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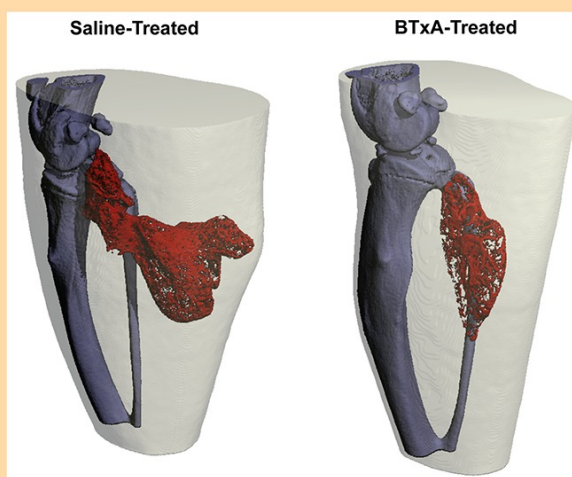
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# Whole-body Vibration at Thoracic Resonance Induces Sustained Pain and Widespread Cervical Neuroinflammation in the Rat

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## Abstract

**Background** Whole-body vibration (WBV) is associated with back and neck pain in military personnel and civilians. However, the role of vibration frequency and the physiological mechanisms involved in pain symptoms are unknown.

**Questions/purposes** This study asked the following questions: (1) What is the resonance frequency of the rat spine for WBV along the spinal axis, and how does frequency of WBV alter the extent of spinal compression/extension? (2) Does a single WBV exposure at resonance induce pain that is sustained? (3) Does WBV at resonance alter the protein kinase C epsilon (PKCε) response in the dorsal root ganglia (DRG)? (4) Does WBV at resonance

alter expression of calcitonin gene-related peptide (CGRP) in the spinal dorsal horn? (5) Does WBV at resonance alter the spinal neuroimmune responses that regulate pain?

**Methods** Resonance of the rat ( $410 \pm 34$  g,  $n = 9$ ) was measured by imposing WBV at frequencies from 3 to 15 Hz. Separate groups ( $317 \pm 20$  g,  $n = 10$ /treatment) underwent WBV at resonance (8 Hz) or at a nonresonant frequency (15 Hz). Behavioral sensitivity was assessed throughout to measure pain, and PKCε in the DRG was quantified as well as spinal CGRP, glial activation, and cytokine levels at Day 14.

**Results** Accelerometer-based thoracic transmissibility peaks at 8 Hz ( $1.86 \pm 0.19$ ) and 9 Hz ( $1.95 \pm 0.19$ , mean difference [MD]  $0.290 \pm 0.266$ ,  $p < 0.03$ ), whereas the video-based thoracic transmissibility peaks at 8 Hz ( $1.90 \pm 0.27$ ), 9 Hz ( $2.07 \pm 0.20$ ), and 10 Hz ( $1.80 \pm 0.25$ , MD  $0.359 \pm 0.284$ ,  $p < 0.01$ ). WBV at 8 Hz produces more cervical extension ( $0.745 \pm 0.582$  mm, MD  $0.242 \pm 0.214$ ,  $p < 0.03$ ) and compression ( $0.870 \pm 0.676$  mm, MD  $0.326 \pm 0.261$ ,  $p < 0.02$ ) than 15 Hz (extension,  $0.503 \pm 0.279$  mm; compression,  $0.544 \pm 0.400$  mm). Pain is longer lasting (through Day 14) and more robust ( $p < 0.01$ ) after WBV at the resonant frequency (8 Hz) compared with 15 Hz WBV. PKCε in the nociceptors of the DRG increases according to the severity of WBV with greatest increases after 8 Hz WBV ( $p < 0.03$ ). However, spinal CGRP, cytokines, and glial activation are only evident after painful WBV at resonance. **Conclusions** WBV at resonance produces long-lasting pain and widespread activation of a host of nociceptive and neuroimmune responses as compared with WBV at a nonresonance condition. Based on this work, future investigations into the temporal and regional neuroimmune response to resonant WBV in both genders would be useful.

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**Clinical Relevance** Although WBV is a major issue affecting the military population, there is little insight about its mechanisms of injury and pain. The neuroimmune responses produced by WBV are similar to other pain states, suggesting that pain from WBV may be mediated by similar mechanisms as other neuropathic pain conditions. This mechanistic insight suggests WBV-induced injury and pain may be tempered by antiinflammatory intervention.

## Introduction

Spinal pain has a lifetime prevalence of 54% to 80% in the United States [51]. Neck and back pain is a major disability for military personnel and is among the most diagnosed illness/injury for active-duty service members [5–9]. Epidemiological reports implicate whole-body vibration (WBV) as the cause of neck/back pain [13, 14, 48, 55, 84] with those who have served in combat settings at greatest risk. Over half (69%) of military helicopter pilots exposed to WBV report pain with the frequency of symptoms correlated with the length of exposure [24, 59]. Because the greatest injury risk exists at resonance [36], studies have defined the resonant frequency of many species, including humans (approximately 4 Hz) [11, 63], Rhesus monkey (5–14 Hz) [72], and rabbit (4.5 Hz) [80]. Although several rat models have examined pain induction and maintenance after either vibration of isolated limbs or WBV [3, 4, 11, 19, 26, 43], the vibration response, and its relevance to injury, in the rat is unknown.

Pain can result from direct injury to the peripheral and/or central nervous system or through nociceptive cascades that are initiated in the dorsal root ganglia (DRG) and/or spinal cord. Workers using vibrating hand tools exhibit nerve damage, pain, and paraesthesia in the hands [34, 66]. Isolated vibration of the limb or tail in the rat produces damage in myelinated fibers [49, 53]. WBV of the rat induces deformation in the cervical spine, which if repeated daily produces long-lasting behavioral sensitivity [11]. Although vibration may induce neural tissue damage, which may be a mechanism of pain, this has not been investigated for WBV.

