate and data are presented as mean pg/µg protein + 1 SD. Serum levels of IL-1α and IL-1β protein were analyzed by one-way ANOVA across weeks for the high and low repetition groups.

RESULTS

Reach Performance Changes

The mean reach rate for the high repetition group in week 1 (baseline) was 8.27 reaches/minute ± 0.66 SEM (Figure 1A). There was a significant decrease in reach rate at the end of week 5 to 6.82 reaches per minute ± 0.66 SEM (p=0.0028, n=31) and week 6 to 6.12 reaches per minute ± 0.51 SEM (p=0.0070, n=26). This decrease in reach rate

continued through week 7 (not significant) and was followed by an increase toward baseline in week 8 (not significant) (Barbe et al, In press). For the low repetition group, there was no significant difference in reach rate across weeks ($p=0.140$) and the mean reach rate was 3.01 reaches per minute $\pm$ 1.03 SEM (Figure 1B).

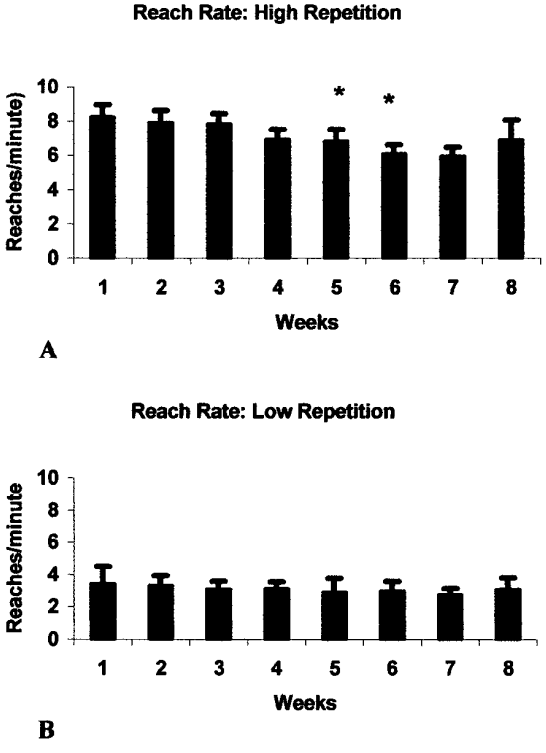

Figure 1: Reach rate (+ SEM) in high repetition group (A) and low repetition group (B) across weeks of task regimen. Asterisks at weeks 5 and 6 in the high repetition group indicate a significant decrease in reach rate ($p < 0.05$).

In the high repetition group, scooping emerged first as an alternative reaching pattern, peaked in 47% of animals by week five, and decreased to 26% of animals by week 8 (Figure 2A). Raking continued to increase beyond 5 weeks and was present in 100% of high repetition group animals in weeks 7 and 8. In the low repetition group, the scooping behavior also preceded that of raking (Figure 2B). However, both alternative movement patterns were present in fewer animals in this group. Scooping remained constant in 20% of animals through week 3 then declined to 0% by week 6. Raking increased across weeks but was present in only 60% of animals in weeks 7 and 8.

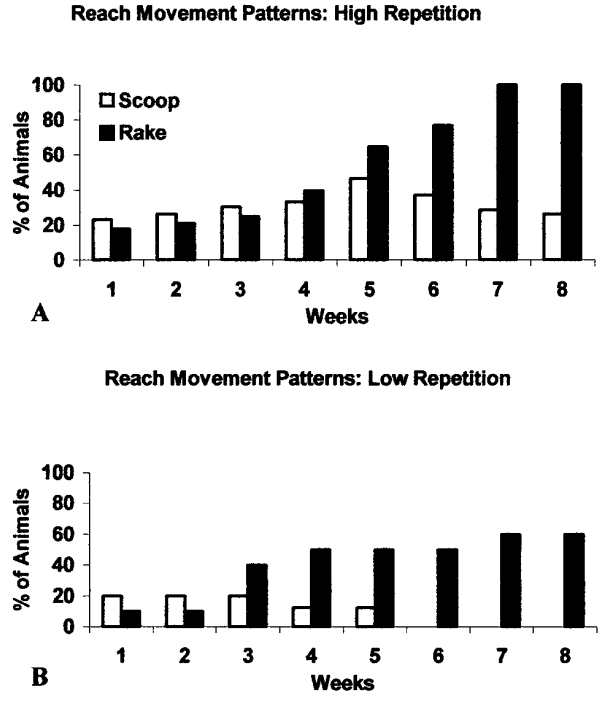

Figure 2: Alternative reach movement patterns in (A) high repetition group (n=39, week 1; n=19, week 8) and in (B) low repetition group (n=8, week 1; n=5, week 8). Data are expressed as the proportion of animals observed to perform the behaviors > once per minute. Note that 100% of all high repetition animals engaged in raking by week 7, whereas only 60% of all low repetition animals engaged in raking by week 7.

