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Title
Aberrant neuronal activity in a model of work-related upper limb pain and dysfunction

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Abstract

Work-related musculoskeletal disorders associated with intense repetitive tasks are highly prevalent. Painful symptoms associated with such disorders can be attributed to neuropathy. In this study, we characterized the neuronal discharge from the median nerve in rats trained to perform an operant repetitive task. After 3-weeks of the task, rats developed pain behaviors and a decline in grip strength. Ongoing activity developed in 17.7% of slowly conducting neurons at 3-weeks, similar to neuritis. At 12-weeks, an irregular high frequency neuronal discharge was prevalent in >88.4% of slow and fast conducting neurons. At this time point, 8.3% of slow and 21.2% of fast conducting neurons developed a bursting discharge, which, combined with a reduction in fast-conducting neurons with receptive fields (38.4%), is consistent with marked neuropathology. Taken together, we have shown that an operant repetitive task leads to an active and progressive neuropathy that is characterized by marked neuropathology following 12-weeks task that mainly affects fast conducting neurons. Such aberrant neuronal activity may underlie painful symptoms in patients with work-related musculoskeletal disorders.

Perspective

Aberrant neuronal activity, similar that reported in this study, may contribute to upper limb pain and dysfunction in patients with work-related musculoskeletal disorders. In addition, profiles of instantaneous frequencies may provide an effective way of stratifying those patients with a painful neuropathy.

Key words

Work-related musculoskeletal disorders, repetitive motion disorder, ongoing activity, inflammation, degeneration, demyelination
Introduction

Work related musculoskeletal disorders (WRMDs), also referred to as repetitive motion and overuse disorders, remain a substantial healthcare challenge. Such disorders are seen in assembly line workers, healthcare professionals, musicians, and office workers, and are the result of repeated trauma to soft tissues. Pathologies often arise where structures such as nerves and tendons pass through fibro-osseous tunnels (for example, the carpal tunnel); anatomical constraints can lead to deformation and irritation of these structures during repetitive limb movements. It is perhaps not surprising that upper limb WRMDs arise insidiously, and consistent with carpal tunnel syndrome (i.e. median nerve injury) being the most common diagnostic category, are often diagnosed as neuropathy.

In 2003, Barbe and Barr first published their novel operant model of WRMDs in rats. The unique feature of this model is that rats are trained to reach and pull a lever for a food reward, a task that was designed to emulate key features of repetitive tasks performed by workers at risk of developing WRMDs. When the task is performed over weeks, the rats develop progressive behavioral and performance declines, but also cutaneous hypersensitivities, which are coupled with widespread inflammation, tissue damage, and neurochemical changes within the dorsal horn and other parts of the central nervous system. Many of these pathological changes resemble those reported and observed in humans with WRMDs. A primary mechanism for the panoply of pathologies seen in this group of disorders seems to be the development of post-inflammatory fibrosis, with a consistent and perhaps key finding being diffuse nerve inflammation (also termed neuritis). Experimental neuritis causes profound changes in affected axons, which includes the development of ongoing activity, and ectopic mechanical and chemical sensitivities in nociceptor axons. Such aberrant activity may provide the neural substrate for the spontaneous and radiating limb pain, and cutaneous hypersensitivities, that are often reported in WRMD. Although ongoing activity induced by
a volitional repetitive task has been reported from slowly conducting afferent neurons at an early outcome point, an extensive electrophysiological assessment of this neuronal activity has yet to be performed.

In this study, we sought to more fully characterize the neuronal activity associated with a task-induced neuropathy. We found clear evidence of pathological primary afferent neuronal discharge, consistent with neuropathy of the median nerve.
Methods

Animals

The Temple University and the University of New England (previous institution for GMB) Institutional Animal Care and Use Committees approved these experiments in compliance with NIH guidelines for the care and use of laboratory animals. Thirty-nine rats were used in this study. Animals were housed in separate cages with a 12-hour light:dark cycle, free access to water, and environmental enrichment in their home cages. All rats were gently handled extensively for 1 week prior to onset of experiments, and then 5 days/week thereafter. Young adult female Sprague-Dawley rats (3 months of age at onset of experiments) from Charles River (Wilmington, MA) were used. Female rats were used to allow comparison to our past studies on female rats using this same model and related treatment. Each rat was inspected weekly and again post-mortem for presence of illness to reduce potential confounders (none were observed), and as previously reported, the animals had no signs of wellbeing issues (such as, weight changes). To further reduce illness-related confounders, additional sentinel rats were examined for presence of illnesses as part of regular veterinary care (none were detected).