The protein kinase C (PKC) pathway and in particular, the PKC $\epsilon$  isoform regulate peripheral nociceptor function [41, 77] and contribute to prolonged pain [1, 25, 26, 28, 43]. PKC $\epsilon$  is responsible for heat and pain sensitivity in peripheral nociceptors [16, 93] and, through PKC pathway signaling, also modulates spinal calcitonin gene-related peptide (CGRP) activity [69, 74]. After injury, CGRP is released by nociceptive fibers in the superficial dorsal horn [56, 58, 74, 76] and can stimulate spinal immune cell activation [15, 64] and cytokine release [69]. These

immune responses not only further exacerbate a host of other inflammatory cytokines and chemokines, but also regulate neuronal responses through modulation at synaptic connections [22, 32, 46, 86].

We have previously shown that WBV at 15 Hz can induce pain in the rat that either resolves or is sustained, depending on how often the exposure is presented, occasionally or daily [11]. Upregulation of neurotrophins in the intervertebral discs is also evident for the case with sustained pain [40]. Although that collection of work supports the notion that WBV causes pain, it does not address whether sustained pain is possible after a single WBV exposure and the relative role of the resonance frequency on pain and injury risk.

Accordingly, we performed two complementary studies in the rat to define the mechanical and physiological outcomes of resonant WBV. First, we asked: (1) What is the resonance frequency of the rat spine for WBV along the spinal axis, and how does frequency of WBV alter the extent of spinal compression/extension? Additional questions derive from those findings: (2) Does a single WBV exposure at resonance induce pain that is sustained? (3) Does WBV at resonance alter the PKC $\epsilon$  response in the DRG? (4) Does WBV at resonance alter expression of CGRP in the spinal dorsal horn? (5) Does WBV at resonance alter the spinal neuroimmune responses that regulate pain?

## Materials and Methods

Procedures were approved by the Institutional Animal Care and Use Committee and performed according to the Committee for Research and Ethical Issues of the International Association for the Study of Pain [95]. Male Holtzman rats (Harlan, Indianapolis, IN, USA) were used in this study to reflect the majority of the population affected [62]. Rats were maintained with a 12-hour/12-hour light/dark cycle and free access to food and water as recommended by the Association for Assessment and Accreditation of Laboratory Animal Care International. All processing and analysis was performed by observers blinded to treatments.

### WBV Transmissibility and Resonance

To answer our first question, of determining the resonance frequency, rats were exposed to WBV over a range of frequencies; the maximum energy transferred from a vibrating platform to the rat spine was determined from the transmissibility profile and taken as resonance. To enable



rigid fixation of sensors directly to the vertebrae of the spine, the study to determine the resonance frequency of the rat spine was performed using dead rats ( $n = 9$ ). Rats ( $410 \pm 34$  g) were anesthetized by intraperitoneal injection of sodium pentobarbital (65 mg/kg) and transcardially perfused with 200 mL phosphate-buffered saline (PBS) (Life Technologies, Carlsbad, CA, USA) followed by 200 mL 4% paraformaldehyde in PBS (PFA; Sigma, St Louis, MO, USA). Rats were placed in the prone position on a rigid vibrating platform to enable WBV along the spine's long axis using straps positioned behind the shoulders and above the pelvis (Fig. 1A) [11]. A sinusoidal input (1.5-mm peak-to-peak amplitude) was applied using a shaker (K2007E01; Modal Shop, Cincinnati, OH) at 3 Hz and increased to 15 Hz in 1-Hz increments for 1 minute each. Uniaxial accelerometers (7521A2; 120 Hz; Dytran, Chatsworth, CA, USA) were affixed to the platform and rigidly pinned between the T9 and T10 vertebrae of the rat (Fig. 1A). A high-speed CCD video camera (0.035-mm resolution; 120 Hz; Phantom Miro eX1; Vision Research Inc, Wayne, NJ, USA;  $640 \times 480$  pixels) tracked markers on the thoracic accelerometer and the platform (Fig. 1A) and relative displacements were digitized using ProAnalyst (Xcitex, Woburn, MA, USA) motion tracking software.

The video-based displacements and accelerometer data were used separately to compute the transmissibility of the thoracic spine at each input frequency [30, 42, 52, 68]. The accelerometer data were filtered using a fifth-order Butterworth bandwidth filter and the root mean square (RMS) acceleration of the thoracic accelerometer was divided by the platform acceleration to compute accelerometer-based transmissibility at each frequency. The video-based transmissibilities were calculated as the ratio of the RMS displacement of the thoracic spine divided by that of the platform at each frequency [12, 45]. A repeated-measures analysis of variance (ANOVA) was used to compare transmissibilities between frequencies and between acquisition approaches. Resonance was determined as the transmissibility that was greater than all other frequencies ( $p < 0.04$ ).

### Pain and Neuroimmune Regulation After WBV at Resonance

We assessed the behavioral and physiological effects of WBV at resonance to answer questions 2 through 5 on pain and neuroimmune regulation. Rats ( $317 \pm 20$  g) were vibrated at resonance (8 Hz,  $n = 10$ ) and also at a frequency (15 Hz,  $n = 10$ ) that has been previously characterized [11, 40]. Vibration was imposed under inhalation anesthesia (4% induction, 2% maintenance) in the two separate groups. To control for anesthetic

effects, a sham group ( $n = 10$ ) underwent the same exposure paradigm but was not vibrated. WBV was imposed as described for the resonance study but with the accelerometer strapped to the rat [11, 40]. Vibration was applied for 30 minutes on Days 0 and 7 for all WBV rats used in this study [11]. Accelerations were kept at 0.5 g for each WBV group with peak-to-peak amplitudes of approximately 5 mm (8 Hz) and 1.5 mm (15 Hz).

