Exposure to Repetitive Tasks Induces Motor Changes Related to Skill Acquisition and Inflammation in Rats

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Abstract

This study elucidates exposure-response relationships between repetitive tasks, inflammation and motor changes with work-related musculoskeletal disorders. Using a rat model of reaching and handle-pulling, we examined effects of performing a high repetition low force (HRLF), low repetition high force (LRHF), or high repetition high force (HRHF) task (2 h/day, 3 days/wk, 12 wks) on reach rate and force, percent success, duration of participation and grip strength. Reach rate and reach force improved with HRLF, and percent success increased in all groups in week 9, and HRLF and HRHF in week 12, indicative of skill acquisition. Duration and grip strength showed force-dependent declines with task performance. A subset of HRHF rats received ibuprofen in weeks 5–12. Ibuprofen significantly improved reach rate, reach force and duration in treated rats, indicative of an inflammatory influence on reach performance. Ibuprofen improved percent successful reaches in week 9, although this increase was not sustained. However, declines in grip strength, a nocifensive behavior, were not prevented by ibuprofen. Examination of cervical spinal cords of untreated and ibuprofen treated HRHF rats showed increased IL-1beta, an inflammatory cytokine, in neurons. These findings suggest that only a preventive intervention could have addressed all motor declines.

Keywords

repetitive task; work-related-musculoskeletal disorders; grip strength, reach performance

Introduction

Work-related musculoskeletal disorders (WMSDs) can be caused by extended performance of repetitive, forceful and/or awkward movements. WMSDs include diagnoses such as tendinopathies, nerve compression syndromes, and muscular and joint disorders (Byl & Melnick, 1997; Hales & Bertsche, 1992; Piligian et al., 2000; Rempel, 1992; Rempel, Harrison, & Barnhart, 1992). The National Occupational Research Agenda (Marras, Cutlip, Burt, & Waters, 2011) emphasizes the need for research to elucidate the exposure-dependent nature of WMSDs and to develop effective preventions.

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Several animal studies of repetitive prehensile tasks have shown that repetitive hand opening and closing induces motor dysfunction (Barbe et al., 2003; Barbe et al., 2008; Byl, Holland, Jurek, & Hu, 1997; Byl et al., 1997; Byl, Merzenich & Jenkins, 1996; Clark, Al-Shatti, Barr, Amin & Barbe, 2004; Coq et al., 2009; Elliott, Barr, Clark, Wade & Barbe, 2010; Elliott et al., 2008; Rani, Barbe, Barr & Litvin, 2010; Sommerich et al., 2007; Topp & Byl, 1999). We have developed a rat model of voluntary, repetitive reaching and grasping. When examining the effects of performing either a high repetition or low repetition food retrieval task (a negligible force task), we observed exposure-dependent decreases in reach performance and grip strength, and the emergence of abnormal movement patterns (Barbe et al., 2003; Barbe et al., 2008; Clark et al., 2003; Coq et al., 2009; Elliott et al., 2008). With both tasks, reach performance declines were transient. However, grip strength declines and abnormal movement patterns were greater with the high repetition, negligible force task. Performance of a high repetition, high force handle-pulling task induced even greater declines in grip strength and reach performance (Clark et al., 2004; Fedorczyk et al., 2010; Rani et al., 2010). This handle-pulling task, in which rats can be trained to perform at a variety of reach rates and force levels, was used in this current study to examine exposure-dependent motor declines and potential contributing mechanisms.

The underlying mechanisms leading to the motor behavior changes associated with WMSDs continue to be insufficiently understood. These mechanisms need to be clarified in order to develop effective interventions. We previously proposed a conceptual model in which three key interrelated pathways (peripheral tissue injury/inflammation, peripheral tissue reorganization, and central nervous system reorganization) can drive further inflammatory responses, adaptive or pathological tissue remodeling, and sensorimotor behavioral changes, with performance of repetitive tasks (Barbe & Barr, 2006).

With regard to inflammation and motor behavior, forelimb grip strength declines occur after intramuscular injections of inflammatory/pro-nociceptive cytokines, such as tumor necrosis factor (TNF), into forelimb muscles (Schafers, Sorkin & Sommer, 2003; Beyreuther, Geis, Stohr & Sommer, 2007). Grip strength also correlates negatively with inflammatory cytokine levels in forelimb muscles of rats performing repetitive tasks for extended periods (Barbe et al., 2008; Fedorczyk et al., 2010; Elliott et al., 2010). However, we have observed that, although a two-week treatment with an anti-TNF alpha drug returned serum and tissue cytokines to baseline levels, it only attenuated, but did not restore, grip strength declines already present at the time of drug administration (Rani et al., 2010). This latter finding suggests that there are contributing factors other than inflammation to declines in grip strength.

Central nervous system changes can contribute to skill acquisition, but also to motor control declines. Movement dysfunction has been attributed to degradation of the somatotopic representation of the forepaw or hand in the primary somatosensory cortex in owl monkeys and rats performing repetitive tasks (Byl et al., 1996; Byl et al., 1997; Topp & Byl, 99; Coq et al., 2009) and human subjects with severe and moderate focal hand dystonia (Byl, McKenzie & Nagarajan, 2000; Byl, Nagarajan, Merzenich, Roberts & McKenzie, 2002). Furthermore, the emergence of nocifensive motor behaviors has been linked to maladaptive changes in the spinal cord, such as increased pro-inflammatory cytokines in neurons or glia, as a consequence of peripheral tissue injury or inflammation (Moalem & Tracey, 2006; Hunt, Winkelstein, Rutkowski, Weinstein & DeLeo, 2001; Elliott et al., 2010).

Therefore, we extended previous studies to examine exposure-dependent changes in several motor behaviors occurring with performance of a handle-pulling task for 9–12 weeks at 3 different repetition and force levels: 1) low repetition with high force, 2) high repetition with low force, and 3) high repetition with high force. We also sought to determine if

administration of a conservative anti-inflammatory drug, ibuprofen, as a secondary intervention would effectively ameliorate motor declines in animals exposed to the HRHF task. Based on our conceptual model, we hypothesize that although some voluntary reach performance outcomes will improve due to skill acquisition, others will decline in an exposure-dependent manner due to underlying inflammatory responses and that ibuprofen treatment will effectively attenuate these latter declines. However, since reduced grip strength can be a nocifensive reflexive behavior, we hypothesize that grip strength declines will persist due to central nervous system changes, such as increased inflammatory cytokines in spinal cord segments, which are not attenuated by ibuprofen treatment.

Methods

Overview

Using our model of a voluntary repetitive upper limb reaching and handle-pulling task in rats, we compared voluntary reach performance outcomes in weeks 9 and 12 of task performance to week 1 (the first week rats began performing the tasks for 2 hours/day, 3 days/week). We also compared reflexive grip strength in weeks 9 and 12 of task performance to baseline naïve values. The independent variable was exposure to 1 of 3 different repetitive tasks: 1) a low repetition with high force task (LRHF), 2) a high repetition with low force task (HRLF), or 3) a high repetition with high force task (HRHF). A subcohort of the HRHF group began receiving ibuprofen treatment at the end of week 4 (HRHF+IBU), and the effects of this treatment to week 12 was compared to untreated HRHF rats. Dependent variables included reach rate (includes successful and unsuccessful reaches), reach force, percent successful reaches, duration of task participation (expressed as a percentage of continuous participation for 120 min), and grip strength.

Animals

All experiments were approved by the Temple University Institutional Animal Care and Use Committee and were in compliance with NIH guidelines for humane care and use of laboratory animals. A total of 96 young adult (14 weeks of age at onset of study) female Sprague-Dawley rats were used (Fig. 1). Female rats were used since human females have a higher incidence of WMSDs than males (Gerr et al., 2002). The rats were housed in a central animal facility in separate cages with a 12 hour light:dark cycle and free access to water. All rats received food pellet rewards and Purina rat chow daily.