Rats at the University of New England were fed ad libitum. The rats at Temple University were food restricted to within 5% of their naïve weights to help motivation towards task performance. Rats randomized to the experimental group (n = 10) went through an initial training period of approximately 5 weeks, in which they were trained to perform a reaching and lever pulling task, before going on to perform a repetitive task, as described further below. Twenty rats were not trained and did not perform the operant repetitive task but served as control (food restricted) rats. All rats were weighed at least weekly throughout the experiment and their food volume adjusted accordingly. In addition to food pellet rewards, all rats received Purina rat chow daily to allow continued gain in weight over time. The control rats received daily
allotments of food pellets and rat chow at matched levels as rats that performed the repetitive task. Once the task rats were transferred to the University of New England, they were fed ad libitum.

**Behavioral task**

The behavioral apparatus was as previously described and depicted. Briefly, custom-designed force apparatuses were used (Custom Medical Research Equipment, Glendora, NJ) that were integrated into an operant behavioral training system (Med Associates, Georgia, VT with Force Lever software, version 3.5.0.0). A portal was located in the wall of the operant conditioning chamber at shoulder height (3.5 cm), so the shoulder had to be fully elevated and the elbow fully extended for the animal to reach through the portal to isometrically pull a custom-designed force handle attached to a force transducer located 1.5 cm away from the portal entrance, outside the chamber wall. An auditory indicator cued the animals to reach in the correct time frame (> 1 reaches/min and <4 reaches/min, considered a high reach rate).

Rats had to grasp the force handle and exert an isometric pull toward the chamber wall with a graded force effort that fell between a minimum force criterion (47% of maximum voluntary pulling force, determined on the last day of training using Force Lever software; 142 cN) and a maximum force criterion of 301 cN, for at least 90 ms. If these force and time criteria were met within a 5 second cueing period, an indicator light was turned on and a 45 mg purified formula food pellet (a mix of banana flavored and grain-based chocolate flavored, F0024 and F0165, respectively, Bio-Serv, Flemington, NJ) was dispensed into a trough located at floor height of the chamber in the wall panel adjacent to the aperture. To obtain the food reward, the animal had to release the handle, withdraw the forepaw from the aperture, and move to the trough to lick up the pellet.

Rats chosen randomly to perform the repetitive task underwent an initial training period for 5-6 weeks in which they learned the task, a training method described previously. All rats
were initially food-restricted for 7 days to no more than 10-15% less than their naive weight to initiate interest in food reward pellets, which was the motivation for performing the repetitive task. After that week, they were given extra rat chow to gain weight. Rats chosen to perform the repetitive task were trained to perform the reaching and handle-pulling tasks during a 5-week period of 10 min/day, 5 days/week, in which they ramped upwards from naive towards the high force task level, which they reached during their last week of training.

At the end of the training period, all trained rats began working towards the target reach rate and force requirement (4 reaches/minute target; 47% maximum voluntary pulling force on the lever bar for 90 to 500 msec in duration) for 2 hours/day, 3 days/week for 3 weeks (n = 5) or 12 weeks (n = 5). The task was divided into 4, 0.5-hour sessions separated by 1.5 hours in order to avoid satiation. A food reward was not given unless they met the force criterion within a 5 second window initiated every 15 seconds. Rats were allowed to use their preferred limb to reach, and their contralateral limb as a support limb, as needed. The side used to reach was recorded in each session.

We have previously shown that rats perform the repetitive task develop discomfort from the task and switch limbs or use both limbs simultaneously to pull on the lever bar in their attempts to garner a food reward, beginning in weeks 2 and 3. Therefore, data from the task are reported without reference to upper limb preference, as there was none from weeks 2-3 to the end of the experiment at week 12.

*Sensorimotor Behavioral Testing*

Testing procedures were conducted after 3- or 12-weeks of the repetitive task, or for controls, at the parallel time. Tests were performed at the same times per day to minimize effects related to diurnal factors, and the technicians carrying out these behavioral tests were naïve to the group assignment and expected outcome.
Reflexive grip strength was measured using a rodent grip strength meter (1027SR-D58, Grip Strength Meter with single sensor and a standard pull bar; Columbus Instruments, Columbus, Ohio). The test was repeated five times on each forepaw, and the maximum grip strength per limb was reported, as previously described.\textsuperscript{11, 16} Maximum grip strength was defined as the value of the peak force recorded from the transducer at the moment at which each animal released its grip from the handle of the grip strength meter.