The transmissibility and spinal deformation were measured during each WBV exposure to define the relative biomechanical severity of the exposures. Accordingly, markers were placed on the platform, the accelerometers, and on the skin at the rostral and caudal ends of the cervical spine and the thoracic spine. Transmissibility was calculated as described previously. Relative extension and compression deformations of the cervical spine were measured by digitizing the distance between the cervical markers and calculating the vector length at the maximum of extension and compression relative to the resting vector length [11]. Biomechanical metrics (transmissibility, extension, and compression) were compared between groups using separate t-tests.

To answer our second question, behavioral sensitivity, a common measurement of pain, was assessed by evaluating the bilateral forepaw withdrawal thresholds to mechanical stimulation. Testing was performed on Day 0 (baseline) immediately before WBV exposure and on Days 1, 7 (before the second WBV exposure), 8, and 14. The withdrawal threshold was defined as the lowest von Frey filament eliciting a response, confirmed by the next higher filament also provoking a response [11, 17, 81]. On each day, three rounds of assessment were performed with at least 10 minutes between rounds. For each day and rat, the average threshold was divided by its baseline response; a repeated-measures ANOVA with Tukey's correction was used to compare groups.

Cervical DRG and spinal cord tissue (C5-C6) was harvested from a subset of rats ( $n = 6$ /group) on Day 14 after behavioral testing for immunohistochemical assessment of neurophysiological and inflammatory responses. Tissue was harvested after procedures described previously for perfusion and postfixed in PFA. Tissue was cryoprotected in 30% sucrose and then embedded in optimal cutting temperature compound. Axial sections (14  $\mu$ m thick) were thaw-mounted on charged slides and dried at room temperature for 48 hours.

To answer our third question on the effect of WBV on neuronal PKC $\epsilon$  levels in the DRG, sections were colabeled for PKC $\epsilon$  and microtubule-associated protein-2 (MAP2). Sections were rinsed and blocked in 10% goat serum in PBS with 0.3% Triton-X100 and then incubated overnight in a rabbit primary antibody to PKC $\epsilon$  (1:500; Santa Cruz, Dallas, TX, USA) with a mouse primary antibody to MAP2

(1:100; Covance, Conshohocken, PA, USA). Sections were washed and incubated in fluorescent goat secondary antibodies for visualization (1:1000; Life Technologies) and, after coverslipping with antifade medium and drying, were imaged at  $\times 200$  using a BX51 microscope (Olympus, Center Valley, PA, USA). Images (three to six per rat) were cropped ( $200 \times 200$  pixels) to include at least 10 nonoverlapping neurons in each section, selected spatially to include the maximum number of cell bodies within the DRG. The percent pixels positive for PKC $\epsilon$  per MAP2-positive area was calculated [28, 47, 81]. The percent of small-diameter (4–20  $\mu\text{m}$ ) or medium-diameter (22–40  $\mu\text{m}$ ) neurons that were positive for PKC $\epsilon$  in each group was also measured [35, 81]. Separate one-way ANOVAs with Tukey's correction were used to compare neuronal PKC $\epsilon$  for each analysis.

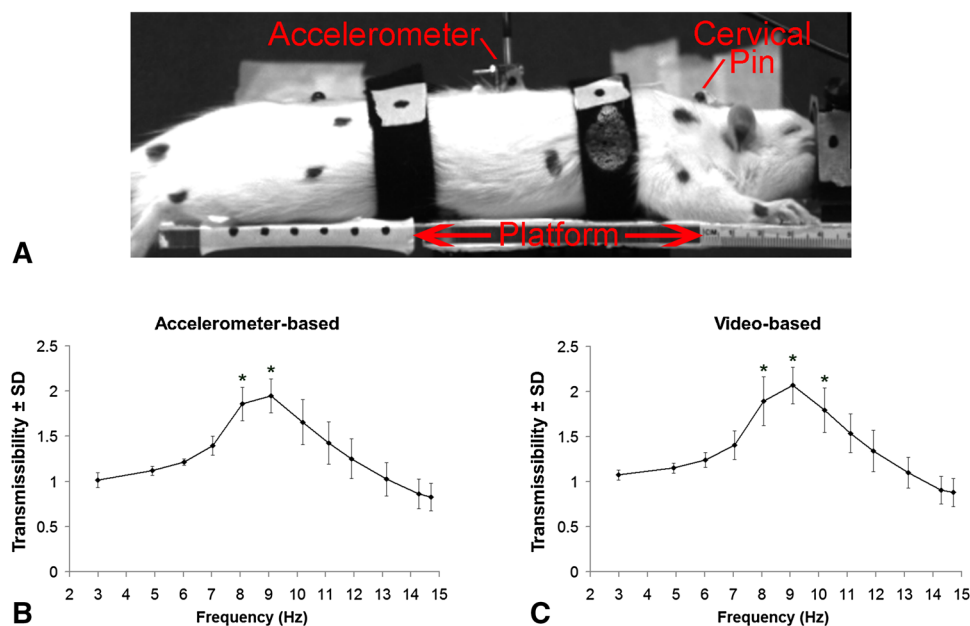
To answer our fourth and fifth questions about the effects of resonant WBV on spinal CGRP and neuroimmune cascades, spinal sections were labeled for the neuropeptide, CGRP, and the glial markers, glial fibrillary acidic protein (GFAP, astrocytic activation), and ionized calcium-binding adapter molecule 1 (Iba1, microglial/macrophage marker). Sections were labeled with rabbit primary antibodies to either CGRP (1:1000; Bachem, San Carlos, CA, USA) or Iba1 (1:1000; Wako, Osaka, Japan) or a mouse primary antibody to GFAP (1:500; Millipore, Billerica, MA, USA) and visualized with fluorescent goat secondary antibodies. Tissue from naïve

rats ( $n = 6$ ), not exposed to any anesthesia, also was included for normalization. Sections (three to six per rat) were imaged and cropped ( $750 \times 200$  pixels) to include only the superficial laminae of the dorsal horn. The percent pixels positive for each protein was calculated [29, 91] and separate one-way ANOVAs with Tukey's correction were used to compare the average expression of each protein.