Cytokine Changes

In the high repetition group, serum levels of IL-1α increased significantly by 27% above control levels in week 8 (Barbe et al, In press) (Figure 3). The serum levels of IL-1β, however, were not significantly different over weeks of task performance ($p=0.3987$) (Figure 4). There was a trend toward decreasing levels of IL-1β in week 8 by 6% as compared to either week 0 or 6. In the low repetition group, serum levels of IL-1α did not change significantly over weeks of task performance ($p=0.5103$) (Figure 3). In contrast to the high repetition group, there was a trend toward a decrease in IL-1α by 34% in week 8 as compared to controls. There was no significant change in

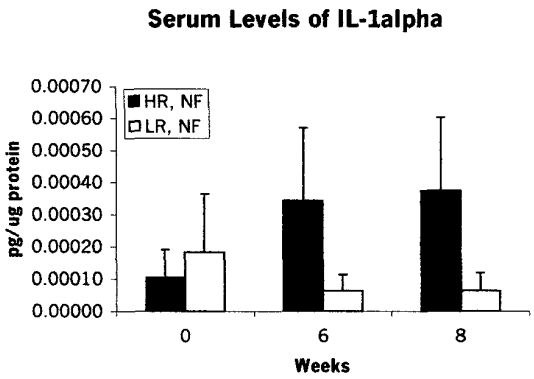

Figure 3: Serum levels of IL-1α expressed as pg IL-1α/μg total protein. HR, NF = high repetition group; LR, NF = low repetition group. IL-1α is significantly greater at 8 weeks (p=0.0188) in the high repetition group as compared to controls (0 weeks). Note the trend towards decreasing IL-1α in the low repetition group.

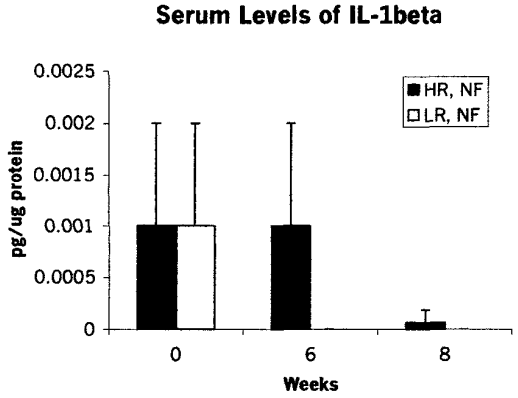

Figure 4: Serum levels of IL-1β expressed as pg IL-1β/μg total protein. HR, NF = high repetition group; LR, NF = low repetition group. Note the decrease in IL-1β levels in the high repetition group in week 8 as compared to controls (p=0.3028). There were no measurable levels of IL-1β at 6 and 8 weeks in the low repetition group.

serum levels of IL-1β over time in the low repetition group (p=0.3028) (Figure 4). However, there was no measurable production of IL-1β in weeks 6 and 8 as compared to the control animals.

DISCUSSION

Our results show a dose-response relationship between reach rate and both behavioral and physiological responses to a repetitive reaching and grasping task in rats. Even in a relatively low repetition reaching task with negligible force and a reach cycle time of 15 to 20 seconds, behavioral changes suggest physical discomfort that coincides with physiological evidence of diffuse and systemic inflammation. Increasing reach cycle time approximately twofold, to 7 to 10 seconds, further increases the magnitude of the behavioral response. The proportion of animals observed to scoop in week 5 was 2.35 times greater and the proportion observed to rake in week 7 was 1.67 times greater in the high repetition group as compared to the low repetition group. It is tempting to conclude from these results that the behavioral response to the performance of a repetitive task may increase linearly with increased reach rate. However, the physiological response observed in these experiments suggests a more complex dose-response relationship.

As has been previously shown, the primary tissue response to repeated, posturally constrained reaching movements in this rat model is inflammation resulting in reduction in physical performance (Barr et al, 2000; Barbe et al, In press). This inflammatory response, which includes an increase in IL-1α in the high repetition group, occurs both locally, in the tissues directly involved in the task, and at more distant tissue sites and in serum. In this study, reduction in the reach rate did not merely attenuate the increasing levels of IL-1α, but showed a decrease in this pro-inflammatory cytokine over weeks in the low repetition group. In both repetition groups, there was a trend toward decreasing levels of IL-1β: in the low repetition group, IL-1β levels were not measurable by week 6. These findings suggest a complex dose-response relationship that requires consideration of the mechanisms underlying inflammation.