Forepaw sensitivity to mechanical stimulation was determined using nylon monofilaments (4 filaments were used: sized 0.16g, 0.4g, 1.0g and 4g; Semmes-Weinstein monofilaments, Stoelting, USA) using previously described methods.\textsuperscript{16} Briefly, each filament was applied 10 times to the rat glabrous forepaw on each side, and the mean number of limb withdrawal responses out of 10 was reported for each monofilament used.

Cold sensitivity was determined using a two-choice temperature preference/aversion assay as previously described.\textsuperscript{10, 32} Briefly, the temperature testing apparatus (T2CT, Bioseb, France) consisted of two plates. One plate was a reference plate at 22°C while the test plate was adjusted from 22-12°C. Rats were timed for how long they preferred to stand on the reference plate, as opposed to the second plate of decreasing temperature, in 2°C steps (3 min per step).

**Electrophysiology**

The 3-week and 12-week task animals were sent to the University of New England for electrophysiological studies as separate groups, separated by one year. Animals that were shipped were acclimated as per institutional guidelines and used within ten days of arrival to minimize possible recovery due to time. Nine naïve control rats were studied using the same methods as described and depicted previously,\textsuperscript{48} and performed in batches prior to the task animals. Rats were anesthetized using isoflurane in pure oxygen and maintained in an areflexic state for the duration of the experiment. The rat was placed supine on a feedback-controlled heating pad
(FHC-Inc, USA), and a water circulating heating pad was wrapped around the torso (Gaymar, USA), to maintain a core temperature of 37°C.

The fur on the axilla and posterior forearm was shaved. The abducted arm was then glued to a small metal platform for stability while also allowing full movement of the wrist. After incising the skin on the medial arm, the median nerve was carefully dissected free of all other structures from the cubital fossa to its intersection with the ulnar nerve near the axilla (Fig 2A). The skin was glued to a metal ring using cyanoacrylate, forming a pool for recording, and the pool was filled with warmed light mineral oil. The median nerve was cut as proximally as possible and draped over a bipolar tungsten stimulating electrode positioned in the cubital fossa, also immersed in the oil. The proximal part of the nerve was placed on a small glass plate used for recording. The epi-perineurium was removed from the proximal 1 mm of the nerve. Fine filaments (8-12 μm) were teased from the nerve and draped over fine gold bipolar electrodes. The distance between the stimulating and recording electrodes was measured using dividers (typically 11-13 mm); this length is sufficient for the identification of C- and Aδ- axons using electric stimulation. The nerve was electrically stimulated near the cubital tunnel using an isolated constant voltage stimulator (Grass, USA), at intensities appropriate to elicit action potentials from these types of axons (up to 0.2ms and 40V). Only neurons with clearly identifiable waveforms, based on shape, amplitude, and duration, were examined; usually one per filament. Action potentials were amplified, band-pass filtered (10–5,000 Hz), and monitored with an oscilloscope. Neuronal activity was digitized, monitored, and recorded with Spike 2 software (Cambridge Electronic Designs, Cambridge, United Kingdom).

Neurons were classified as having either C- or Aδ- fiber axons by their conduction velocity, which was determined by dividing the conduction distance by the response latency of individual neurons. Due to the limited length of available nerve (10-12 mm), it was not possible to calculate the conduction velocities of Aαβ- fiber axons with conduction velocities faster than
10 m/s. However, these nerve fibers could usually be evaluated using their responses to innocuous mechanical stimulation and by the very different characteristics of their action potentials (shorter time base and much greater signal to noise ratio).

Mechanical responsiveness was evaluated using only innocuous stimulation, to avoid any possibility of sensitization of the receptive fields of the neurons under study as well as subsequent neurons. This included gentle flexion and extension of the wrist, and light touching of the hand and forearm with fingers and blunt probes. The presence or absence of a mechanical response was recorded. Although neurons that innervate muscle spindles were identified, they were excluded from the analysis. Twelve or fewer slowly conducting neurons were recorded per arm.

It was not possible to record from neurons with conduction speeds faster than could be detected, no ongoing activity, and no mechanically sensitive receptive field. Because rats show symptoms on both arms regardless of their preferred reaching limb, neurons innervating both arms were recorded in each experiment.

After identification, the neuron was recorded for up to three minutes for ongoing (spontaneous) activity; the rate of faster and regular discharge was capturable in shorter epochs. A secondary analysis of high-rate erratic and bursting discharge was performed as described below.