In separate groups ( $n = 4/\text{group}$ ), as part of our fifth question about the effect of WBV on the spinal neuroimmune response, C6 spinal cord was harvested at Day 14. Tissue was flash-frozen on dry ice and stored at  $-70^\circ\text{C}$  until assayed. Tissue from each group was pooled to minimize variation between individual rats. Protein was extracted by homogenization in PBS with protease inhibitors. A commercial proteome profiler array (ARY008; R&D Systems, Minneapolis, MN, USA) was used to detect a panel of cytokines and chemokines, according to the manufacturer's instructions. The integrated pixel intensity after background subtraction was quantified for each spot. Duplicate spots were averaged and expressed as fold change over sham and were compared between WBV groups using separate t-tests.

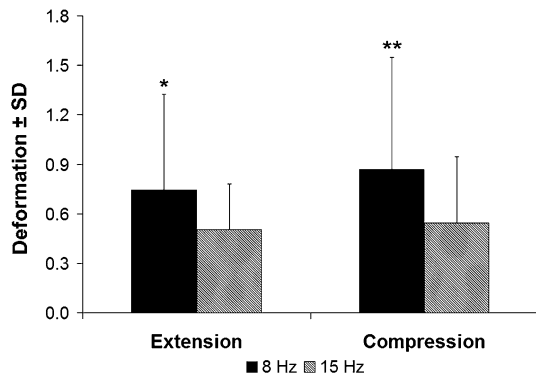
## Results

The transmissibility response in the rat peaked between 8 Hz and 10 Hz (Fig. 1). Additionally, the transmissibility



**Fig. 1A–C** A representative image (A) showing a rat on the test setup with the platform and the cervical pin and the accelerometer that are both rigidly inserted into the spine. The arrow indicates that the direction of vibration is along the long axis of the rat's spine. The transmissibility profiles are separately calculated using data derived

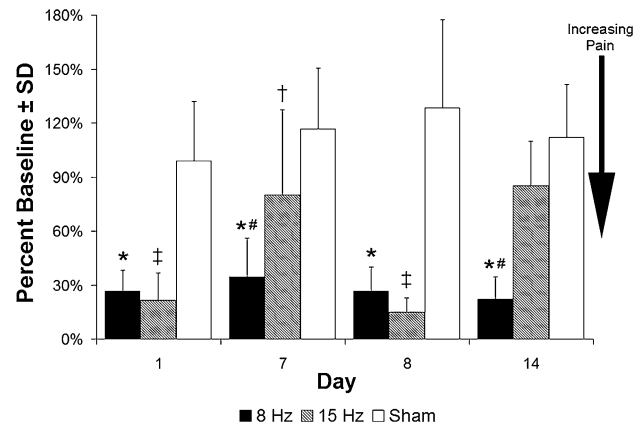
from the accelerometer (B) and the video imaging system (C); the peak transmissibility is between 8 Hz and 10 Hz, which are greater than all other frequencies ( $*p < 0.03$ ) but not different from each other.



**Fig. 2** The extent of deformation measured in the cervical spine is greater for an 8-Hz WBV exposure than a 15-Hz WBV exposure. The neck exhibits more cervical extension (\* $p < 0.03$ ) and compression (\*\* $p < 0.02$ ) during an 8-Hz vibration than a 15-Hz one and also exhibits greater variability in that group.

profile determined by the accelerometer (Fig. 1B) was not different from that calculated using video data (Fig. 1C). Using accelerometer-based data, the peak thoracic transmissibility is at 8 Hz ( $1.86 \pm 0.19$ ) and 9 Hz ( $1.95 \pm 0.19$ ), which are not different from each other but are higher (mean difference with 95% confidence interval [CI],  $0.290 \pm 0.266$ ;  $p < 0.03$ ) than any other frequency. Similarly, the peaks (mean difference with 95% CI,  $0.359 \pm 0.284$ ;  $p < 0.01$ ) of the video-based thoracic transmissibility profile are evident at 8 Hz ( $1.90 \pm 0.27$ ), 9 Hz ( $2.07 \pm 0.20$ ), and 10 Hz ( $1.80 \pm 0.25$ ) with no difference among them. These same trends are evident in the physiologic study with thoracic transmissibility at 8 Hz greater than that at 15 Hz regardless of measurement approach (mean difference with 95% CI,  $0.370 \pm 0.141$ ;  $p < 0.01$ ) (Fig. S1 [Supplemental materials are available with the online version of CORR®.]). Moreover, WBV at 8 Hz produces more cervical extension ( $0.745 \pm 0.582$  mm; mean difference with 95% CI,  $0.242 \pm 0.214$ ;  $p < 0.03$ ) and compression ( $0.870 \pm 0.676$  mm, mean difference with 95% CI,  $0.326 \pm 0.261$ ;  $p < 0.02$ ) than 15 Hz (extension,  $0.503 \pm 0.279$  mm; compression,  $0.544 \pm 0.400$  mm) (Fig. 2).