Behavioral Apparatuses

The behavioral apparatuses used were as previously described (Clark et al., 2004; Fedorczyk et al., 2010). Briefly, custom-designed force apparatuses (Custom Medical Research Equipment, Glendora, NJ) were integrated into an operant behavioral training system (Med Associates, Georgia, VT). Animals reached through a shoulder height portal and then isometrically pulled a force handle attached to a force transducer (Futek Advanced Sensor Technology, Irvine, CA) located outside the chamber wall. The load cell was interfaced with Force-Lever software (version 1.03.02, Med Associates, St. Albans, VT). An auditory indicator cued the animals to reach once every 15 seconds, i.e. 4 reaches/min for high repetition tasks, or to reach once every 30 seconds, i.e. 2 reaches/min, for the low repetition task. The rats had to grasp the force handle and exert an isometric pull for at least 50 ms with a graded force effort of either 15% ($\pm$ 5%) of maximum voluntary pulling force for the low force task, or 60% ($\pm$ 5%) for high force tasks. If these criteria were met within a 5 second cueing period, a 45 mg purified formula food pellet (Bioserve, NJ) was dispensed into a trough located at floor height for the animal to lick up. Animals were allowed to use their preferred limb to reach. The contralateral limb was used as a postural support limb throughout the task, with the rats often pushing with this limb against the chamber wall as

they performed the isometric pull with the reach limb (as depicted in Fedorczyk et al., 2010).

Initial training to learn the tasks

All rats except for normal controls were food-restricted for a short period (no more than 7 days) to 85–95% of their naive weight to initiate interest in the food pellets. After that first week, rats were given extra rat chow, weighed weekly, and maintained thereafter as closely as possible to within ± 5% of age matched normal control rats (used for weight comparison only) until euthanasia. Thee food restricted rats went through an initial training period of 10–15 min/day, 5 days/week, for 5–6 weeks (see Fig. 1). During this period, they were trained to perform the reaching and handle-pulling tasks at the appropriate reach rate and force requirements for a particular task, as previously described (Clark et al., 2003; Elliott et al., 2010).

HRLF, LRHF, HRHF Task regimens

After the training period, 92 rats began the task regimens for 2 hrs/day, 3 days/wk for up to 12 weeks: LRHF, n=18; HRLF, n=26; HRHF, n= 48 (Fig. 1). The task was divided into 4, 0.5-hr sessions separated by 1.5 hrs in order to avoid satiation. HRLF rats were cued to reach at a target rate of 4 reaches/min, grasp the force handle and exert an isometric pull for at least 50 ms at a force effort of 15% (± 5%) of the average maximum pulling force, as previously described (Elliott et al., 2010). LRHF rats were cued to reach at a target rate of 2 reaches/min, and grasp the force handle at a force effort of 60% (± 5%). HRHF rats were cued to reach at a target rate of 4 reaches/min, and grasp the force handle at a force effort of 60% (± 5%), as previously described (Clark et al., 2004; Driban, Barr, Amin, Sitler & Barbe, 2011). If they either undershot the minimum criterion or overshot the maximum criterion, no food reward was delivered. Because the inherent nature of our task is voluntary, the rats tended to reach more frequently than the target rate, as described further in the results. In addition, they were not prevented from reaching at a higher or lower force than their target force. Thus, the animals were allowed to self-regulate their participation in task performance, making these voluntary tasks. However, the food reward was not given unless they met the force criterion within a 5 second window initiated every 15 (high repetition) or 30 seconds (low repetition), and held the handle with the correct force for 50 ms.

Ibuprofen Treatment of a cohort of HRHF group

At the end of the 4th week of task performance, a subcohort of 22 (Fig. 1), of the HRHF animals were administered ibuprofen (Children's Motrin Grape Flavored, Johnson & Johnson) (HRHF+IBU) in drinking water daily (45 mg/kg body weight) as previously described (Driban et al., 2011). The HRHF+IBU animals continued to perform the HRHF task regimen with ibuprofen treatment for the remainder of the 12 week task period (i.e., throughout an 8 week course of ibuprofen treatment). The amount of medicated water consumed/day was tracked for each animal and serum levels of ibuprofen were confirmed using National Medical Services (Willow Grove, PA). Based on these assessments, the average weekly ibuprofen dose was 48.8 ± 6.3 mg/kg body weight.

Determination of Reach Performance Behaviors

Force lever data were recorded continuously during each task session for later calculation of the dependent variables (reach rate, reach force, percent of successful reaches, and duration) via an automated script (MatLab; Mathworks, Natick, MA). A reach was defined as a force deflection that exceeds 2.5% of the baseline (or zero) force until the next sample in which the force falls below 2.5% of baseline. In this way, unsuccessful reaches that did not meet the food reward criteria were recorded and analyzed along with successful reaches. Thus,

reach rate was defined as the average number of both successful and unsuccessful reaches performed per minute. Reach force was the average force (expressed as a percentage of maximum pulling force) applied to the force handle for all reaches on a given day. The average percent of successful reaches was determined by dividing the number of successful reaches (in which the food reward criteria were met) by the total number of reaches, then multiplying by 100. Duration was defined as the number of minutes (expressed as a percentage of the target of 120 minutes) the rats participated in the task. Data for each variable was calculated on the last day of week 1, 9 and 12.

Force lever data were obtained from: a) 15 HRLF animals at weeks 1, 9, and 12 of HRLF task performance; b) 12 LRHF animals at weeks 1, 9, and 12 of LRHF task performance, c) 26 untreated HRHF animals at week 1 of HRHF task performance, with 18 animals continuing to week 9 and 10 animals continuing to week 12 (the number reduces across weeks due to euthanasia for other analyses); and d) 8 HRHF+IBU animals at week 1 (prior to ibuprofen treatment), and at weeks 9 and 12 (after 5 and 8 weeks of ibuprofen treatment, respectively). Week 1 was used as the baseline for reach performance variables since that was the first week rats actually performed the task regimens for 2 hours/day, 3 days/week.

Grip Strength Analysis

Grip strength of the reach and support forelimb was tested in all task rats (HRLF n=26, LRHF n=18, untreated HRHF n=26, and HRHF+IBU n=22; Fig. 1), at baseline before training and task performance (the naïve time point), and in weeks 9 and 12, as previously described, using a grip strength meter for rodents (Stoelting, Wood Dale, IL, USA) (Elliott et al., 2010). Naïve data were used for baseline grip strength values since we have previously reported a training effect for grip strength (Elliott et al., 2010). Maximum grip strength was defined as the value of the peak force (in grams) recorded from the transducer at the moment that forepaw grip strength is overcome by the examiner. Importantly, the moment at which each animal released its grip from the handle of the grip strength meter was self-determined. Therefore, the amplitude of force generated were influenced by several factors, e.g. muscle inflammation, connective tissue changes, or spinal cord or brain neuroplasticity, that can influence behavioral performance, as described previously (Schafers et al. 2003; Elliott, Barr & Barbe 2009a; Coq et al, 2009). The test was repeated five times per forelimb, in a randomized fashion for right versus left limbs, and the maximum grip force (strength in grams) per trial was included in the statistical analysis. The person carrying out the testing was blind to treatment.

Examination of Spinal Cord

Subcohorts of the HRHF and HRHF+IBU rats (n=4/group), as well as normal control rats (n=4) that were not exposed to the training or tasks, were euthanized with an overdose of sodium pentobarbital (120 mg/kg body weight), perfused intracardially with 4% paraformaldehyde in phosphate buffer, and the cervical segments of the spinal cords collected and sectioned as previously described (Elliott et al., 2010). The spinal cord sections were then immunostained for interleukin-1 beta (IL-1beta; a pro-inflammatory cytokine) and NeuN (a neuronal cell body marker), and then incubated in appropriate secondary antibodies tagged to green (Cy2) or red (Cy3) fluorescent markers, using previously described methods (Elliott et al., 2010). The ventral horns of the cervical spinal cord were examined qualitatively for presence of cells immunostained for IL-1beta.

Statistical Analyses

To determine the effect of task performance on reach performance variables, two-way ANOVAs were used with the following factors: week (weeks 1, 9 and 12 of task performance) and group (HRLF, LRHF, HRHF and HRHF+IBU). To determine the effect of

task performance on grip strength, two-way ANOVAs were used with the following factors: week (naïve, and weeks 9 and 12 of task performance) and group (HRLF, LRHF, HRHF and HRHF+IBU). A repeated measures ANOVA could not be used since the number of animals per week differed across weeks and groups due to euthanasia and tissue collection for other studies. The Bonferroni post-hoc method for multiple comparisons was used to compare behavioral results in week 9 and 12 to week 1 or naive data (within group comparisons), and to compare between groups at matching temporal endpoints (inter-group comparisons). Adjusted *P*-values are reported. Data are expressed as mean ± standard error of the mean (SEM).