**Secondary analysis of high-rate erratic and bursting discharge**

Instantaneous frequencies (IF) were determined using Spike 2 software. Normalized (percent) histograms of the IF were plotted (in 5Hz bins) for each unit, and a moving average (over 15 Hz) was overlaid on each graph. Histograms of neurons with a bursting discharge typically had a bimodal or polymodal distribution, whereas neurons without this bursting pattern of activity had a unimodal distribution (Fig. 4a-c). From the moving average, the IF at each peak was determined. For neurons with a bursting discharge, the reciprocal of the slowest IF peak value corresponded to the interval between bursts; the reciprocal of the faster IF peak value
corresponded to the interspike interval during the bursting discharge. From these parameters, the
duration of the burst was determined using the following formula:

\[ \text{Duration of burst (ms)} = \frac{m \cdot n}{(1000 - m \cdot n)/p} \]

where \( m \) = interspike interval during the bursting discharge (ms), \( n \) = mean ongoing activity rate (Hz) and \( p \) = interval between bursts (ms).

**Statistical Analyses**

GraphPad PRISM version 8.4.3 was used for the statistical analyses and figure design. Exact \( p \) values are reported for all data with a minimum of 0.05 being considered statistically significant. The average number of reaches per minute per week for each animal were compared using paired t-tests. Grip strengths were compared using a Mann-Whitney t-test. Forepaw mechanical sensitivity and temperature sensitivity were compared using a two-way analysis of variance (ANOVA). The teased nerve electrophysiological data are presented qualitatively and quantitatively; ongoing activity rates were compared using Chi-square tests, and conduction velocities were compared using Kruskal-Wallis tests followed by Dunn's tests (for three groups) or Mann-Whitney tests (for two groups). A linear regression analysis was performed between discharge characteristics.
Results

Behavioral signs of neuropathy

From 3-weeks after commencing the operant repetitive task, rats showed signs of a neuropathy (n = 5 at 3- and 12-weeks; n =10 control animals; Fig. 1A-H). The number of reaches per minute declined significantly at 3-weeks (p <0.05 [3-weeks], p>0.01 [12-week], Paired t-test; Fig. 1A-B). Reflexive grip strength also declined at 12-weeks in both forelimbs compared to the control animals (p = 0.27 [3-weeks], p<0.0001 [12-weeks], Mann-Whitney test; Fig. 1C-D). These rats also developed hypersensitivity to mechanical stimuli applied to their forepaws from 3-weeks ($F(1, 112) = 34.79, p<0.0001$ [3-weeks], $F(1, 112) = 25.47, p<0.0001$ [12-weeks], main effect for group; two-way ANOVA; Fig. 1E-F), as well as hypersensitivity to cold temperatures ($F(1, 78) = 5.39, p<0.05$ [3-weeks], $F(1, 78) = 11.40, p<0.01$ [12-weeks], main effect for group; two-way ANOVA; Fig. 1G-H).

Median Nerve Electrophysiology

Slow conducting neurons

Recordings were made from 95 slowly conducting neurons in control rats (18 forelimbs; 9 animals), 79 neurons in rats that performed the repetitive task for 3-weeks (7 forelimbs; 5 animals), and 40 neurons in rats that performed the task for 12 weeks (10 forelimbs; 5 animals; data from the 3-week task rats were reported previously$^{16}$). The conduction velocities were higher for the recordings from the 3-week (median 2.17 ms [IQR 2.71]) and 12-week groups (2.10 ms [IQR 1.54]) compared to those from controls (median 1.30 ms [IQR 1.60]; p < 0.001; Kruskal-Wallis test; Fig. 2B).

Ongoing activity was only observed in 3% (3/95) of neurons from control rats (Fig. 2C). In contrast, ongoing activity was more prevalent following 3-weeks of the repetitive task (17.7%; 14/79) and highly prevalent in the 12-week group (90%; 36/40 neurons; p <0.0001, Chi Square
The ongoing activity rates were significantly higher in recordings from the 12-week group (median 21.8 Hz [IQR 39.8]) than from the 3-week task-performing group (1.3 Hz [IQR 3.8]; p < 0.0001; Kruskal-Wallis test; Fig. 2D). Sample recordings of ongoing activity from control, 3-week and 12-week groups are provided in Figure 2E-I. The pattern of firing was irregular for most neurons, although three of the neurons (8.3%) from the 12-week group exhibited bursting discharge (Fig. 2I-J), not observed in neurons from 3-week or control rats that exhibited ongoing activity. The conduction velocities of the three neurons with a bursting discharge were 2.12, 2.50, and 3.24 m/s. The rate of ongoing activity was significantly higher in A-fiber neurons (n = 36; median 21.75 Hz [IQR 40.79]) compared to C-fiber neurons (i.e. neurons with conduction velocities ≤ 1.3m/s; n = 14; median 0.94 [IQR 7.6]) in the 3- and 12-week task rats (p < 0.001, Mann-Whitney test); segregation based on previously reported classification58).