Forepaw sensitivity, a method of measuring behavioral sensitivity (and commonly taken as a measure of pain), was induced at Day 1 after each of the WBV magnitudes ( $p < 0.01$ ), whereas sham anesthesia exposure did not alter responses from baseline at any day (Fig. 3). On Day 7 after the first exposure, only the 8-Hz group exhibited a reduction in withdrawal threshold from baseline and compared with the sham group ( $p < 0.01$ ); the response of the 15-Hz group was not different from its corresponding baseline response. After the second WBV exposure, behavioral sensitivity was again produced in both groups on Day 8 and the withdrawal threshold was decreased (indicating



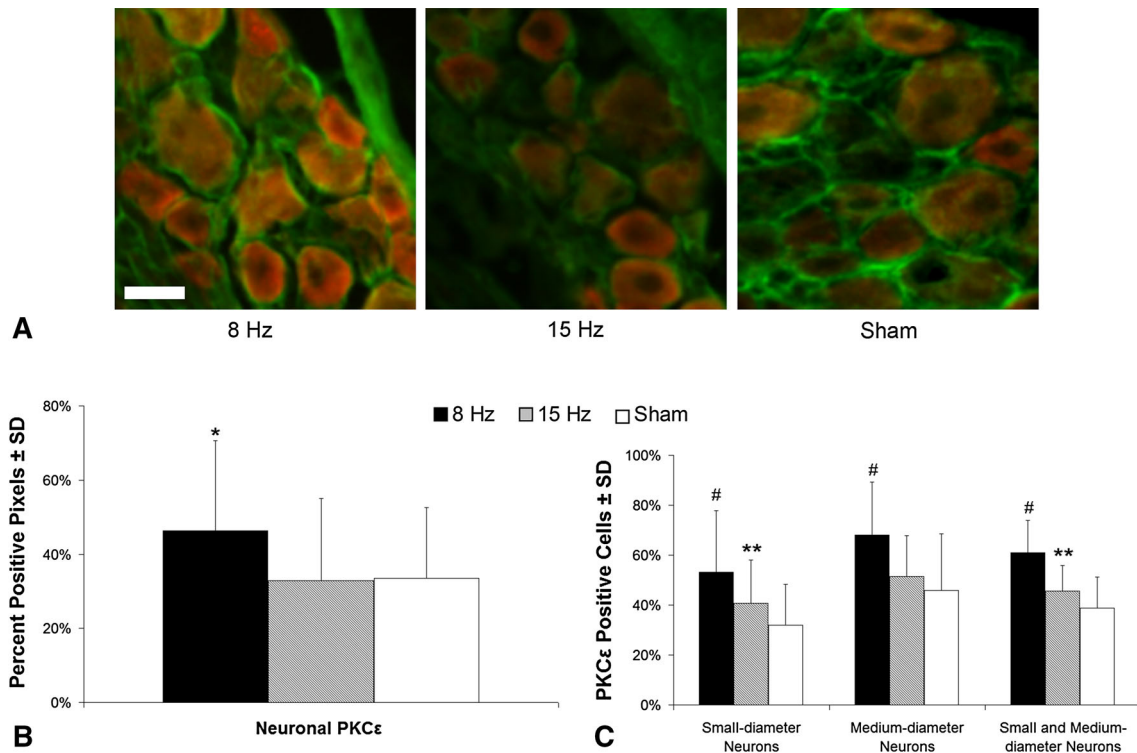
**Fig. 3** Exposure to either WBV profile on Day 0 and Day 7 induces a robust decrease in the percent of baseline withdrawal thresholds, taken as increased behavioral sensitivity (ie, a pain response) in the forepaw on Days 1 and 8. On all days, the withdrawal threshold for 8 Hz is less than sham and baseline (\* $p < 0.01$ ), and on Days 7 and 14, it also is lower than the withdrawal threshold for the 15-Hz WBV ( $\#p < 0.01$ ). In contrast, a 15-Hz WBV exposure lowers the withdrawal threshold compared with sham only on Day 7 ( $\dagger p < 0.03$ ) and lower than both baseline and sham on Days 1 and 8 ( $\ddagger p < 0.01$ ) after exposure.

increasing pain) compared with both the corresponding baseline ( $p < 0.01$ ) and sham responses ( $p < 0.01$ ). By Day 14, sensitivity was resolved in the 15-Hz group but remained in the 8-Hz group compared with both baseline and sham levels ( $p < 0.01$ ) (Fig. 3).

PKC $\epsilon$  expression in the DRG at Day 14 followed the behavioral responses (Fig. 4). There was more ( $p < 0.01$ ) neuronal PKC $\epsilon$  in DRGs after 8 Hz WBV than after 15 Hz and sham exposures (Fig. 4B). There were also more small- and medium-diameter neurons that were positive for PKC $\epsilon$  after an 8-Hz WBV than after a 15-Hz ( $p < 0.01$ ) and sham ( $p < 0.01$ ) exposure (Fig. 4C). There were more PKC $\epsilon$ -positive small-diameter neurons after a 15-Hz WBV than sham ( $p < 0.03$ ) (Fig. 4C).

Spinally regulated mediators in the superficial dorsal horn increased only in the 8-Hz WBV group. CGRP was more than doubled after an 8-Hz WBV ( $p < 0.02$ ) and unchanged from sham levels after 15-Hz WBV (Fig. 5). Similarly, both GFAP (Fig. 6A) and Iba1 (Fig. 6B) exhibited an increase ( $p < 0.01$ ) only after painful WBV at resonance. WBV at 15 Hz did not alter the extent of either astrocytic or microglial activation, as measured by GFAP and Iba1, respectively, from sham levels (Fig. 6).

