Pain after whole-body vibration exposure is frequency dependent and independent of the resonant frequency: lessons from an in vivo rat model

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Abstract

Occupational whole-body vibration (WBV) increases the risk of developing low back and neck pain; yet, there has also been an increased use of therapeutic WBV in recent years. Although the resonant frequency (f_r) of the spine decreases as the exposure acceleration increases, effects of varying the vibration profile, including peak-to-peak displacement (s_{ptp}), root mean squared acceleration (a_{rms}) and frequency (f), on pain onset are not known. An established in-vivo rat model of WBV was used to characterize the resonance of the spine using sinusoidal sweeps. The relationship between a_{rms} and f_r was defined and implemented to assess behavioral sensitivity – a proxy for pain. Five groups were subjected to a single 30-minute exposure, each with a different vibration profile, and a sham group underwent only anaesthesia exposure. The behavioral sensitivity was assessed at baseline and for 7 days following WBV-exposure. Only WBV at 8Hz induced behavioral sensitivity, and the higher a_{rms} exposure at 8Hz led to a more robust pain response. These results suggest that the development of pain is frequency-dependent, but further research into the mechanisms leading to pain are warranted to fully understand which WBV profiles may be detrimental or beneficial.

Keywords

Whole-body vibration; pain; in-vivo; frequency; resonance

Introduction

Low back and neck pain are common in the general population [1], a leading cause of disability [2], and account for 70% of all years lived with disability due to a musculoskeletal disorder [3]. Although whole-body vibration (WBV) is not the most common source of low back and neck pain [4], it remains a significant health risk to those subjected to regular vibration exposures [5]. In fact, a meta-analysis showed that people regularly exposed to WBV are twice as likely to suffer low back pain and sciatica compared to people who do not routinely experience WBV [6]. Conversely, WBV has also emerged as a therapy for treating low back pain [7, 8], reducing fall risk and increasing bone mineral density [9-11]. Despite the increased attention of WBV, the biomechanical mechanisms by which it induces pain and injury are not well defined, nor are the mechanisms by which it may be beneficial [12].

In order to control and minimize the detrimental effects of WBV in occupational settings, a daily vibration exposure normalized to an 8-hour reference period (A(8)) has been defined using the root mean squared acceleration (a_{rms}) with a frequency-weighting [13]. Using this standard method, occupational exposure action and limit values of 0.5m/s^2 and 1.15m/s^2 respectively, have been established [14, 15]; the EU Directive on WBV [14] states that if the action value of 0.5m/s^2 is exceeded, employers are required to establish and implement measures to reduce exposures to a minimum, and if the limit value of 1.15m/s^2 is exceeded, immediate action is required to reduce the exposure. In cases of combined vibration and shock loading, the vibration dose value (VDV) is considered a more suitable measure of exposure [13], with action and limit values of 9.1m/s$^{1.75}$ and 21m/s$^{1.75}$, respectively [14]. Helicopter pilots are exposed to vibrations along the spine's axis, including at resonance during routine flights, with an A(8) exposure that is regularly above the exposure action value [16]. Military helicopter pilots exhibit spinal degeneration at a higher rate than military clerical personal [17], have greater pain, and their

degeneration is correlated with increased flight-hours [17, 18]. Similarly, train drivers routinely experience WBV above the A(8) action value [19, 20]; and are at increased risk of low back and neck pain compared to a reference cohort [19]. The evaluation of WBV in professional drivers of heavy industrial machinery, forklift trucks, and container trucks has also shown that exposures routinely reach A(8) or VDV action values [21, 22], and that the cumulative exposure to WBV is associated with low back pain [21] and possibly an increased risk of cervical disc herniation [22]. A rat model of WBV has been established to study the biomechanical, behavioral, and biological effects of vibration applied along the long-axis of the spine [23-26]. A single sinusoidal WBV exposure at approximately the A(8) daily exposure limit of 1.15m/s^2 using a peak-to-peak displacement (s_{ptp}) of 5.0mm at 8Hz (a_{rms} of 4.47m/s^2) is sufficient to induce sustained behavioral sensitivity (i.e. pain) and neuroinflammation in both the lumbar and thoracic regions of the spinal cord [23, 24]. In-vivo animal models like that provide valuable tools to investigate the mechanisms of pain development following WBV, as well as the effects of vibration profiles typical of both occupational and therapeutic exposures.