The proportion of slowly conducting neurons with receptive fields that responded to innocuous mechanical stimuli were comparable between control (16.8% [16/95]), 3-week (19.0% [15/79]) and 12-week (15% [6/40]) task-performing rats (p = 0.85, Chi Square Test; Fig. 2K). Two additional slowly conducting neurons in the control group were identified with deep receptive fields that responded to joint movement. The proportion of neurons with characterized receptive fields that had ongoing activity was 6.3% (1/16), 20% (3/15) and 83.3% (5/6) in control, 3-week and 12-week groups respectively. None of the neurons that exhibited a bursting discharge had receptive fields that responded to innocuous mechanical stimuli.

Fast conducting neurons

Single fiber recordings were obtained from 72 neurons in control rats and 32 and 112 neurons from rats that had performed the repetitive task for 3 and 12 weeks respectively (data were collected during the same experiments as the slow fiber recordings).
Ongoing activity was more prevalent in the recordings from the 12-week group (88.4%; 99/112) than from the 3-week group (0%; [0/32]) and control rats (15.3%; 11/72; p < 0.0001, Chi Square Test Fig. 3A). The ongoing activity rates were higher in the task-performing rats (median 26.8 Hz [IQR 38.1]) than in the control rats (median 11.3 Hz [IQR 32.8], p < 0.05, Mann Whitney Test; Fig. 3B). Figure 3C is a representative recording from a control neuron, and Figures 3D-G are representative recordings from three neurons from the 12-week group. The pattern of firing was irregular for most neurons. In addition, twenty-one neurons (21.2%) from the 12-week group exhibited bursting discharge, not observed in neurons from control rats that exhibited ongoing activity (Fig. 3F-G).

The number of fast conducting neurons recorded with receptive fields was significantly higher in control rats (87.5% [63/72]) and 3-week rats (100%; [32/32]) compared to that rats that performed the repetitive task for 12 weeks (38.4% [43/112], p < 0.0001, Chi Square Test; Fig. 3H). The proportion of neurons with receptive fields that had ongoing activity was 9.5% (6/63), in control and 74.4% (32/43) in the 12-week group (p < 0.0001, Fisher’s Exact Test). Only one of the neurons that exhibited a bursting discharge had a receptive field that could be detected.

**Secondary analysis of high-rate erratic and bursting discharge**

We performed a secondary analysis of those neurons with a bursting pattern of ongoing activity. Example traces of bursting activity and normalized histograms of the instantaneous frequency (IF) are shown in Figure 4A-C. Discharge characteristics were determined from histogram profiles of the instantaneous frequencies (refer to Figure 4). For most units, the duration of each burst was short (median = 13.6 ms (11.3 IQR); Fig. 4D), although a small number of fast conducting units had a prolonged activity period (e.g. Fig. 4B). A significant feature was the high rate of activity during the bursting discharge for all units (mean = 290 Hz; 83.6 SD; Fig. 4E). The median number of action potentials within each burst was 3.24 (2.27 IQR;
Fig. 4F), and the median interval between bursts was 28.7 ms (34.2 IQR; Fig. 4G). As the data show, discharge patterns are comparable between fast and slow conducting neurons. A regression analysis of the combined data for fast and slow conducting neurons is summarized in Table 1. There were significant positive correlations between the duration of the burst, number of action potentials in the burst and the interval between bursts.

**Discussion**

In this study, rats that performed an operant repetitive task that emulates work performed by humans developed marked pathological afferent discharge from neurons of the median nerve, which significantly progressed from 3- to 12-weeks. Such pathological discharge, together with widespread neuroinflammatory changes reported in this model, is consistent with a neuropathy. The pain behaviors observed in these animals have been previously reported and are consistent with the mechanical and cold allodynia often described by humans with peripheral neuropathies and WRMDs.

**Slow fibers**

Although only neurons that responded to innocuous mechanical stimulation were characterized, it is likely that many of the slow conducting neurons were nociceptors. In response to inflammation, the normally quiet nociceptors present erratic discharge to the CNS, similar to the pattern of activity reported here. In the current results, the discharge patterns of the slowly conducting neurons at 3 weeks are consistent with an active neuritis causing ectopic ongoing activity in intact nociceptor axons (this phenomenon occurs in the absence of axonal injury / axonotmesis). However, at 12 weeks, the increased rate of ongoing activity, and bursting discharge in a small proportion, are more consistent with demyelination or degeneration (see below). Based on the neuritis model, and evidence of neuroinflammation within the median
nerve in this model, axons could generate ectopic activity in response to the repetitive task along much of their length. This is also supported by previous studies where a proximal nerve injury, which induces Wallerian degeneration, leads to ongoing activity and mechanical sensitivity in intact nociceptors.