Results

Concerning reach rate, because the inherent nature of our task is voluntary, the rats tended to reach and pull more frequently than their target rates (Fig. 2), albeit not all of these were successful reaches (see Fig. 4). The actual reach rate of the HRLF rats in week 1 was 20.7 reaches/min, approximately five times the target rate. The actual reach rate of the LRHF rats in week 1 was 7.2 reaches/min, and the actual reach rate of the HRHF rats in week 1 was 13.42 reaches/min, each over 3 times their target rate. Since the reach rate variable includes both successful and unsuccessful reaches, these results suggest that the rats did not rely completely on the auditory prompts, and did not effectively learn that a food reward could only be obtained 2 or 4 times per minute (for the low repetition or high repetition tasks, respectively) despite more frequent pulling of the handle, although they did improve with time (as reported next).

Reach rate declined (improved) towards target levels across weeks of task performance in the group with the lowest force requirements (HRLF) and with ibuprofen treatment (HRHF +IBU) (week, p<0.0001; group, p<0.0001; interaction, p=0.03) (Fig. 2). Specifically, reach rate declined in HRLF rats in weeks 9 and 12, compared to their week 1 (p<0.01), suggesting skill acquisition since it declined towards their target reach rate (from 5 to 3 times their target rate). However, reach rates in LRHF and untreated HRHF rats did not differ with week into the protocol, and remained approximately 3 times more than their target. In contrast, reach rate declined in 12-week HRHF+IBU rats, compared to week 1 (p<0.01), again suggesting skill acquisition since it declined towards their target rate (from 3 to 1.8 times their target). Compared across groups, reach rate was lower in week 1 in the high force groups (LRHF, HRHF and HRHF+IBU) than in HRLF (p<0.01 each). Also in week 1, LRHF rats had, appropriately, the lowest reach rate compared to the other groups (p<0.01 each).

The reach force target of 15% was met by the low force group (HRLF) in week 12, while the reach force target of 60% was met only by the ibuprofen treated group (HRHF+IBU) in week 9, although they neared it in week 12 (week, n.s., p=0.16; group, p<0.0001; interaction, p=0.007) (Fig. 3). Specifically, reach force declined towards the target force level of 15% in HRLF rats in weeks 9 and 12, compared to week 1 (p<0.01 each). Since they met target force level in week 12, this is indicative of skill acquisition. In contrast, reach force did not change significantly in LRHF or HRHF rats in weeks 9 and 12 from week 1 levels, remaining below their target of 60% throughout the experiment (their reach force ranged from 38–46%). However, reach force was greater in HRHF+IBU rats in weeks 9 and 12, than in week 1 (p<0.01 each). They reached their target force level of 60% in week 9, declined to 52% in week 12 (Fig. 3). Concerning group differences, all high force rats (LRHF, HRHF, HRHF+IBU) had, appropriately, higher reach force than HRLF in each week (p<0.01 each). Lastly, in week 9, the HRHF+IBU rats had higher reach force than LRHF (p<0.05) and untreated HRHF (p<0.01), the latter indicating an inflammatory influence on reach force.

Percent successful reaches increased in all groups with continued task performance, except in the ibuprofen treated group (HRHF+IBU), which increased in week 9 but decreased in week 12 (week, p=0.001; group, p<0.0001; interaction, p=0.001) (Fig. 4). Specifically, compared to their week 1 levels, percent successful reaches increased in week 9 in HRLF, LRHF, HRHF and HRHF+IBU (p<0.05 for HRLF, p<0.01 for the others), and in week 12 in HRHF, each suggestive of skill acquisition. Surprisingly, HRHF+IBU rats had fewer successful reaches in week 12, compared to their week 1 (p<0.01), indicating that ibuprofen was only effective short-term. There were also differences by group, with the HRLF group having lower percent success than each of the high force groups in week 9 (p<0.01 each), and lower than HRHF in week 12 (p<0.01 each). Also, 12-week HRHF+IBU rats had fewer successful reaches than 12-week HRHF rats (p<0.01).

The duration of voluntary task participation neared the target of 120 min per day only in the low force group (HRLF) in week 1, and in the ibuprofen treated group (HRHF+IBU) in weeks 9 and 12 (week, n.s., p=0.98; group, p<0.0001; interaction, p<0.0001) (Fig. 5). Specifically, the duration of task participation declined in HRLF rats across weeks slightly, but not significantly. Also, the duration of task participation in the LRHF rats decreased in week 12 (p<0.01), and in the HRHF rats in weeks 9 (p<0.01) and 12 (p<0.05), compared to their week 1 values, indicative of exposure-dependent declines (exposure defined here as number of weeks exposed to the task). In contrast, duration increased in HRHF+IBU rats in weeks 9 and 12, compared to week 1 (p<0.01 each), indicating an inflammatory influence on duration. Concerning group differences, in week 1, the high force groups (LRHF, HRHF and HRHF+IBU) had lower duration levels than the HRLF (p<0.01 each), as did LRHF in week 12 and HRHF in weeks 9 and 12 (p<0.01 each), indicating a force level influence on duration. In contrast, duration increased in HRHF+IBU rats in weeks 9 and 12, compared to LRHF and HRHF rats in weeks 9 and 12 (p<0.01), indicating that there was also an inflammatory influence on duration.

Grip strength declined, bilaterally, in all groups with continued task performance and with higher force requirements, and was only nominally attenuated by ibuprofen treatment (both limbs: week, p<0.0001; group, p<0.0001; interaction, p=0.0009) (Fig. 6). Specifically, in the preferred reach limb (Fig. 6A), grip strength declined in all groups in weeks 9 and 12, compared to baseline naïve levels (p<0.01 each). Concerning group differences, grip strength was lower in the reach limbs of the high force groups (LRHF, HRHF and HRHF+IBU) in week 9 compared to 9-week HRLF rats, and in 12-week HRHF rats compared to 12-week HRLF (p<0.01 each), indicative of a force level influence on grip strength. Ibuprofen treatment did not improve grip strength in the reach limb in HRHF+IBU rats above HRHF levels (p>0.05, n.s.), although they were equivalent to HRLF rats by week 12 rather than lower, as seen in week 9.

In the contralateral support limb, a limb used for postural support against the chamber wall, grip strength also decreased in all groups in weeks 9 and 12, compared to baseline levels (p<0.01 each, Fig. 6B). Concerning group differences, grip strength was lower in the HRHF group in this limb in 9-week HRHF rats than 9-week HRLF (p<0.05), and lower than all other groups in week 12 (p<0.01 each). Two-tailed t-tests confirmed that 9-week LRHF and 9-week HRHF+IBU rats did not have grip strengths that were significantly different from 9-week HRLF rats. These findings indicate that the level of postural support "push" used by HRHF rats affected their grip strength more than in the other groups. Grip strength was higher in 12-week HRHF+IBU rats than 12-week HRHF rats (p<0.01), although still lower than baseline levels, indicating that grip strength was only partially influenced by inflammation in this limb.

We next examined cervical spinal cord segments for evidence of increased IL-1beta, a pro-inflammatory cytokine, and found increased IL-1beta immunoreactivity in neurons (NeuN+) in the intermediate and ventral horns of 12-week HRHF rats (Fig. 7A,B), but not in normal control rats (Fig. 7C). Ibuprofen treatment for 8 weeks did not attenuate IL-1beta immunoreactivity in 12-week HRHF+IBU rat (Fig. 7D), indicating that oral dosing with this anti-inflammatory drug did not attenuate this central nervous system inflammatory response. Similar results were observed in all animals examined (n=4/group). This finding suggests that neuroplastic changes may be contributing to the grip strength declines.

Discussion

Our results show that some behaviors, such as reach rate and reach force improved, at least partially, in rats performing the lower force task (HRLF) and in ibuprofen treated rats (HRHF+IBU). Percent successful reaches also improved in all groups, except the 12-week HRHF+IBU rats. Percent success decreased less with the lower force task, in general, than with the high force tasks. Other behaviors, such as the ability or willingness to participate fully in the task (duration) and grip strength, showed exposure-dependent declines, with greater declines in the untreated high force groups. Ibuprofen treatment greatly improved reach rate, reach force and duration, indicating an inflammatory influence on these behaviors. However, ibuprofen treatment did not rescue grip strength declines to baseline levels, and only nominally improved grip strength above untreated HRHF levels. There was an increase in IL-1beta immunoreactivity in neurons located in cervical spinal cords of both HRHF and HRHF+IBU rats that was not responsive to ibuprofen treatment that might have contributed to the persistent declines in grip strength.