WBV at the resonance of the spine has been suggested to damage spinal structures [12]. The first principal resonant frequency (f_r) along the long-axis of the spine for seated human subjects was defined at 4-6.5Hz [27-29], and the resonance remains within this range even for changes in posture [30]. The f_r in other species has been estimated at 4.5Hz in rabbits [31], 5Hz in rhesus monkeys [32], and 8-9Hz in rats [24]. Together, these studies have defined resonance using vibration exposures of either a single a_{rms} or s_{ptp}. Yet, the human spine exhibits a 'softening' effect, with f_r in seated subjects decreasing from 5.4Hz to 4.2Hz as the a_{rms} of a random vibration signal increases over 0.25-2.5m/s^2 [33]. Similar variability in resonance is reported for sinusoidal WBV exposures in human subjects, with f_r decreasing from 6.5Hz to 4.5Hz as a_{rms} increases from 0.1-1.6m/s^2 [29]. Mechanical testing of isolated human cadaveric intervertebral discs

has also demonstrated non-linear behavior under vibration loading, with a jump phenomenon at resonance that varies with the magnitude of displacement, preload simulating the upper body mass, and whether the frequency increases or decreases [34]. Despite these efforts, the f_r of the spine has not been defined in terms of the exposure acceleration, nor has it been characterized over the wide range of frequencies that may be encountered during occupational WBV exposures [12, 27]. Additionally, there are limited data concerning the effects of different WBV exposures on pain onset.

Despite the detrimental aspects of occupational WBV, there is increasing recent interest in WBV as a therapeutic intervention, particularly low-amplitude, high-frequency WBV to treat low back pain [7, 8], improve balance [9-11], and increase bone mineral density in populations at risk of osteoporosis [9-11, 35, 36]. WBV therapy has shown little [37] or no [38, 39] efficacy on bone mineral density. However, interpreting therapeutic WBV studies can be problematic due to a lack of standardization in the application or documentation of the direction, frequency, displacement, and acceleration of the WBV exposures.

In-vitro and in-vivo studies have reported gene expression in the disc to be altered by both frequency and acceleration [40, 41]. However, those studies also show that, compared to controls, the modulation of both anabolic and catabolic genes is variable over time, and with the vibration exposure. A single 30-minute exposure to sinusoidal vibration at 15Hz along the long-axis of the spine at an a_{rms} of 2.09m/s^2 transiently induces anabolic gene expression in a mouse model [41]. Comparable results are observed in the discs at 6hrs after vibration is applied vertically to free-standing mice on a platform (a_{rms} of 2.09m/s^2 at 45Hz) [41]. However, the same in-vivo exposure applied repeatedly for 30min/day over 5 days each week for 4 weeks led to disc degeneration and knee osteoarthritis [42, 43]. Similarly, repeated (30min/day for 7 days) WBV exposures at 15Hz and an a_{rms} of 4.71m/s^2 applied along the rat's spinal axis induces sustained behavioral sensitivity and expression

of brain-derived neurotrophic factor and nerve growth factor in the discs [25]. Together, these studies highlight not only the potentially detrimental effects of repeated WBV exposure, but also that different frequencies or accelerations, even in a single exposure, may have effects on cellular physiology.

The differences between the frequency response and acute and repeated exposures across models and species, combined with large variations in vibration profiles and exposure methods in the literature complicates efforts to define the relative benefits and detriments of various WBV exposures. Accordingly, a sine-sweep analysis was used to characterize the resonant frequency of the spine at different s_{ptp} in an established in-vivo rat model [23-26] to understand the mechanical effects of displacement and acceleration on the resonance of the spine over a range of frequencies commonly encountered in occupational and military environments. We sought to implement the relationship between the a_{rms} of WBV exposure and the f_r defined from those sine-sweep tests to assess the behavioral pain response to resonant and non-resonant WBV over a range of frequencies and accelerations equivalent to both occupational and therapeutic exposures.

Methods

Procedures were approved by our Institutional Animal Care and Use Committee and followed the recommendations of the Association for Assessment and Accreditation of Laboratory Animal Care International. Male Holtzman rats (Envigo; Indianapolis, IN, USA) weighing 285-399g at the start of the study were housed with a 12-hour/12-hour light/dark cycle and free access to food and water.

All rats were exposed to sinusoidal WBV under isoflurane inhalation anaesthesia (4% induction, 2% maintenance). Rats were positioned prone on a platform with straps behind the shoulders and above the pelvis, and WBV was applied along the long-axis of the spine [23-26]. The platform applied vibration as controlled by an electromechanical

shaker (K2007E01; The Modal Shop; Cincinnati, OH, USA), with its displacement measured using a laser displacement sensor (LTC-050-10; MTI Instruments; Albany, NY, USA). A uniaxial accelerometer (5g; 49.0m/s^2 capacity; 7521A2; Dytran Instruments; Chatsworth, CA, USA) was affixed to the platform and another mounted on a Velcro strap that was firmly strapped around the thoracic region of the rat [23-25]. Both the control, and acquisition, of the displacement transducer and accelerometers were performed using a custom control system in Labview software (Version 12.0.1; National Instruments; Austin, TX, USA), with all data acquired at 1250Hz.