The increased and erratic discharge recorded from the slowly conducting neurons (i.e. neurons with C- and Aδ-fiber velocities), represents a substantial increase in nociceptive input into the spinal cord. Such activity, if present in humans with work-related musculoskeletal disorders, may underlie spontaneous pain or drive spinal mechanisms that lead to central sensitization. Evidence of central sensitization was reported following localized neuritis, and is consistent with the development of mechanical and cold cutaneous hypersensitivities in our animals.

**Fast fibers**

We have previously shown that neuritis without axonal damage does not evoke ongoing activity in faster fibers, nor does it cause bursting discharge. As such, the large number of fast conducting neurons with high frequency bursting discharge following 12-weeks of the repetitive task indicates a more marked neuropathology. Since the faster conducting neurons represented many sensory modalities and probably included motor neurons, the possible effect of their ectopic discharge is complicated to predict. The erratic discharge of neurons with mechanically responsive receptive fields seems consistent with the sensation of "pins and needles" so often reported by patients with work-related disorders. It is likely that our sampling included axons that transmit information about position. High frequency and erratic ectopic discharge in position-encoding neurons would be expected to give rise to poor judgements of arm position and reduced coordination. This would be consistent with the alteration of the fine motor
skills that are required for obtaining the food rewards that are reported in this model, which changes to include less efficient strategies.\textsuperscript{24}

\textit{Demyelination and degeneration}

A similar pattern of high frequency bursting discharge was reported in a model of chemically-induced demyelination.\textsuperscript{20} Since myelin disruption is a feature of our model,\textsuperscript{10, 16} the fast conducting neurons with such activity may be myelinated axons that are undergoing demyelination. Furthermore, the increase in the mean conduction velocity of the slowly conducting axons at 3- and 12-weeks could also reflect demyelination. Although this seems incongruous, the increase in conduction velocity is likely to reflect the presence of demyelinated nerve fibers with conduction velocities that are slowed to enough to be included in our recording window. Furthermore, we encountered two neurons with slow conduction velocities that responded like muscle spindles, and were likely demyelinated.

A similar pattern of bursting discharge was reported in models of acute neuromata.\textsuperscript{37, 56} In such models, the number of neurons displaying high frequency bursting discharge decreases sharply after 2 weeks.\textsuperscript{37} This suggests that if axons are damaged due to the repetitive task, they are being damaged as an ongoing process. In our data, the reduction in the proportion of fast conducting neurons with identifiable receptive fields at 12-weeks, and an absence of identifiable receptive fields in neurons with bursting discharge, is consistent with axonal damage. Further evidence for axonal damage is the report of axonal swelling, which is considered to precede axonal degeneration,\textsuperscript{44} in axons within the median nerve following 12-weeks of the repetitive task.\textsuperscript{31}

Taken together, our findings suggest that 12-weeks of repetitive task performance leads to a neuropathy that is associated with significant damage to fast conducting axons, whereas the slow conducting axons are relatively spared, despite abnormal firing patterns. We have previously
reported an increase in the expression of activating transcription factor-3, a marker of neuronal stress and injury, in neuronal nuclei within the cervical ventral horn but not the cervical dorsal root ganglia.\textsuperscript{10} suggesting that fast-conducting motor neurons may be differentially targeted. The observed reduction in grip strength is consistent with a partial loss of motor axons. The relative sparing of slowly conducting axons is also emulated by the similarities in the proportion of sensory neurons that responded to innocuous mechanical stimulation in each group, i.e. this population was intact after 12-weeks.

\textit{Discharge characteristics}

In this study we used histogram profiles of the instantaneous frequencies to identify and quantify the discharge characteristics of the bursting activity. Whereas non-bursting irregular firing neurons had a unimodal distribution, neurons with a bursting pattern of activity had a bimodal or polymodal distribution. A notable finding from this analysis was the high rate of activity during the burst. Similar rates were not observed from those neurons that had a continuous irregular pattern of firing. This high rate of activity is reported to be triggered by enhanced sinusoidal oscillations in the membrane potential of axotomized neurons, sufficient to cause depolarization,\textsuperscript{3} and maintained by depolarizing afterpotentials.\textsuperscript{4} Termination of each burst may be the result of a reduction in amplitude of these afterpotentials. Based on this mechanism, the positive correlation between the duration of the burst (as well as number of action potentials during the burst) and length of the interval between bursts is probably the result of a longer recovery of the membrane potential following prolonged high frequency activity, and subsequent delayed development of the sinusoidal oscillations.