Behaviors that improved with skill acquisition

Reach rate, the average number of both successful and unsuccessful reaches per minute, showed improvement only in the low force (HRLF) and ibuprofen treated (HRHF+IBU) groups. The HRLF rats dropped from exceeding their target rate of 4 reaches/min by five fold to three fold (although still in excess), and the HRHF+IBU rats dropped from 3 fold to 1.8 fold. We have previously reported a transient declines in reach rate after exposure to the HRHF handle pulling task, compared to week 1 (Clark et al., 2004). In retrospect, although we previously described these decline as impairments, perhaps declines in reach rate with the handle-pulling task are due to skill acquisition. In HRLF rats, a 1.6 fold decline in reach rate was matched by a 1.8 fold improvement in percent successful reaches. Over time, rats presumably learned the meaning of the auditory cues that preceded the start of each reach phase, increased their ability to control the applied force, or a combination of the two. The lower reach rates in the high force groups, in general, than the low force group, may also be due to a greater ease of force modulation when near maximum voluntary force levels. However, reach rates did not improve in the high force groups, except in the HRHF+IBU group, presumably due to reduction in discomfort from task-induced tissue inflammation. Discomfort likely prevented the LRHF and untreated HRHF from modulating their reaching effectively.

Reach force also showed improvement in the HRLF and HRHF+IBU groups. The HRLF rats, for example, met their target requirement of 15% maximum pulling force by week 12. Ballermann, Thomkins, and Whishaw (2000) reported that rats can modify and correct their reach force with experience when learning a skilled reaching and grasping task. Apparently, though, there is a limit to rats' ability to correct their reach force, as we observed no improvement in the LRHF or untreated HRHF groups with continued task performance. These findings combined suggest that it is easier to reach the 15% force level than the 60% force level. Only HRHF+IBU rats were able to reach their target of 60% maximum pulling

force in week 9. However, despite continued ibuprofen treatment, they were unable to maintain this hypothesized correction, and declined to 52% in week 12.

Each group showed an increase in percent successful reaches in week 9, with or without improvements in reach rate or force, an improvement maintained through week 12 in HRLF and HRHF rats. Since no food reward was delivered if the rats undershot their minimum criterion (−5% of their target force) or overshot their maximum criterion (+5% of their target force), and since only HRLF and HRHF+IBU rats showed improved reach force, an ability to correct reach force with experience was not the key factor contributing to success. The rats also had to reach within the right time frame and hold the handle for 50ms to receive a food reward. That skill was achieved by all but the 12-week HRHF+IBU rats. Their ability to pull at a reach force of 52% in week 12 rules out muscle fatigue. Although this may seem counterintuitive, perhaps the rats with the greater levels of exposure (and thus the greatest amount of tissue injury and inflammation), were honing their attention in order to maximize their efficiency of movement to avoid any unnecessary movements and/or discomfort. Ibuprofen treatment in the HRHF+IBU rats may have reduced that attention by week 12. This idea may also explain the low percent success in the HRLF rats. Whatever the reason for the lower success in 12-week HRHF+IBU and HRLF rats, consistent maintenance of a reach force of approximately 40–46% by the LRHF and untreated HRHF rats throughout the weeks of task performance was a more successful strategy with respect to percent successful reaching.

Behaviors that decline with exposure (continued task performance and/or level of task)

Despite the acquisition of greater skill, decreased duration in the two high force groups (LRHF and HRHF) suggests fatigue and/or diminished tolerance for continuation of activity. This is consistent with findings in our prior studies in which rats performing high force tasks show exposure-induced declines in duration (Clark et al., 2004; Elliott et al., 2009b) declines not observed in rats performing negligible force tasks (Coq et al., 2009; Elliott et al., 2008). These findings combined indicate that high force tasks are more difficult to sustain than low force tasks. Also, the more pronounced declines with HRHF than with the HRLF or LRHF groups suggest that force magnitude and repetition have a combined effect. Ibuprofen treatment in the HRHF+IBU rats ameliorated this decline in duration. In fact, duration was close to 100% in the HRHF+IBU group, even higher than the HRLF group. This suggests that there is an inflammatory component to declines in duration.

We also observed grip strength declines in all groups in weeks 9 and 12, with greater declines in the high force groups than the low force group. In general, HRHF rats had the lowest grip strengths. These findings are indicative of force-dependent declines in grip strength. We have also reported repetition-dependent declines in the past, with lower grip strengths in rats performing a high repetition, negligible force task than a low repetition, negligible force task (Barbe et al, 2008). The declines in grip strength were bilateral, since, as shown previously, the contralateral (nonreach) limb is used for postural support against the chamber wall (Fedorczyk et al., 2010). The push activity of the support limb apparently decreased grip strength more than the isometric pull by the reach limb, indicating that the use of other body regions for postural support can also affect function in that limb.

Behavioral declines linked to inflammatory responses

Several of the reach performance variables improved in the HRHF+IBU rats, including reach rate, reach force, duration and percent success, albeit the latter only in 9-week HRHF rats. These findings provide additional evidence that inflammation is one of the mechanisms related to declines in motor function with repetitive tasks. This hypothesis is further supported by studies from our lab and others that report reduced success, duration, grip

strength, and reflexive limb withdrawal to mechanical stimuli are associated with increased inflammatory cells, cytokines, and nociceptor-related neurochemicals in peripheral tissues and serum (Barbe et al., 2008; Clark et al., 2004; Elliott et al., 2009a,b; Elliott et al., 2010; Fedorczyk et al., 2010; Schafers et al., 2003; Beyreuther et al., 2007). It has also been shown that declines in grip strength occur after injection of TNF into forearm muscles (Beyreuther et al., 2007). Inflammation linked declines in grip strength have been termed muscle hyperalgesia, and may be a form of myalgia. In our model, grip strength declines improved after treatment with an anti-rat TNF drug in weeks 5–6 of a 6 week HRHF task, although similar to these current findings, grip strength did not return to baseline levels (Rani et al., 2010).

On the other hand, we recently reported persistent declines in grip strength in young adult HRLF rats in which the circulating inflammatory response had resolved (Xin et al., 2011). Those results combined with our current findings indicate that anti-inflammatory medication, such as ibuprofen, may be effective only during the early management of repetitive motion injuries. This is particularly true since ibuprofen use may affect muscle negatively (Machida & Takemasa, 2010; Soltow et al., 2006). Thus, factors other than inflammation must also contribute to persistent grip strength declines.

This is a concern since non-steroidal anti-inflammatory drugs (NSAIDS), including ibuprofen, are commonly used (self-care and prescribed) for both acute and chronic musculoskeletal pain (Anthony, Martin, Avery & Williams, 2010; Beebe, Barkin & Barkin, 2005; Bouef-Calnan, Lapeyre-Mestre, Niezborala & Montastruc, 2007; Curatolo and Bogduk, 2011; Krause, Scherzer & Rugulies, 2005; Lashuay, Burgel, Harrion, Israel, et al, 2002; Martin, Levenson, Hollingworth, Kliot, et al, 2005). The first line of treatment of upper extremity limb pain by practioners usually entails a prescription of NSAIDS (Clarke, 2001; Lashuay et al, 2002). Krause et al (2005) found in a survey study that 84% of 941 workers used NSAIDS, including ibuprofen, for pain. Forty percent of 2213 French workers reported the regular use of ibuprofen in a one month period (Bouef-Cazou et al, 2009). Back and shoulder injuries and other musculoskeletal strains are largely self-treated by migrant farm workers with rest and over-the-counter drugs, such as ibuprofen (Anthony et al, 2010). However, as best stated by Curatolo and Bogduk (2011), "the results [for commonly used drugs} are sobering, if not disconcerting". Our results match those of several clinical trials, that NSAIDS have short-term effectiveness, but not long-term, for chronic musculoskeletal conditions with pain.

Behaviors that decline due to maladaptive central nervous system neuroplasticity

We postulate that the increased IL-1beta immunoreactivity in ventral horn neurons observed here contribute to the persistent grip strength declines. These observations match a recent report from our lab in which we observed that two pro-inflammatory cytokines, TNF-alpha and IL-1beta, increase in neurons in the dorsal horns of cervical spinal cord segments of aged rats performing a HRLF task (Elliott et al., 2010). These increases correlated positively with enhanced nocifensive behaviors, including declines in grip strength (Elliott et al., 2009b). We have also reported that Substance P and its receptor, neurokinin 1, increase in cervical spinal cord segments, in conjunction with increased peripheral inflammation, in rats performing repetitive tasks (Elliott et al., 2008; Elliott et al., 2009a; Elliott et al., 2009b; Elliott et al., 2010). Again, these increases correlated with declines in grip strength. Increased neurochemicals and inflammatory cytokines in spinal cord neurons are characteristics of central sensitization, an enhancement of neuronal sensitivity and neuropathic pain (Woolf and Salter, 2000). Thus, pain or discomfort as a result of central sensitization is likely contributing to declines in grip strength.