Resonance characterization study

A group of rats (n=8; 353±11g) was exposed to vibrations with peak-to-peak s_{ptp} of 1.5mm and 5.0mm to match those used in previous studies [23-26]; a second group (n=8; 381±33g) was exposed to sine sweeps with s_{ptp} of 0.435mm, 0.735mm, and 2.5mm in order to define the resonant frequency (f_r) over a wider range of displacement exposures. Vibration at each displacement was performed from 3-15Hz at 1Hz intervals. Due to limitations of the power capacity of the shaker, for the 2.5mm and 5.0mm s_{ptp}, the maximum frequency was limited to 12Hz and 9Hz, respectively. For each s_{ptp}, WBV was performed in increasing frequency. Each frequency was applied for 1min, with approximately 2min between each exposure to minimize any residual effects of the prior test(s).

Analysis of each 1min exposure was completed using the last 40sec to ensure that the exposure was stable. The actual s_{ptp} for each exposure was calculated using a fast Fourier transform of the displacement transducer data, and the accelerometer data were filtered with a 5th order Butterworth filter (Matlab 2010; Mathworks; Natick, MA, USA) with a cut-off frequency of 1.5 times the exposure frequency [44]. The transmissibility (T) was calculated at each frequency for each s_{ptp} by dividing the a_{rms} of the rat by that of the platform [24]. The f_r at each s_{ptp} was defined as the frequency at which the peak

transmissibility (T_p) occurred. To assess the effect of resonance on T, T_p was compared to T at all other frequencies with paired t-tests, and a Ryan-Holm-Bonferroni step-wise multiple comparison procedure was used to preclude the inflation of type I error rate [45]. A significance level of 0.05 was used in all comparisons. The relationship between the f_r and the a_{rms} was determined using a power regression analysis.

Behavioral response study

The relationship between the WBV exposure a_{rms} and the f_r defined from the resonance characterization study was used to determine exposures to assess the behavioral response for resonant and non-resonant WBV over a range of frequencies and accelerations. Since a single exposure at 8Hz at the A(8) limit value of 1.15m/s^2 has been shown to induce behavioral sensitivity [23, 24], exposures were performed at 8Hz to probe whether sensitivity would be induced within occupational limits at that frequency. Additional exposures were based on studies reporting long-term degenerative effects of high-frequency, low-amplitude WBV in mouse [42, 43]; our study aimed to determine if a *single* exposure of the same WBV profile, and exposures at the *same* s_{ptp} or a_{rms} at resonant frequency, would induce sensitivity.

Five groups of rats underwent different WBV exposures, three of which were predicted to be resonant exposures, and three of which had an a_{rms} exposure of 2.09m/s^2 to match the a_{rms} that induces disc degeneration after repeated WBV in an in-vivo mouse model [42, 43] (Table 1). All rats (weighing 340±27g at start of procedures) were exposed to a single WBV or sham exposure for 30min. One group (n=6) was exposed to s_{ptp} of 0.074mm at 45Hz to match previous studies [42, 43], equating to an a_{rms} of 2.09m/s^2. A second group (n=6) was exposed to s_{ptp} of 8.1mm at 4.3Hz, to match the predicted f_r for an exposure with an a_{rms} of 2.09m/s^2; a third group (n=6) was exposed to s_{ptp} of 0.074mm at 17.1Hz, to match the predicted f_r for an exposure with a s_{ptp} of 0.074mm. Two groups were exposed to 8Hz WBV: one group had s_{ptp} of 0.974mm at 8Hz (n=4), since this

displacement was predicted to be resonant at 8Hz, and another group (n=4) was exposed to s_{ptp} of 2.338mm at 8Hz, to match the a_{rms} of 2.09m/s^2 used in the 4.3Hz and 45Hz groups. The sham group (n=6) underwent the same anaesthesia and behavioral test protocols but no WBV. Analysis of accelerometer data for the 30min exposures was performed over the last 29min using the same method as described in the resonance characterization study above.

Behavioral sensitivity was taken as the proxy for pain and determined by separately measuring the forepaw and hind paw withdrawal threshold to mechanical stimulation [26]. Behavioral testing was performed by the same tester at baseline (D0) immediately before WBV, and on days 1, 3, 5, and 7. The withdrawal threshold was defined as the lowest von Frey filament to elicit a paw withdrawal response, which was confirmed by a response to the next highest filament [26, 46]. This procedure was repeated separately for the forepaw and hind paw three times for each rat, with at least 10min between assessments. The mean value of all rounds was taken as the withdrawal threshold for the either forepaw or hind paw at each day.