Histogram profiles of the instantaneous frequencies may be a useful tool for characterizing ongoing activity in other models. The presence of a bimodal or polymodal distribution could provide ‘fingerprint’ for identifying a more progressive painful neuropathy. Since
microneurography can be used to record ongoing activity from peripheral nerves in humans, such profiling could potentially be used to stratify patients with painful WRMDs.

**Limitations**

The electrophysiology method we used allows characterization of individual neurons but comes with limitations that must be considered for proper interpretation of the results. Our primary goal was to look at the neural response to nerve inflammation and degeneration, and thus we focused on recording ongoing activity. As we have previously described, nociceptors respond to noxious stimulation by developing increasing ongoing activity that correlates to both the development of inflammation and the time frame of these experiments. Therefore, while the methods can allow more comprehensive characterization of the neurons in terms of receptive fields, doing so would have inflamed the preparation and sensitized other nociceptors, thus confounding the results. Alternately, we could have recorded from one nociceptor per experiment, which would have reduced the yield dramatically. While this is possible, we must consider that each rat who completed 12 weeks of task performance was a product of 200-250 person-hours of effort, and as such were valuable. Further, we were not able to determine whether faster conducting neurons without receptive fields were sensory or motor neurons. To address this possibility, we attempted to record from the cervical dorsal and ventral roots, but the methods proved unfeasible.

Only female rats were included in this study, which is in part because there is a higher incidence of work-related musculoskeletal disorders in females compared to males, but also because the force transducer sensitivity of the model is tailored to a pulling strength of female rats. The inclusion of male rats would have made the interpretation of findings substantially more difficult.
Summary

We have shown that an operant repetitive task performed by rats can have profound effects on neurons within the median nerve. Our observations of erratic discharge in neurons after only 3-weeks of a repetitive task, with more intense high frequency bursting activity following 12-weeks, is consistent with an active and progressive neuropathy. As the neuritis develops, and fibrosis progresses, nerve fibers undergo demyelination and degeneration, which most likely results in the development of a neuroma in continuity by 12-weeks. Furthermore, the present data indicates a neuropathy that is associated with marked neuropathology affecting fast conducting neurons. The increase in erratic discharge from sensory neurons will undoubtedly contribute to the development of more severe painful symptoms. Since the behaviors parallel those observed in humans, these results give substantial insight into the pathophysiology of humans suffering similar conditions. Importantly, this study has highlighted the importance of ongoing activity in the pathophysiology of repetitive motion disorders. Treatments that diminish or prevent the neuropathy in this rat model may provide effective therapies in patients with repetitive motion disorders.

Author contributions: G.M. Bove and M.F. Barbe designed the research, performed experiments, analyzed data, and wrote the paper. A. Dilley designed and contributed analytical tools, analyzed data, and wrote the paper. M. Harris contributed to the development of the model, as well as analysis of the work task behavioral.

Data and materials availability: Detailed methods, protocols, and raw data are available without restriction through direct contact of G.M. Bove or M.F. Barbe.
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<th>Burst frequency</th>
<th>Burst duration</th>
<th>Burst frequency</th>
<th>Number of action potentials in burst</th>
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<td>Number of action potentials in burst</td>
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<td>0.12</td>
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<td>Interval between bursts</td>
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<td>0.13</td>
<td>0.97</td>
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<td>ns</td>
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Table 1. Summary of a regression analysis between discharge characteristics. Fast and slow conducting neurons were combined for the analysis. Correlation coefficients, r, are shown. **** p<0.0001. ns = not significant.
FIGURE LEGENDS

**Figure 1. Behavioral signs of neuropathy.** (A, B) Reaches per minutes, (C, D) Reflexive grip strength, (E, F) mechanical and (G, H) cold sensitivity in 3- and 12-week task-performing and control groups. \( n = 5 \) 3-week and 5 12-week task performing rats compared to 10 control rats. Individual values are shown (from both limbs in C-F). In A to D, mean ± SEM [A and B] and median ± interquartile range [C and D] are also given. In A and B, * \( p < 0.05 \), ** \( p < 0.01 \) (paired t-tests). In D, **** \( p < 0.0001 \) (Mann-Whitney test). In E-H, p < 0.0001 [E and F], p<0.5 [G], P<0.01 [H]; main effect for group; two-way ANOVA).