Several studies also indicate that maladaptive neuroplasticity in the somatosensory cortex contributes to motor declines (Ballermann, McKenna & Whishaw, 2001; Byl & Melnick, 1997; Byl et al., 1997; Byl et al., 1996; Coq et al., 2009). Disruption of the forepaw map and the emergence of large receptive fields in the somatosensory cortex resulted in involuntary movements characteristic of focal hand dystonia in a monkey model of repetitive prehensile tasks (Byl et al., 1997; Byl et al., 1996). In our model, performance of a repetitive negligible force reaching task for 8 weeks also resulted in disruption of the forepaw map in the somatosensory cortex, as well as the emergence of large receptive fields encompassing several forepaw subdivisions, such as digits-pads and forepaw-forearm (Coq et al, 2009). The larger receptive fields correlated negatively with percent successful reaches. Thus, somatosensory cortical changes likely result in ambiguous interpretation of tactile cues and contribute to declines in grasp control.

Changes in the motor cortex were also detected in the Coq study mentioned above (Coq et al., 2009). These changes included enlarged motor forepaw maps and increased representation of digit and multi-joint movements. Some of these changes seemed more adaptive than deleterious, and likely contribute to our observed skill acquisition. This is supported by studies showing that motor skill learning is associated with expansion of the representation of distal forelimb movements in the motor cortex (Kleim, Barbay & Nudo, 1998; Plautz, Milliken & Nudo, 2000). However, we also observed increased pathological multi-joint movements when stimulating motor cortex neurons in our past study (Coq et al, 2009), and that the amount of current needed to evoke these multi-joint movements was lower than in controls. These changes correlated positively with increased inflammatory cytokines in the forearm muscles, suggestive of a peripheral tissue inflammation influence on the motor cortex. These results combined provide strong evidence that peripheral inflammation, spinal cord neuroplasticity and cortical neuroplasticity contribute to the development of motor declines as well as skill acquisition observed with chronic repetitive motion tasks.

Conclusions

Using a rat model of voluntary reaching and handle-pulling, in which rats perform repetitive tasks for 12 weeks (2 h/day in 30 min sessions, 3 days/wk) with low or high repetition requirements, combined with high or low force requirements, we observed that repetitive motion tasks have adverse, exposure-dependent effects on some indicators of motor behavior. However, these changes were likely to be superimposed upon other changes in motor behavior, such as those indicative of skill acquisition. It is clear that when greater force requirements were combined with a highly repetitive task, the adverse effects on motor performance were magnified. Treatment with ibuprofen helped to ameliorate some of the adverse effects on motor performance, providing further support that inflammation is a critical component of the pathophysiology of repetitive motion injury. However, anti-inflammatory medication was not a complete solution to a complex multi-system problem. Although the findings of animal models of WMSDs cannot be directly generalized to human workers, the findings of such studies provide strong evidence of a complex pathophysiological process that involves multiple systems and tissues and leads to motor decline. Because the pathophysiology of repetitive motion injury is complex, the most effective therapeutic approach needs to be multifaceted. However, it is quite clear that earlier or preventive interventions are needed to avoid persistent motor dysfunction.
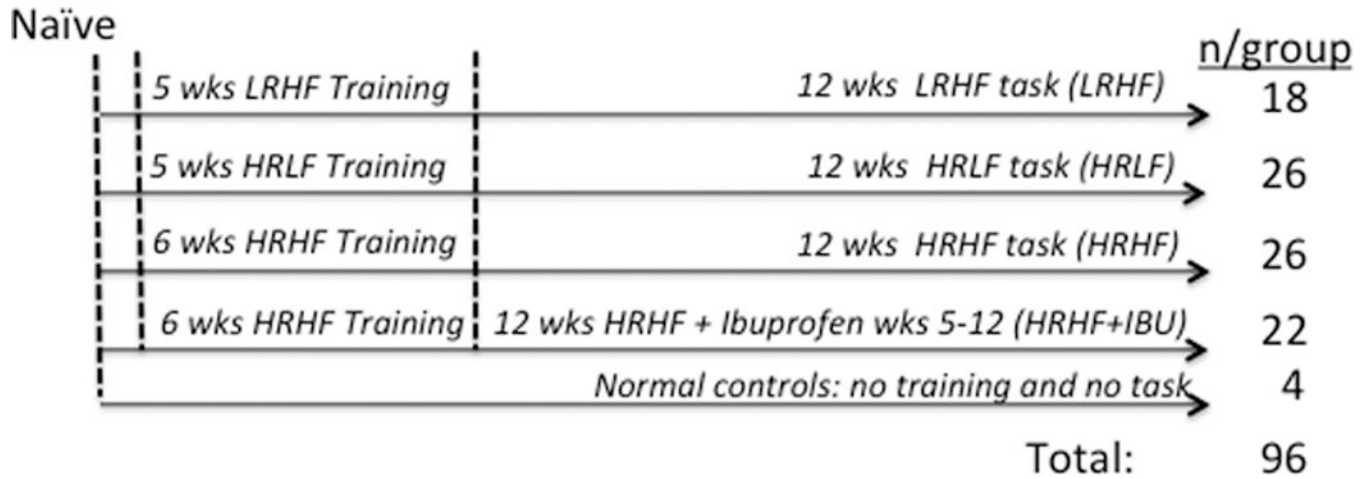
Acknowledgments

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Naïve

		n/group
5 wks LRHF Training	12 wks LRHF task (LRHF)	18
5 wks HRLF Training	12 wks HRLF task (HRLF)	26
6 wks HRHF Training	12 wks HRHF task (HRHF)	26
6 wks HRHF Training	12 wks HRHF + Ibuprofen wks 5-12 (HRHF+IBU)	22
	Normal controls: no training and no task	4
	Total:	96