Global comparisons of behavioral sensitivity and WBV were completed using a repeated-measures ANOVA with exposure as a covariate; a Greenhouse-Giesser correction was applied for violations of sphericity, and Bonferroni post-hoc analysis made global comparisons between groups (SPSS Version 22.0.0.0; IBM; Armonk, NY, USA). Comparisons between groups at each time point were completed using independent t-tests and a Ryan-Holm-Bonferroni step-wise multiple comparison procedure to preclude the inflation of type I error rate [45]. Results were confirmed by also completing paired t-tests and Ryan-Holm-Bonferroni step-wise multiple comparison procedures at each follow-up time relative to baseline within each group.

Results

Characterization of resonant frequency as a function of vibration amplitude

The applied s_{ptp} during the sine-sweeps were within 2.5% of target values and were 4.960±0.092mm, 2.478±0.034mm, 1.486±0.060mm, 0.739±0.025mm, and 0.442±0.019mm. Sine-sweeps at all s_{ptp} demonstrated a typical transmissibility (T) curve, with an initial value of approximately 1, rising to a peak at resonance, and then decreasing beyond the resonant frequency (Fig. 1). The transmissibility displacements of 2.5mm, 0.735mm, and 0.435mm continued to decrease as the frequency increased beyond resonance, whereas displacements of 5.0mm and 1.5mm exhibited a second peak at 9Hz and 14Hz respectively (Fig. 1), which may correspond to secondary resonance, as has been reported with human volunteers [27, 33].

The sine-sweep tests demonstrated that f_r decreased with increasing WBV displacement magnitude; T_p occurred at 10Hz, 9Hz, 7Hz, 6Hz, and 5Hz for s_{ptp} of 0.435mm, 0.735mm, 1.50mm, 2.50mm, and 5.00mm, respectively (Fig. 1). This reduction in the f_r also corresponded to an increase in the a_{rms} (Fig. 2). Mean T_p values for each s_{ptp} ranged between 1.57 and 1.89 (Fig. 1). For exposures with a s_{ptp} of 1.5mm and 2.5mm, the T_p was significantly greater than the T at all other frequencies ($p>0.026$), suggesting resonance at or close to the T_p frequencies of 7Hz and 6Hz respectively. The exposures at other s_{ptp} led to the T_p not being significantly different from either or both adjacent T values (Fig. 1); suggesting that the true resonance may lie close to, but not exactly at, the integer frequencies used for these sine sweeps. Only the exposure with a s_{ptp} of 5.0mm resulted in the T_p not being significantly different from a non-adjacent T value. However, the transmissibility curve suggests this may be due to a secondary resonance (Fig. 1). A power regression of the a_{rms} based on the mean s_{ptp} at f_r, plotted against the f_r resulted in an excellent fit ($R^2=0.970$) for the equation $a_{rms}=15.708f_r^{-1.392}$ (Fig. 2), providing good

confidence in being able to predict the resonance for different vibration exposures based on the a_{rms}.

Behavioral response to resonant and non-resonant vibration exposures

Similar to the resonance characterization study, the system was able to apply vibrations close to the target s_{ptp} values for each exposure (Table 2). Based on the characterization study, exposures predicted to be at resonance were expected to have T values greater than 1.5, and this was the case (Table 2). In contrast to the T value of 1.61±0.10 at the resonant exposure of 2.09m/s^2 at 4.3Hz, the non-resonant exposures of the same a_{rms} at 8Hz and 45Hz had T values of only 1.55±0.24 and 0.23±0.14, respectivelySimilarly, while both 8Hz exposures had a T value above 1.0, the resonant 0.974mm exposure was greater (1.85±0.32) than the 2.338mm exposure (1.55±0.24) (Table 2).

The withdrawal threshold after both 8Hz WBV exposures, with s_{ptp} of either 2.338mm or 0.974mm, was significantly reduced compared to sham and baseline values (Fig. 3), indicating a pain response to that WBV. The global analyses showed that both 8Hz exposures led to significantly different withdrawal thresholds compared to all other groups in both the forepaw ($p<0.042$) and hind paw ($p<0.001$). There were no differences detected between the 4.3Hz, 17.1Hz, 45Hz or sham groups in either the forepaw or hind paw ($p>0.999$).