**Figure 2. Electrophysiology of slowly conducting axons passing within the median nerve.** A. Diagram of methods used. In anesthetized rats, the median nerve was exposed from the cubital fossa to the axilla, where it was disconnected and placed upon a small platform for recording. B. Conduction velocities of neurons in the control group and after the 3- and 12-week repetitive task (**\( P < 0.01 \), ***\( P = 0.001 \); Kruskal-Wallis with Dunn's tests). One data point was outside of the x-axis and its value noted. Blue symbols indicate those neurons with ongoing activity. C. Numbers of neurons with ongoing activity in each group (p <0.0001, Chi Square Test). D. Ongoing activity rates in each group (* \( p < 0.05 \), *** \( p < 0.001 \); Kruskal-Wallis with Dunn's tests). One data point was outside of the range of the x-axis and its value noted. E. Representative recording from a control neuron with ongoing activity (0.3 Hz). F. Representative recording from a neuron after 3 weeks of the repetitive task (4 Hz). G-I. Representative recordings from three neurons after 12 weeks of the repetitive task (3 Hz, 26 Hz and 58 Hz, respectively). J. Detail of the neuron in I, showing firing in triplets. K. Numbers of neurons with and without receptive fields (RF) that responded to innocuous mechanical stimuli in control, 3-week and 12-week animals (p = 0.85; Chi Square Test). N = 5 3-week and 5 12-week task performing animals; n = 9 control animals.
Figure 3. Electrophysiology of fast-conducting axons passing within the median nerve in control and working rats. A. Numbers of neurons with ongoing activity in each group. B. Ongoing activity rates in each group (* p < 0.05; Mann-Whitney test). C. Representative recording from a control neuron (14 Hz). D-G. Representative recordings from four neurons after 12 weeks of the repetitive task with increasing rates of ongoing activity (15 Hz, 49 Hz, 72 Hz, and 90 Hz, respectively). Neurons in F and G had a bursting pattern of firing. H. Number of neurons with and without receptive fields (RF) in control, 3-week and 12-week animals. N = 5 3-week and 5 12-week task performing animals; n = 9 control animals (same animals as figure 2).

Figure 4. Analysis of the bursting discharge. A-C. Examples traces of bursting activity from fast (A-B) and slow (C) conducting neurons. In both A and C, a second neuron is present that does not display a similar erratic discharge pattern (taller unit in A and shorter unit in C). Normalized histograms of the instantaneous frequency (IF) for each of these neurons are shown. A1, B1 and C1 correspond to those neurons with a bursting discharge pattern, whereas A2 and C2 correspond to the neurons with a more regular firing pattern. Note the different IF histogram profiles. Histograms of neurons with bursting activity have a bimodal or polymodal distribution. The reciprocal of the slower IF peak value (*) corresponds to the interval between each burst, whereas the reciprocal of the faster IF peak value (†) corresponds to the interspike interval during the burst. Histograms of neurons without this bursting pattern of activity typically have a unimodal distribution. The reciprocal of the peak value (‖) for these neurons corresponds to the interspike interval. D-G. Discharge characteristics for each neuron with bursting discharge, calculated from the histogram profiles (see methods). Vertical lines correspond to the median duration of burst, number of spikes within the burst and interval between bursts, and the mean frequency during the burst. Data for both fast and slow conducting neurons was combined. Empty circles = fast conducting neurons (n=22); filled circles = slow conducting neurons (n=3).
There is no clear difference in bursting characteristics between fast or slow conducting neurons. Bin size = 5 ms. Line on histograms = moving average (over 15 Hz).

References


13. Barbe MF, White AR, Hilliard BA, Salvadeo DM, Amin M, Harris MY, Cruz GE, Hobson L, Popoff SN. Comparing Effects of Rest with or without a NK1RA on Fibrosis and
Sensorimotor Declines induced by a Voluntary Moderate Demand Task. *Journal of Musculoskeletal and Neuronal Interactions.* 19:396-411, 2019


Figure 2

A

B

C

D

E

F

G

H

I

J

K

Number of recordings

Control 3-Week 12-Week

With RF Without RF

Conduction velocity (m/s)

Rate (Hz)

Time (sec)

Time (sec)

Time (sec)

Time (sec)

Time (sec)
Figure 3

A

Number of recordings

OA Pos

OA Neg

Control 3-Week 12-Week

B

Control

12-Week

Rate (Hz)

C

D

E

F

G

H

Number of recordings

With RF No RF

Control 3-Week 12-Week