IL-1 is a multifunctional cytokine secreted by a variety of cell types including monocytes/macrophages, osteoblasts and fibroblasts. IL-1, in combination with several other cytokines, is involved in the regulation of the immune response, hematopoiesis, inflammation and bone resorption. The induction of IL-1 is orchestrated by a variety of stimuli including

autocrine stimulation, other cytokines (both pro- and anti-inflammatory) and chemokines. The cellular mechanisms regulating the production and activities of cytokines is still under investigation. There are two members of the IL-1 family, IL-1α and IL-1β. Their physiological differences appear to be related to differential affinities of receptors for these two cytokines on their target cells (Chole et al, 1994).

In our experiments, the serum levels of IL-1β decreased with task performance in both the high and low repetition groups. IL-1α increased in the high repetition group, but decreased in the low repetition group. These discrepancies are probably due to different mechanisms of the overall inflammatory response elicited by tissue injury. This response includes the production of both pro- and anti-inflammatory proteins. The IL-1 cytokines are pro-inflammatory proteins and are synthesized and secreted by fibroblasts, myoblasts, synoviocytes, infiltrating monocytes and macrophages in response to mechanical tissue injury. The macrophages also produce pro-inflammatory cytokines through autocrine mechanisms. Inflammatory mediators such as the cytokines also induce the production of anti-inflammatory cytokines as part of homeostatic repair and regeneration elicited after injury. Upregulation of these anti-inflammatory cytokines usually stimulates a down regulation of the pro-inflammatory cytokines (Tachimoto et al, 2000). We know from our histological examination of tissues from these animals that several anti-inflammatory cytokines have been upregulated by 8 weeks of task performance (data not shown). We hypothesize that the net cytokine production in the low repetition group allows for the maintenance of homeostasis through the resolution of an acute inflammatory response. The level of repeated incidents of mechanical injury to the tissues in the high repetition group, on the other hand, leads to a net production of IL-1α, which is increasing in the blood serum and is indicative of a chronic and systemic inflammatory phase in which some pro-inflammatory cytokines remain elevated as task performance continues. Furthermore, animals in the high repetition group are not able to maintain a consistent reaching pace over time.

Our findings indicate that performance of highly repetitive tasks is associated with both local and systemic inflammatory responses that may result in decrements in task pace and movement efficiency. At higher repetition rates, a chronic inflammatory response may develop. These findings support a preventive approach to MSDs in which exposure to risk factors, such as repetitiveness, are reduced through job redesign. They also suggest a role for the management of systemic inflammation in workers with severe or chronic symptoms.

ACKNOWLEDGMENTS

This project was supported by NIAMS Grant # R03 AR 46426, NIOSH Grant # R01 OH 03970, Foundation for Physical Therapy NIFTI Award Program and Temple University.

REFERENCES

Barbe MF, Barr AE, Gorzelany I, Amin M, Gaughaugn J, Safadi, FF. Chronic repetitive reaching and grasping results in decreased motor performance and widespread tissue responses. *J Orthop Res*, In press.

Barr AE, Barbe MF. Pathophysiological Tissue Changes Associated with Repetitive Movement: A Review of the Evidence. *Phys Ther* 82: 173 –187, 2002.

Barr AE, Safadi FF, Garvin RP, Popoff SN, Barbe MF. Evidence of progressive tissue pathophysiology and motor behavior degradation in a rat model of work related musculoskeletal disease. Proceedings of the *IEA 2000/HFES 2000 Congress*. 5: 584 - 587, 2000.

Bornefalk E, Ljunghall S, Johansson AG, Nilsson K, Ljunggren O. Interleukin-1beta induces cyclic AMP formation in isolated human osteoblasts: a signaling mechanism that is not related to enhanced prostaglandin formation. *Bone Mineral* 27: 97-107, 1994.

Bureau of Labor Statistics. *Safety and Health Statistics*. U.S. Department of Labor, http://www.bls.gov/datahome.htm. 2001.

Chloe RA, Tinling SP, Faddis BT. Human recombinant interleukin-1 receptor antagonist blocks bone resorption induced by interleukin-1beta but not interleukin-1alpha. *Calcif Tissue Int* 55: 12-15, 1994.

Silverstein BA, Fine LJ, Armstrong TJ. Hand wrist cumulative trauma disorders in industry. *Br J Ind Med* 43:779-84, 1986

Tachimoto H, Ebisawa M, Hasegawa T, Kashiwabara T, Ra C, Bochner BS, Miura K, Saita H. Reciprocal regulation of cultured human mast cell cytokine production by IL-4 and IFN-gamma. *J Allergy Clin Immunol* 106: 141-149, 2000.

Whishaw IQ, Pellis SM. The structure of skilled forelimb reaching in the rat: a proximally driven movement with a single distal rotatory component. *Behav Brain Res* 41: 49-59, 1990.