The withdrawal thresholds were also compared between groups at each day and within groups relative to their pre-exposure baseline responses. There was no difference between any group at baseline in either the forepaw ($p>0.395$) or hind paw ($p>0.634$), and there was no difference between baseline and any follow-up time in the sham group with no WBV exposure, in the forepaw ($p>0.999$) or hind paw ($p>0.999$). This suggests all groups were responding similarly at baseline, and that the sham group was not affected by anaesthesia or handling. In agreement with the global analysis, the withdrawal

threshold did not change relative to respective baseline or sham at any time in either the forepaw ($p>0.053$) or hind paw ($p>0.137$) for the 8.100mm at 4.3Hz WBV, the 0.074mm at 17.1Hz WBV, or the 0.074mm at 45Hz groups. Also in agreement with the global analysis, both exposures at 8Hz significantly lowered the withdrawal thresholds (i.e. induced pain) compared to sham and their respective baseline values (Fig. 3). The withdrawal thresholds from WBV exposures of 2.338mm at 8Hz decreased by day 1 to 3.28±1.69 in the forepaw and 5.00±1.28 in the hind paw, which were significantly lower than sham (9.83±1.81 and 19.39±6.57 respectively; $p<0.010$) and baseline (9.33±0.54 and 17.33±1.74 respectively; $p<0.008$), and remained decreased at all follow-up times in both the forepaw ($p<0.010$) and hind paw ($p<0.010$) (Fig. 3). Although WBV exposure of 0.974mm at 8Hz did decrease the withdrawal threshold compared to sham and baseline at all follow-up times in the hind paw ($p<0.039$) and at day 1 and day 3 in the forepaw ($p<0.033$), sensitivity was not sustained at later times in the forepaw ($p>0.082$) (Fig. 3).

Discussion

Although the increased risk of back pain and injury due to occupational WBV has been widely documented in epidemiological studies, and has been supported by studies using in-vivo animal models [23-26, 42, 43], this is the first study to define the non-linear resonance of the spine in relation to the exposure acceleration (Fig. 2). Further, a single WBV exposure at 8Hz, which is in the range of a low-frequency (<20Hz) occupational exposure, leads to the development of pain, which is sustained at through day 7 (Fig. 3). In contrast, a single WBV at a high-frequency (>20Hz) in the reported-therapeutic range does not lead to the development of pain (Fig. 3). These findings suggest a frequency-dependent behavioral response (Fig. 3), which is not related to resonance or transmissibility (Table 2). Interestingly, exposures of the same acceleration but *different* frequencies induce different pain outcomes, while exposures at the same frequency but

different acceleration produce differences in the maintenance of pain following the exposure (Fig. 3).

Previous studies have shown that WBV at 8Hz with an approximate acceleration of 4.47m/s^2 induces sustained pain in both the forepaw [24] and hind paw [23], while similar exposures at 15Hz induce less robust, short-lasting forepaw [24, 26] and hind paw [23, 26] sensitivity. The current results show that at 8Hz, exposures with acceleration as low as 0.775m/s^2 induce sustained pain in the hind paw and transient pain in the forepaw (Fig. 3), while exposures with a 1.863m/s^2 acceleration induce sustained pain in both the paws through day 7 (Fig. 3). Although the WBV at 4.3Hz and an a_{rms} of 1.893m/s^2 is along the resonance curve (Fig. 2), and the T value was higher in these exposures than non-resonance exposures of the same acceleration magnitude (8Hz and 45Hz) it does *not* induce behavioral sensitivity (Fig. 3). This finding suggests that pain from WBV is frequency-dependent but does not require that the vibration be at resonance, which is in contrast to the notion that the greatest risk for damage to the spine is from WBV at resonance [12].

The determination in the resonance characterization study that the resonant frequency decreases as the vibration acceleration increases is consistent with previous human studies [29, 33], and cadaveric isolated disc units in-vitro [34]. It has been suggested that such a softening effect is caused by multiple factors relating to material properties, spinal buckling, and muscle properties and/or activation [29]. However, for this investigation, since the rats were anaesthetised during the vibration exposure, muscle activity was not present. The same softening effect was reported by Marini et al [34] in the majority of cases of isolated disc specimens tested in-vitro at 0.1-0.2mm amplitudes with varying compressive preloads, where muscle function was not involved, and buckling was prevented due to testing with single-level specimens constrained to allow movement only along the spine's long-axis. So, while the effects of muscle action and buckling cannot be

discounted in the in-vivo spine, and may affect the response to vibration, the softening effect that is observed is likely primarily due to the material properties of the intervertebral disc. Marini et al [34] reported that the softening response was observed after endplate damage in some specimens and suggested that the collagenous structure of the annulus fibrosus leads to the non-linear vibration response. Large increases in intervertebral disc stiffness, and moderate increases in intervertebral disc damping coefficients have been estimated by comparing quasistatic loading to impact loading [47], which could lead to an increase in the resonant frequency. Yet, it is possible that these conditions do not replicate the continued nature of vibration exposures, which may affect the solid and fluid phases of the disc in a different way. However, an in-vivo study does enable investigating these possible mechanisms. Further studies specifically focusing on the solid/fluid phases and effects of loading rate and exposure type would provide greater understanding of the mechanisms leading to such non-linear behaviour.