All of the cytokines/chemokines probed were detected in the spinal cord, and many were altered after 8 Hz WBV (Fig. 7). However, many were not different between the WBV groups: cytokine-induced neutrophil chemoattractant (CINC)-1, CINC-2 $\alpha/\beta$ , CINC-3, ciliary neurotrophic factor (CNTF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon- $\gamma$ , interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ ,



**Fig. 4A–C** Representative images and quantification of PKCε labeling (red) in cervical DRGs colabeled with MAP2 (green), as a neuronal marker, at Day 14. PKCε is increased most robustly after WBV at the painful resonance frequency (8 Hz). Neuronal PKCε (orange) is evident in all groups (A), but more extensively after an 8-Hz vibration, and when quantified, there is more neuronal PKCε (B) evident after an 8-Hz exposure than after a 15-Hz or sham

exposure (\**p* < 0.01). In addition, there are more small- and medium-diameter neurons that are positive for PKCε (C) in the 8-Hz group than in either the sham or 15-Hz groups (#*p* < 0.01). There are also more PKCε (C) positive small-diameter neurons in the 15-Hz group than the sham group (\*\**p* < 0.03). The scale bar in A is 25 μm and applies to all panels.

IL-13, IL-17, L-selectin, and regulated-on-activation, normal T-cell expressed and secreted (RANTES). Most of the pro- and antiinflammatory cytokines and chemokines increased by between 1.5- to threefold after an 8-Hz WBV compared with a 15-Hz exposure (*p* < 0.05) (Fig. 7). CXCL7 was the only chemokine probed that decreased in response to WBV at resonance (Fig. 7).

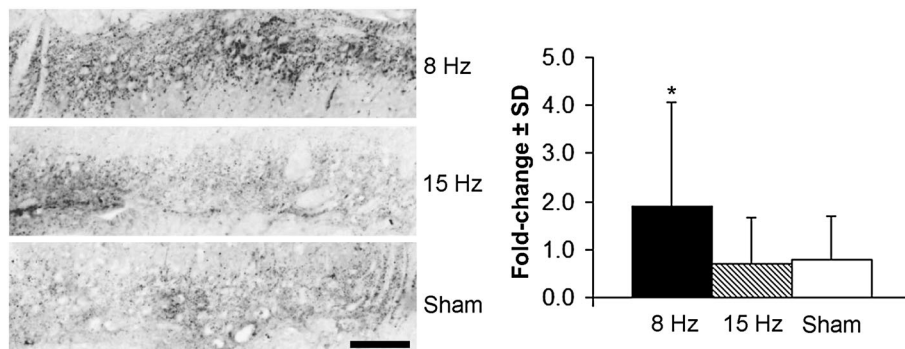
**Discussion**

Despite having high incidence and economic burdens, and impacting the quality of life of both military and civilian populations [5–9, 24, 51, 59], vibration-induced pain is not well understood. Although epidemiological studies link WBV and pain [14, 48, 55], they do not provide mechanistic insight. Rodent models are useful to investigate the mechanisms of injury and pain [23, 39, 78, 85, 86]. Vibration of isolated limbs or the tail induces local myelin damage, endothelial cell death, growth factor upregulation, and increased vulnerability to reinjury [3, 21, 26, 49, 53].

More recently, it has been found that WBV induces widespread pain and aberrant nerve fiber growth [11, 40, 71], but the resonance condition has not been investigated. Accordingly, we defined the resonance frequency of the rat spine, because that species is used to study pain and evaluate potential therapeutics [23, 39, 60, 78, 85, 86]. We hypothesized that vibration at resonance produces more robust pain than nonresonant WBV [11, 40] and that the neuroimmune cellular and molecular pathways are more extensively activated for that WBV exposure.

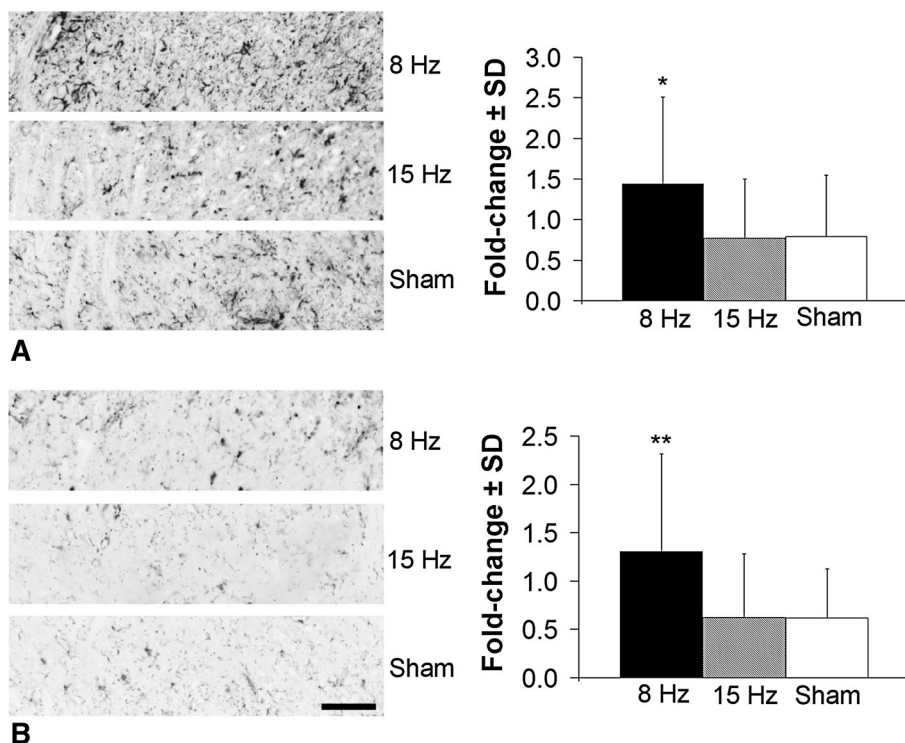
Like with any animal study, ours has limitations. The resonant frequency of the spine was defined using only dead rats to enable instrumenting the spine (Fig. 1A). In both live and deceased studies, 8 Hz resulted in greater biomechanical changes (eg, transmissibility and cervical spine deformation) than 15 Hz (Figs. 1, 2, S1). Cervical transmissibility also peaks at 8–9 Hz (data not shown), supporting the larger cervical spine deformations that are observed here (Fig. 2). Because the rat’s head is free to translate within the nose cone in the test setup, possible contributions of that cannot be fully discounted. However,





**Fig. 5** Representative images showing CGRP immunoreactivity in the cervical superficial dorsal horn and quantification as fold change over normal expression for each group at Day 14. There is more

CGRP labeling in the 8-Hz group than in each of the 15-Hz and sham groups (\* $p < 0.02$ ). The scale bar is 100  $\mu\text{m}$  and applies to all panels.