Figure 1.
Experimental design and number of animals per group. Ninety-two rats were trained for 10–15 min/day for 5–6 weeks to learn one of four tasks, and then performed this task for 2 hr/day, 3 days/week for 12 weeks. LRHF = low repetition with high force task group. HRLF = High repetition with low force task group. HRHF = high repetition with high force task group. HRHF+IBU = HRHF rats that received ibuprofen treatment in last 8 weeks of task performance. Four normal control rats were included that were not exposed to the training or to the task. The training period that preceded the task period is indicated by dashed lines.
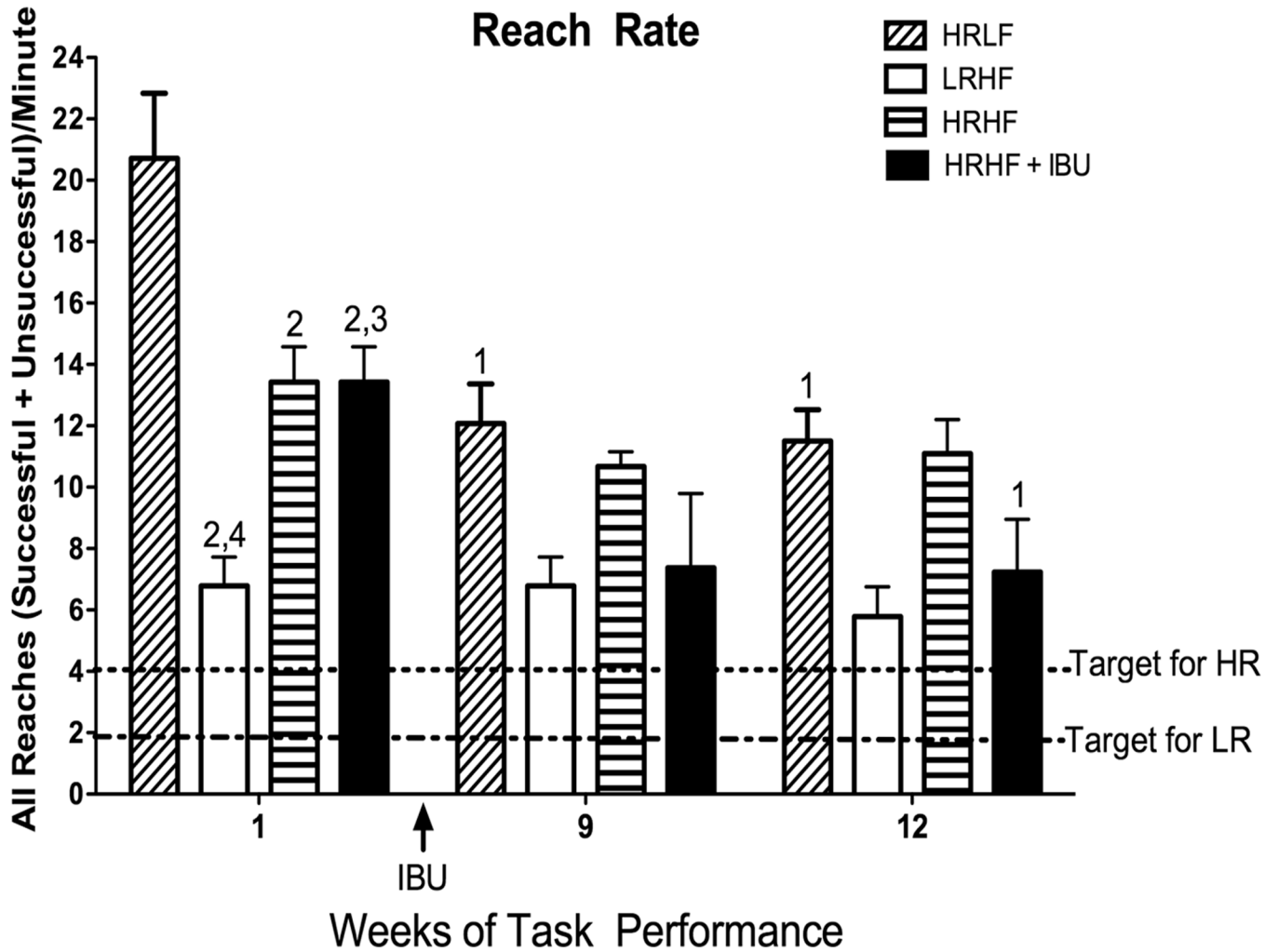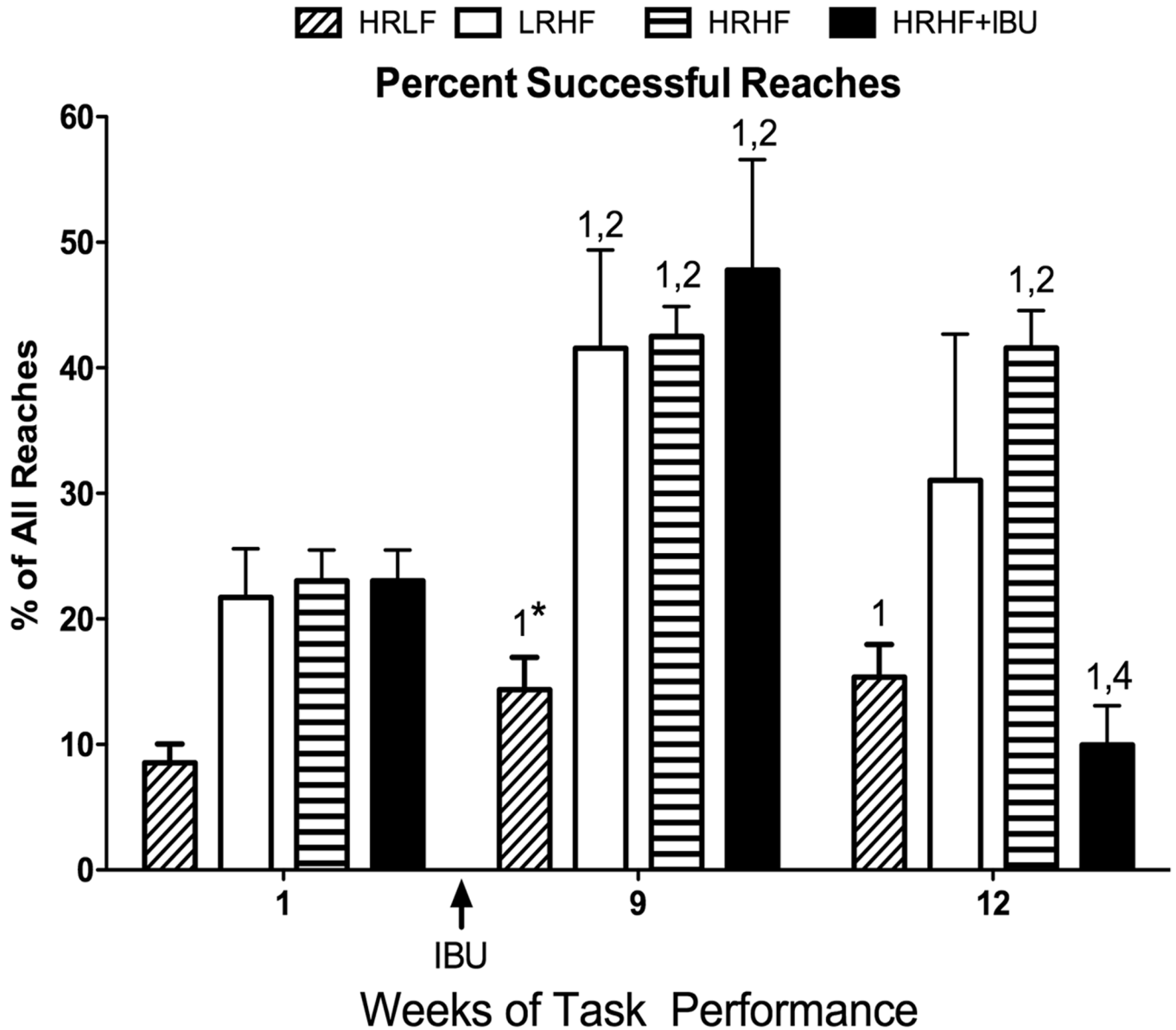
Figure 2.
Reach Rate (number of all successful and unsuccessful reaches/minute). Across weeks of task performance, reach rate declined towards the target of 4 reaches/min in the HRLF and HRHF+IBU groups, but not in the LRHF and HRHF groups. Group differences were also detected: In week 1, HRHF and HRHF+IBU groups had lower reach rates than HRLF, and the LRHF group (the group with the lowest target reach rate), had lowest rate. Dashed lines show target reach rates for high repetition (HR, 4 reaches/min) and low repetition (LR, 2 reaches/min), as indicated. Arrow indicates onset of ibuprofen treatment (IBU) at end of week 4. 1 = $p<0.01$ compared to week 1 of same group; 2 = $p<0.01$ compared to same week HRLF; 3 = $p<0.01$ compared to same week LRHF, 4 = $p<0.01$ compared to same week HRHF.
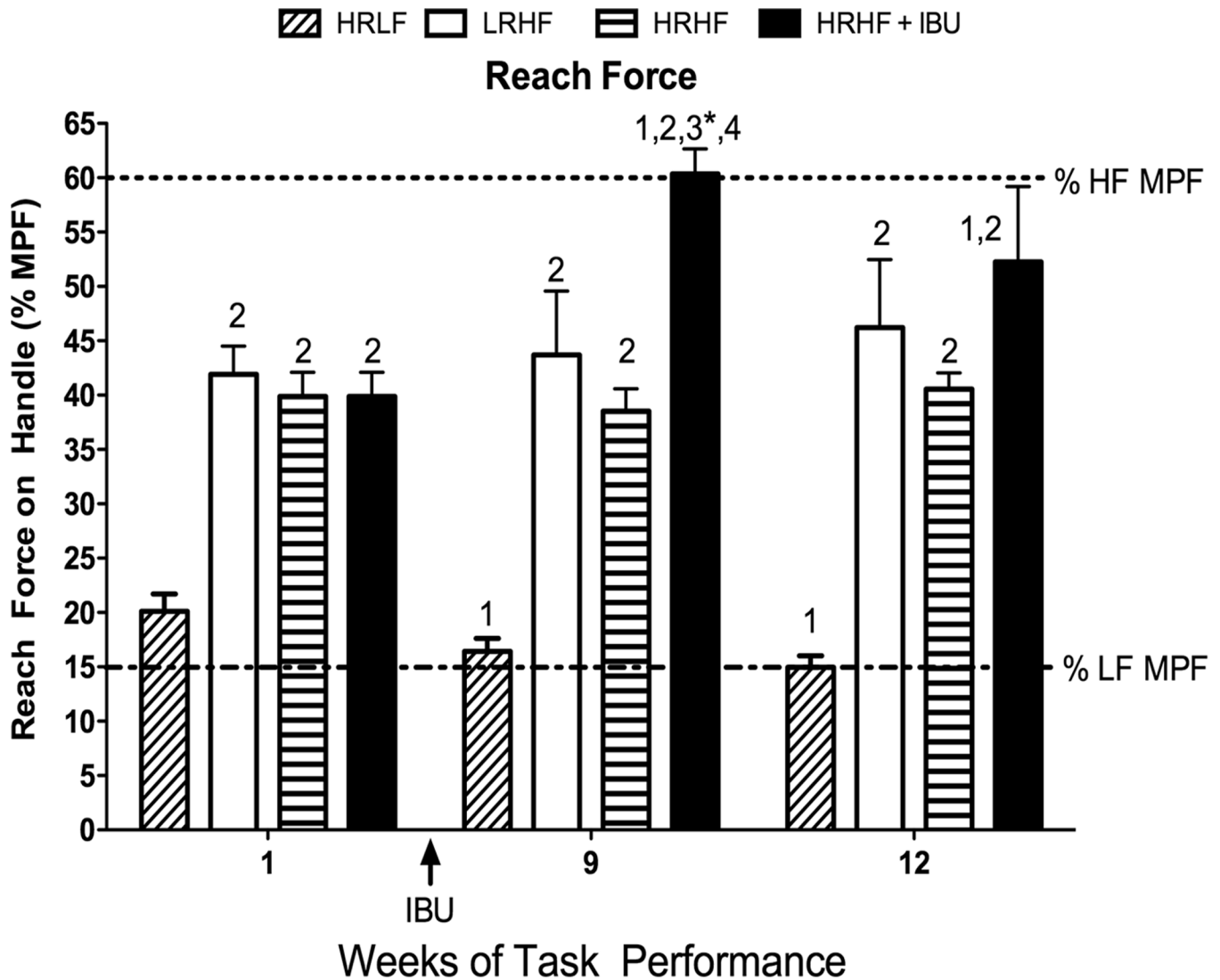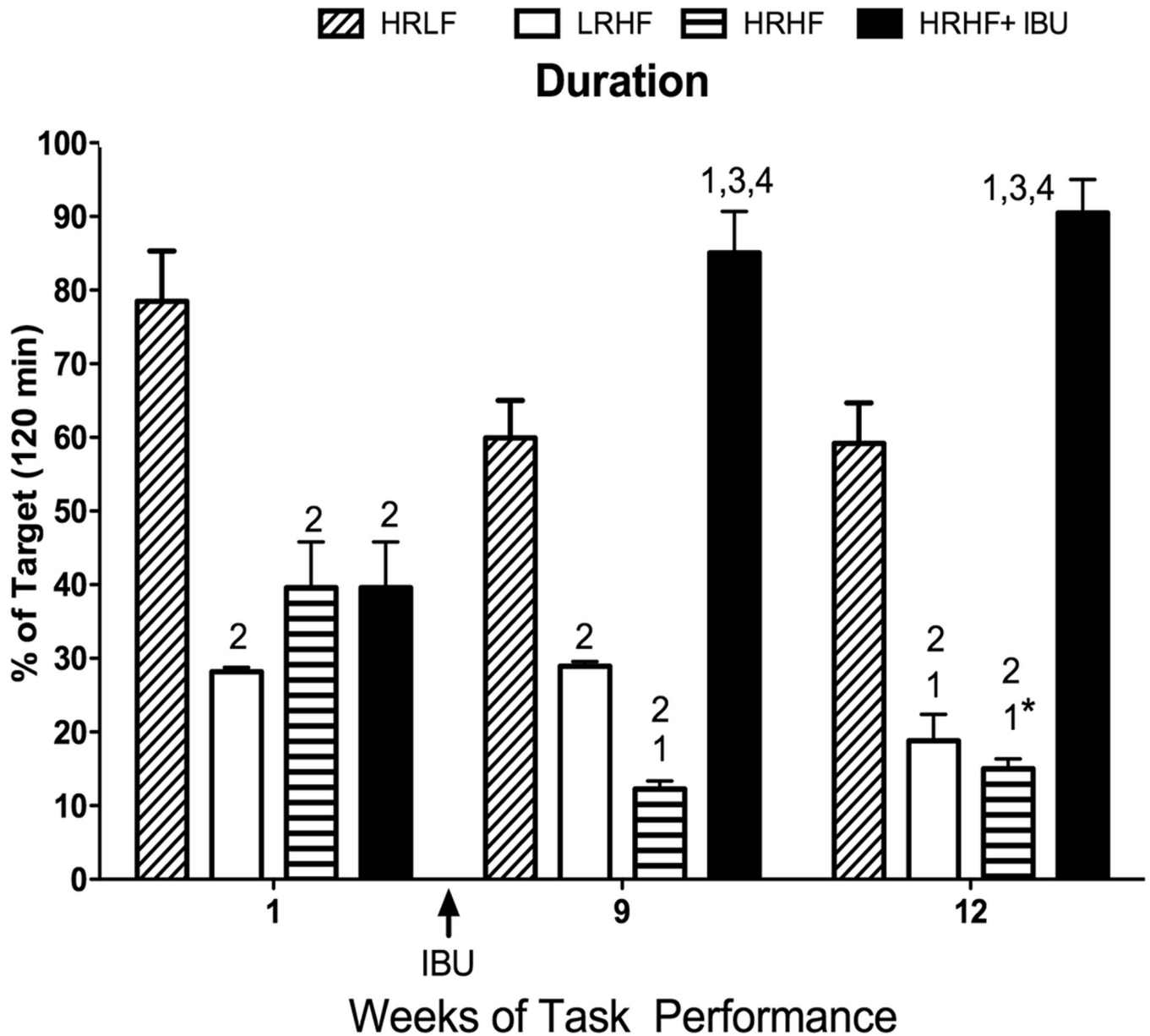
Figure 3.
Reach Force on handle (percent of maximum pulling force, MPF). Across weeks of task performance, reach force declined in HRLF towards their target of 15% and met that target in week 12. Likewise, reach force increased in HRHF+IBU rats to their target of 60% in week 9, although it dropped to 52% in week 12, which was still significantly higher than in week 1. The reach force did not change across weeks in the LRHF and HRHF groups, and remained lower than the target. Group differences