As stated above, it is possible that the natural muscle tension, or muscle activation present during normal exposures to WBV may affect the response of the spine to vibration, and whether or not pain develops. However, to ensure that the vibration exposure was directed along the length of the spine, the present study constrained rats on the vibration platform. In order to balance technical and ethical requirements for such research, anesthesized rats were used but such studies are limited by not including muscle activity that would otherwise be present. Future research studies addressing this limitation would be beneficial.

Resonance is measured here at the thoracic spine, using an accelerometer strapped around the rat. This may introduce inaccuracies in the accelerometer measurements due to movement of the skin and/or tissue relative to the spine. However, prior work with this same model and approach has shown that the response measured at the thoracic region adequately captures the true response of the spine when compared to

an accelerometer rigidly fixed to the vertebrae [24]. In addition, the variable resonant frequency identified here, which decreases as vibration acceleration increases, is consistent with previous human studies using an accelerometer attached to the skin over the lumbar region [29, 33]. Together, these findings suggest that the resonance as a function of the a_{rms} identified in this study (Fig. 2) is an accurate measurement of the transfer of vibration along the spinal column. In addition to the peaks in transmissibility used to determine the primary resonant frequency, the resonance characterization study identified possible secondary resonance during exposures at 5mm and 1.5mm. It is likely that secondary resonance was not identified at other amplitudes due to the secondary resonance being beyond the frequency range of 3-15Hz used for amplitudes of 0.735mm and 0.435mm, or beyond the 3-9Hz range used at the amplitude of 1.5mm. The vibration exposure of 0.074mm at 45Hz led to an actual acceleration of 2.809±0.119m/s^2 (Table 2), which was higher than that calculated (2.09m/s^2) for a sinusoidal signal. In addition, the actual s_{ptp} was 0.069±0.003mm (Table 2), suggesting that the quality of the sine wave may have been less well-maintained at the higher than lower frequency exposures, where divergence from the theoretical a_{rms} was not as pronounced (Tables 1 and 2). Nevertheless, despite this increased acceleration compared to other groups (Table 2), behavioral sensitivity was not induced at any time point in either paw (Fig. 3), emphasizing the finding that specific frequencies may be more likely to result in pain, even when compared to exposures of higher acceleration magnitude (Table 2).

The frequency-dependent effect of WBV on pain observed here may be due to a host of physiological mechanisms, among them vibration-induced inflammation [23, 24]. Previous WBV studies using the same 8Hz and 15Hz WBV have shown that only 8Hz WBV induces sustained pain and upregulation of kinase pathways in the dorsal root ganglia and spinal cord, as well as activated inflammatory cells in the dorsal horn of the spinal cord [23, 24], which have been linked to pain onset and maintenance, nociceptor

sensitization, and even chronic pain following peripheral injury [48, 49]. Increased expression of the inflammatory cytokine IL-1β in the intervertebral disc was also found after repeated exposures to high-frequency WBV [43], which were of the same 45Hz, 0.074mm profile used in this study. In-vivo studies of vibration training in older adults have reported mixed results of inflammatory markers in the blood, with one study finding an exposure of 20-35Hz at an amplitude of 4mm used over an 8-week period having no change in either the pro-inflammatory or anti-inflammatory cytokines [50], but exposures of 30-45Hz at s_{ptp} of 2mm over 9 weeks led to an upregulation of IL-10, and a decrease in the concentration of the pro-inflammatory TNFα [51]. Since those studies adopted similar exercises, and time periods of 30-60 seconds per exercise, it is possible that the differences in inflammatory responses may be due to differences in the vibration frequency and/or acceleration.

It is important to note that this in-vivo rat model of the present study used only male Holtzman rats, in order to enable comparison with prior studies using the same model [23-26]. DeLeo et al [52] reported no difference in mechanical allodynia between male and female Holtzman rats after an L5 spinal nerve transection, but found allodynia to be significantly greater in female Sprague-Dawley rats than males of the same strain. In contrast, Gaudet et al [53] reported that male Sprague-Dawley rats exhibited mechanical allodynia after spinal cord injury, but female rats of the same strain did not despite both sexes exhibiting thermal hyperalgesia. As such, caution must be taken in generalizing findings of the present study to females, and further research comparing sex effects in response to WBV is necessary.