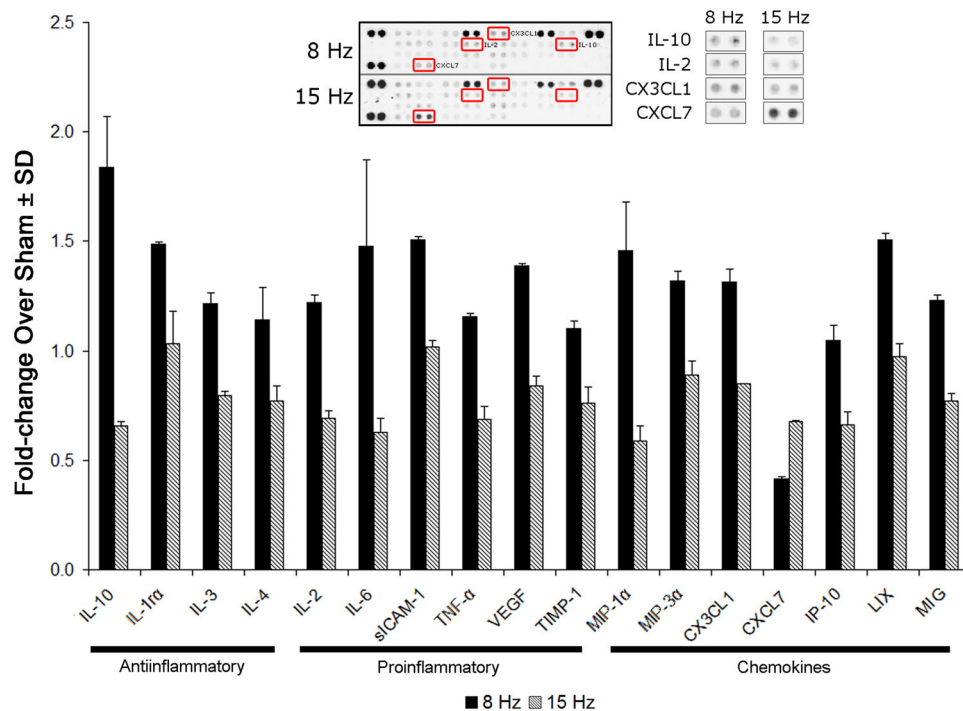


**Fig. 6A–B** Glial cell activation in the superficial spinal dorsal horn at Day 14 increases only after WBV at the resonance frequency (8 Hz). Representative images show increased GFAP immunoreactivity (A), indicative of activated astrocytes, and quantification for each group expressed as fold change over normal at Day 14. There is more GFAP

labeling in the 8-Hz group than in either the 15-Hz or sham groups (\* $p < 0.01$ ). The same is observed with Iba1 immunoreactivity (B), which is greater in the 8-Hz group than in each of the 15-Hz and sham groups (\*\* $p < 0.01$ ). The scale bar in (B) is 100  $\mu\text{m}$  and applies to all panels in both A and B.

because those relative movements are the same in both exposure groups (data not shown), this is not likely. To apply a consistent acceleration (0.5 g) to both WBV groups, the displacement amplitude was different, yet pilot studies (unpublished) varying accelerations and displacements produce similar behavioral outcomes, supporting WBV frequency as a potent factor. Behavioral sensitivity as assessed in this study is a commonly accepted

measurement of pain in both laboratory animals and humans; however, this metric does not capture spontaneous or affective components of pain. Given that approximately 85% of the active-duty military population is male [62], that sex of rats was used; because sex differences have been reported in pain and cellular cascades [73], additional studies are needed using female rats. Although Day 14 as an endpoint was selected using previous studies [11, 40],



**Fig. 7** Bar chart showing those pro- and antiinflammatory cytokines and chemokines that are altered after resonant 8 Hz WBV compared with vibration at 15 Hz ( $p < 0.05$ ) (shown as fold change over sham levels). Although 10 cytokines and six chemokines are upregulated ( $p < 0.05$ ), CXCL7 is lower ( $p < 0.01$ ) after 8-Hz WBV related to 15-Hz WBV. sICAM-1 = soluble intercellular adhesion molecule 1;

TNF- $\alpha$  = tumor necrosis factor alpha; VEGF = vascular endothelial growth factor; TIMP-1 = tissue inhibitors of metalloproteinases metalloproteinase inhibitor-1; MIP = macrophage inflammatory protein; IP-10 = interferon gamma-induced protein 10; LIX = lipopolysaccharide-inducible CXC chemokines; MIG = monokine induced by interferon-gamma.

tissue responses are only a snapshot in time (Figs. 4–7). Although this study limited assessments to DRG and the spinal cord, other nonneural tissues are susceptible to WBV injury [10, 40]; regardless, the largest modifications are expected after WBV at resonance.