were also detected: HRHF+IBU had the highest reach force in week 9. Reach force was appropriately higher in the high force groups than the low force group. Dashed lines show target reach forces for low force (LF; 15%) and high force (HF; 60%), as indicated. Arrow indicates onset of ibuprofen treatment (IBU) at end of week 4. 1 = $p<0.01$ compared to week 1 of same group; 2 = $p<0.01$ compared to same week HRLF; 3* = $p<0.05$ compared to same week LRHF; 4 = $p<0.01$ compared to same week HRHF.
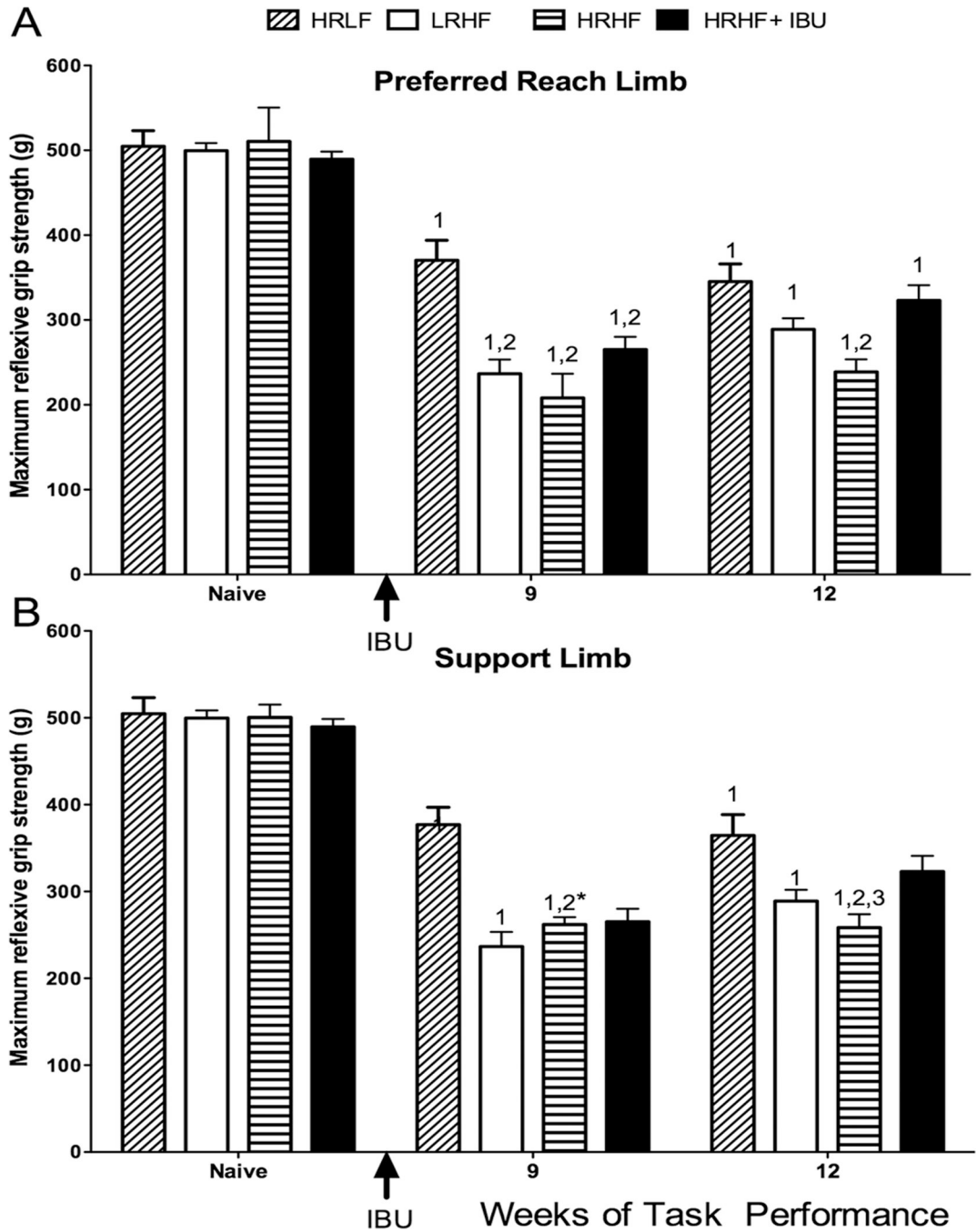
Figure 4.
Percent Successful Reaches (of all reaches). Percent successful reaches increased in all groups in week 9, and in HRLF and HRHF in week 12, compared to week 1. In contrast, HRHF+IBU rats had decreased success in week 12. Group differences were also detected: percent success was greater in all high force groups than the HRLF in week 9, but lower in HRHF+IBU rats than HRHF in week 12. Arrow indicates onset of ibuprofen treatment (IBU) at end of week 4. 1 and 1* = p<0.01 and p<0.05, respectively, compared to week 1 of same group; 2 = p<0.01 compared to same week HRLF; 4 = p<0.01 compared to same week HRHF.
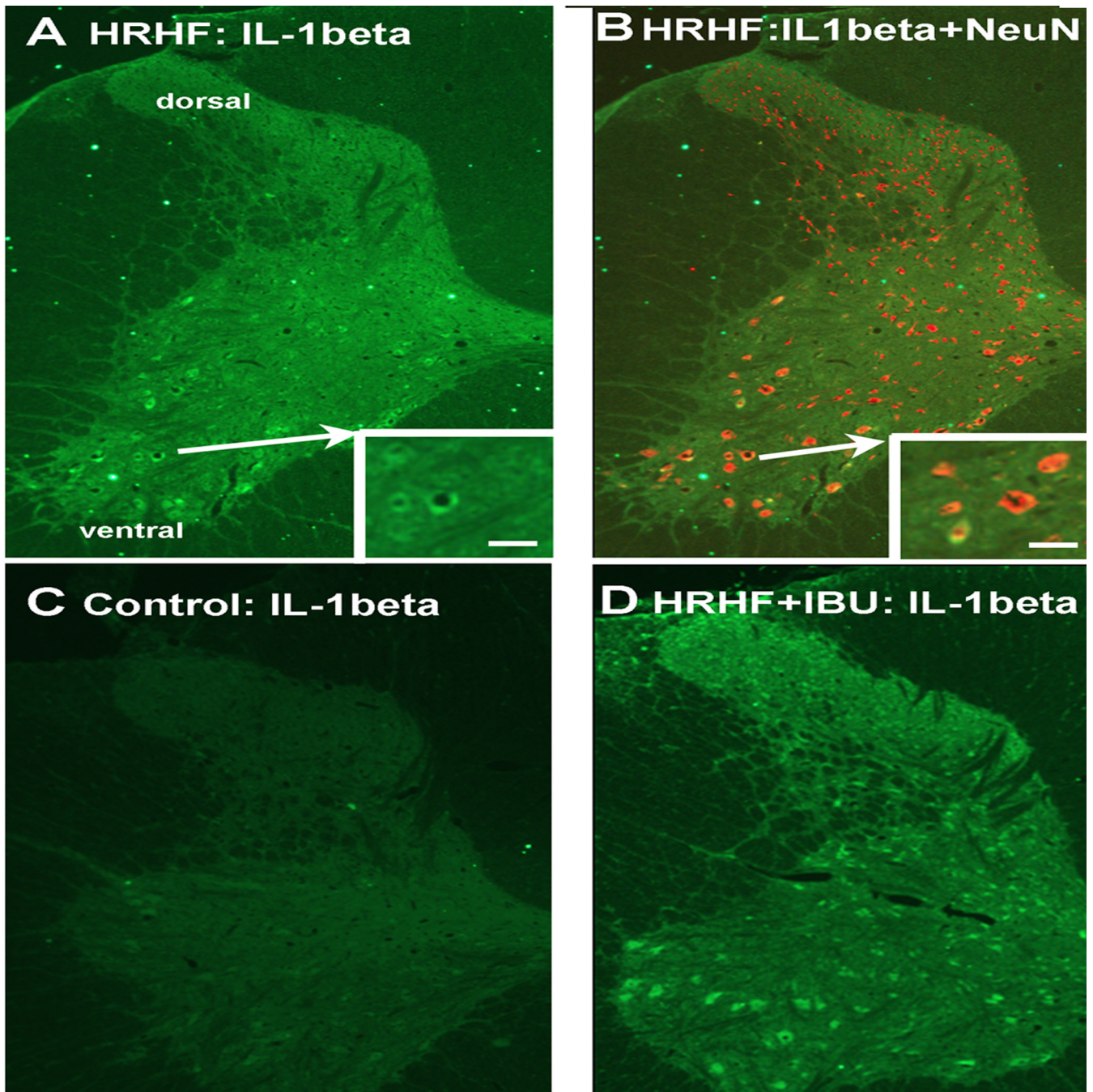
Figure 5.
Duration of task participation (as a percent of the target of 120 min/day). Across the weeks, duration decreased in the HRHF and LRHF groups. In contrast, duration dramatically increased in HRHF+IBU rats in weeks 9 and 12, compared to their week 1. Group differences were also detected: Duration was lower in all high force groups than the HRLF group in week 1, and lower in the LRHF and HRHF groups than the HRLF group in weeks 9 and 12. In contrast, duration was dramatically increased in HRHF+IBU rats in weeks 9 and 12, compared to the other high force groups. Dashed line indicates target duration of 120 minutes (100%). Arrow indicates onset of ibuprofen treatment (IBU) at end of week 4. 1 and 1* = p<0.01 and p<0.05, respectively, compared to week 1 of same group; 2 = p<0.01 compared to same week HRLF; 3 = p<0.01 compared to same week LRHF; 4 = p<0.01 compared to same week HRHF.