While the EU Directive on WBV [14] has adopted the use of the A(8) exposure to ensure that the health risk due to vibration is minimized in the workplace, including the use of the action and limit values of 0.5m/s^2 and 1.15m/s^2 respectively, there is limited

evidence for the establishment of these values. Both exposures at 8Hz in this study produced an A(8) exposure within the action limit of 0.5m/s^2 (Table 2), but even the lower A(8) exposure of 0.200±0.001m/s^2 from a displacement of 0.974mm leads to transient pain in the forepaw and sustained pain in the hind paw (Fig. 3). Despite having similarities in anatomy [54], there are limitations in scaling such exposures between the rat and the human. However, if pain arises from a frequency-dependent cellular response, it is possible that similar accelerations could also induce pain in the human. This requires further research in order to update frequency-weightings and occupational guidelines to minimize pain and injury resulting from WBV.

Although pain was not produced for the high-frequency (0.074mm at 45Hz) WBV (Fig. 3), which had an A(8) exposure within the action value of 0.5m/s^2 (Table 2), the same vibration exposure leads to disc degeneration and knee osteoarthritis in mice exposed daily for 4 weeks [42]. This suggests that repeated high-frequency WBV may lead to musculoskeletal damage, even when single exposures do not induce discomfort or pain. The harm that high-magnitude accelerations used in therapeutic WBV may cause has been reported [55], and an assessment of the vibration profiles used in 27 random, controlled studies evaluating WBV in human subjects [9, 11, 35, 36, 56-78] shows that the majority (18/27) used exposures in excess of the 1.15m/s^2 A(8) limit value [11, 35, 36, 56-70] (Figure 4). The acceleration transmitted to the lumbar spine for vibrations applied through the feet to standing human subjects is greater than 50% of the platform acceleration, even with the knees in a flexed position [79]. Considering the large accelerations used in many therapeutic WBV studies (Fig. 4), it is possible that the vibrations at the lumbar spine would still exceed the A(8) limit value, and have the potential to cause long-term damage.

The findings in this study suggest that the development of pain following even a single WBV exposure is frequency-dependent. Defining the relationship(s) between

mechanics of WBV, the potential or real peripheral injury, and the central sensitization cascades that drive long-term pain will provide insight into the relative trade-offs of different WBV profiles, specifically as they relate to both occupational and therapeutic WBV. However, this research also demonstrates the importance of defining and understanding the potential long-term sequelae of therapeutic exposures. Although the nature of inflammation and pain due to WBV across the range of both occupational and therapeutic exposure profiles is not yet fully understood, this research clearly shows that considering the accelerations that subjects are exposed to is critical in minimizing exposure to harmful vibrations in light of occupational standards and regulations.

Data Availability

Supporting datasets have been uploaded as part of the supplementary material.

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Conflict of interest statement

The authors declare no conflicts of interest.

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Figure Captions

Figure 1. The resonant frequency decreases as s_{ptp} increases. For 5.00mm displacement, T_p at 5Hz is significantly higher than at all other frequencies ($^{+}p<0.020$), except 6Hz and 9Hz ($p=0.194$). For a 2.50mm displacement, the T_p at 6Hz is significantly greater than T_p at all other frequencies ($^{*}p<0.004$). Similarly, the T_p for 1.50mm is at 7Hz, which is significantly greater than all other frequencies ($^{\#}p<0.026$). T_p for 0.735mm is at 9Hz and is significantly greater than at all other frequencies ($^{\wedge}p<0.004$) except 10Hz ($p=0.66$). For 0.435mm the T_p is at 10Hz and is significantly higher than at other frequencies ($^{*}p<0.004$), except 9Hz and 11Hz ($p=0.102$).

Figure 2. The relationships of a_{rms} with respect to the f_r is defined by a power regression and the resulting regression curve describes the predicted resonance. The decreasing f_r with increasing a_{rms} of the WBV exposure is evident. The power regression provides an excellent fit over the 5-10Hz range of measured f_r.

Figure 3. Behavioral sensitivity is measured by withdrawal threshold at times immediately before (day 0; D0) and for 7 days after WBV in the forepaw and hind paw. Exposures of 2.388mm or 0.974mm at 8Hz significantly lower withdrawal thresholds from responses before exposure (D0) and relative to sham exposure at all days in the hind paw ($^{+}p<0.039$), and at days 1 (D1) and 3 (D3) in the forepaw ($^{*}p<0.033$). Only the exposure of 2.338mm at 8Hz induces sensitivity in the forepaw at days 5 (D5) and 7 (D7) ($^{\#}p<0.010$). 8Hz groups are only different at day 5 (D5) in the forepaw ($^{\wedge}p=0.049$).