The peak transmissibility of the thoracic spine of the rat for spinally directed WBV is between 8 and 10 Hz with greater cervical deformations at 8 Hz than 15 Hz when acceleration is constant and displacement is varied (Figs. 1, 2, S1). This resonance is consistent in terms of order of magnitude with spinal responses of other species: human (approximately 4 Hz) [10, 64], primate (5–14 Hz) [72], and rabbit (4.5 Hz) [80]. Spinal muscle activity in the human is also maximal at resonance [10]. Although concomitant muscle activation suggests a compensatory mechanism that may stabilize the spine, it also presents a potential for local tissue injury.

WBV at the thoracic resonance induces sustained pain, but 15 Hz WBV produces only transient sensitivity (Fig. 3). This is similar to reports that repeated exposure to near-resonant frequencies causes long-lasting pain, numbness, and loss of dexterity in humans [34, 66, 83]. When WBV at 15 Hz is imposed daily for 1 week in the rat using the same mechanical conditions as the current study, the

pain is similar to a single exposure at resonance [11]. The second 15-Hz WBV exposure does not exacerbate the pain response (Fig. 3), whereas a second direct compression injury to the nerve root induces more severe and longer lasting pain [38]. This difference in pain after repeated tissue insults may reflect the difference between direct injury to neural tissue and dampened effects from WBV.

Because the biomechanically more severe injury produces pain that is sustained (Fig. 3), increased PKC $\epsilon$  in the nociceptive DRG neurons is expected (Fig. 4). The PKC pathway is important in transducing painful stimuli from those nociceptors, both activating and modulating downstream signaling [44, 77, 94]. PKC $\epsilon$  is also upregulated in the DRG after painful joint injury, peripheral inflammation, or heat application [16, 28, 81, 93]. In fact, increased PKC $\epsilon$  is evident days after WBV in our current study and also after isolated spinal joint injury [28, 81], suggesting ongoing pain signaling from the periphery even after trauma is removed (Figs. 3, 4). PKC $\epsilon$  increases over sham levels in the small-diameter neurons after a 15-Hz WBV, which does not exhibit pain (Figs. 3, 4), suggesting PKC $\epsilon$  regulation is sensitive to tissue loading magnitude in the periphery but may not be sufficient to modulate pain responses.

The sensitivity to pain and injury severity is also evident in spinal CGRP expression (Fig. 5), which also increases after a host of painful peripheral traumas and inflammatory stimuli [18, 50, 56, 69, 92]. In contrast, it decreases after a painful root compression [37, 92], most likely from axonal degeneration in the root decreasing trafficking to the dorsal horn. Because CGRP enhances PKC-mediated signaling to increase neuronal hyperexcitability [44, 58, 74] and because only peripheral PKC $\epsilon$  is upregulated after non-painful 15 Hz WBV, it is likely that several responses sustain pain from WBV. CGRP is also proinflammatory in the dorsal horn, recruiting immune cells [15, 69] and modulating cytokine release [69].

Spinal glia are activated only after the acceleration inducing 8 Hz in this study (Fig. 6). Also, a host of inflammatory cytokines and chemokines are altered by 8 Hz WBV compared with 15 Hz (Fig. 7). Neuroinflammation is associated with pain [22, 75, 79], and spinal glial activation occurs after a variety of painful injuries [18, 22, 25, 31, 32, 38, 60, 79, 82, 87, 88, 90]. The proinflammatory cytokines, IL-6 and tumor necrosis factor- $\alpha$ , nearly double after acceleration that was painful and occurred at the resonance of 8 Hz, like painful nerve injury [67, 88, 89]. However, both pro- and antiinflammatory mediators contribute to spinal hyperexcitability and pain [39, 78, 88, 89]. The antiinflammatory cytokines, IL-10 and IL-1ra, also increase only after resonant WBV (Fig. 7). CX3CL1/fractalkine increases after painful 8 Hz WBV (Fig. 7) and provides positive feedback to further stimulate glial cells, which can lead to long-term spinal inflammation and pain [22, 32, 39, 54]. These data suggest similar inflammatory cascades may be initiated after painful WBV like in other neuropathic conditions. Only the chemokine CXCL7 decreases, but little is known about its role in pain; although it has been reported to recruit leukocytes, it also has actions different from prototypical chemokines [33, 70]. Nonetheless, additional investigations are needed to understand its apparent discordant response to painful 8 Hz WBV.

To our knowledge, this is the first study defining the resonant frequency of the rat under WBV and demonstrating that condition produces greater spinal deformations, pain, and widespread cellular neuroimmune activation. Responses persist for days after a single exposure. PKC $\epsilon$  expression in small-diameter afferents is the only mediator in this study that increases after each WBV magnitude with greater increases in the 8-Hz group. For all other neuroimmune responses, only 8 Hz WBV exhibits changes compared with control levels. Of note, PKC $\epsilon$  is also the only peripheral mediator that was probed. There is strong evidence across a variety of pain models of different tissue injuries that spinal neuroimmune responses are correlated with pain [2, 27, 29, 60, 61, 65, 67, 81, 87–89]. Importantly, similar pain pathways are initiated by WBV,

suggesting potential therapeutic targets and/or helping to identify treatments for patients with these symptoms. Our results identify potential questions leading to further investigation of the temporal and anatomical neuroimmune response to resonant WBV. That work would not only help shape potential sites and times for therapeutic interventions, but also help determine if similar effects exist for low back pain. Given the robust inflammatory response after WBV, antiinflammatory and/or nonsteroidal antiinflammatory drug treatment could prove to be potentially effective therapeutic avenues. Findings are important given that spinal pain is one of the most common causes for evacuation from military operation with 86% of those not returning to deployment [20, 57]. Although our study provides important physiologic context for exposure to vibration at resonance and there is anecdotal evidence supporting the assertion that service members are regularly exposed to spinally resonant frequencies [10], there still remains a need for field data relating exposures to pain.

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