Figure 6.
Grip strength (maximum reflexive grip strength in grams (g)). (A) Preferred reach limb. In weeks 9 and 12, grip strength was decreased in this limb in all groups, compared to baseline naïve levels. Grip strength was also lower in week 9 in all high force groups, and in the HRHF group in week 12, than in the HRLF group. (B) Contralateral support limb. In weeks 9 and 12, grip strength was decreased in this limb in all groups, compared to baseline naïve levels. Grip strength was also lower in the HRHF group in weeks 9 and 12 than in the HRLF group, and lower than in HRHF+IBU in week 12. Arrow indicates onset of ibuprofen treatment (IBU) at end of week 4. 1 = p<0.01 compared to week 1 of same group; 2 and 2*

= p<0.01 and p<0.05, respectively, compared to same week HRLF; 3 = p<0.01 compared to same week LRHF; 4 = p<0.01 compared to same week HRHF.

Figure 7.
Inflammatory cytokine immunohistochemical expression in spinal cords. (A) Representative photograph of a cervical spinal cord in cross section showing IL-1beta staining (green staining) in the intermediate and ventral horns of HRHF rats. Inset shows higher power stained cells indicated. (B) Same section as shown in panel A showing that IL-1beta stained cells (green staining) are double-labeled for NeuN, a neuronal cell body marker (red staining). (C) A cervical spinal cord segment from a control rat showing a near absence of staining for IL-1beta. (D) A cervical spinal cord segment from a HRHF+IBU rat showing the presence of IL-1beta stained cells. Scale bar = 50 micrometers. Similar staining was observed in 4 animals/group.