Figure 4. WBV exposures of both the current behavioral study using an in vivo rat model, and random controlled in-vivo human studies of therapeutic WBV converted to A(8) exposures are shown together. Over half (18/27) of the human studies used an exposure greater than the A(8) limit. Studies include a wide variety of displacements, frequencies, and durations.

Table 1. Vibration exposures of groups of rats for behavioral analysis

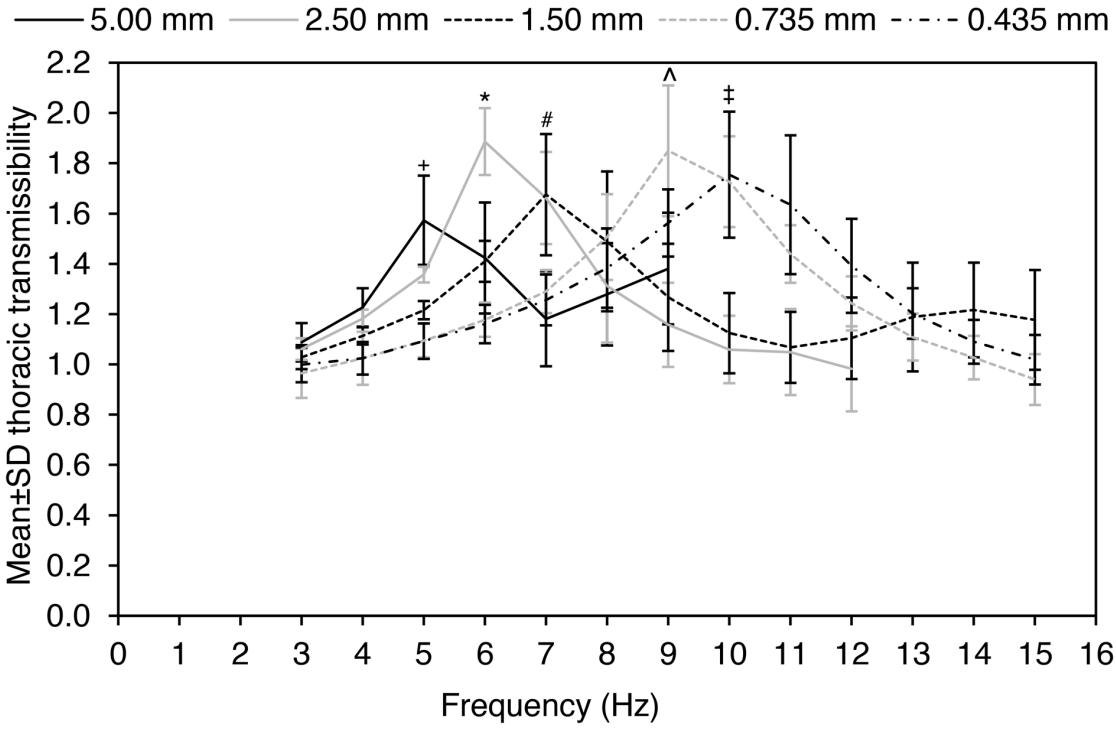
s_{ptp} (mm)	f (Hz)	a_{rms} (m/s^2)	Group size	Predicted resonance	A(8) (m/s^2)
8.100	4.3	2.09	6	Yes	0.522
2.338	8.0	2.09	4	No	0.541
0.974	8.0	0.87	4	Yes	0.225
0.074	17.1	0.30	6	Yes	0.055
0.074	45.0	2.09	6	No	0.145

Note: The frequency weighting (W_k) used to calculate the A(8) exposure was calculated according to Annexe A of ISO 2631-1:1997 [13].

Table 2. Mean±SD s_{ptp} during the behavioral response study were applied within 10% of the target values, and all exposures predicted to be at resonance had a T>1.5.

Freq. (Hz)	Target s_{ptp} (mm)	Actual s_{ptp} (mm)	Actual a_{rms} (m/s^2)	Actual A(8) (m/s^2)	T
4.3	**8.100**	**8.261±0.093**	**1.892±0.032**	**0.472±0.008**	**1.61±0.10**
8.0	2.338	2.336 ±0.054	1.865±0.051	0.483±0.013	1.55±0.24
8.0	**0.974**	**0.964±0.005**	**0.772±0.003**	**0.200±0.001**	**1.85±0.32**
17.1	**0.074**	**0.071±0.005**	**0.311±0.019**	**0.057±0.004**	**1.51±0.38**
45.0	0.074	0.067±0.003	2.809±0.119	0.194±0.008	0.23±0.14

Note: The frequency weighting (W_k) used to calculate the A(8) exposure was calculated according to Annexe A of ISO 2631-1:1997 [13]. Rows shown in bold represent exposures predicted to occur at resonance.



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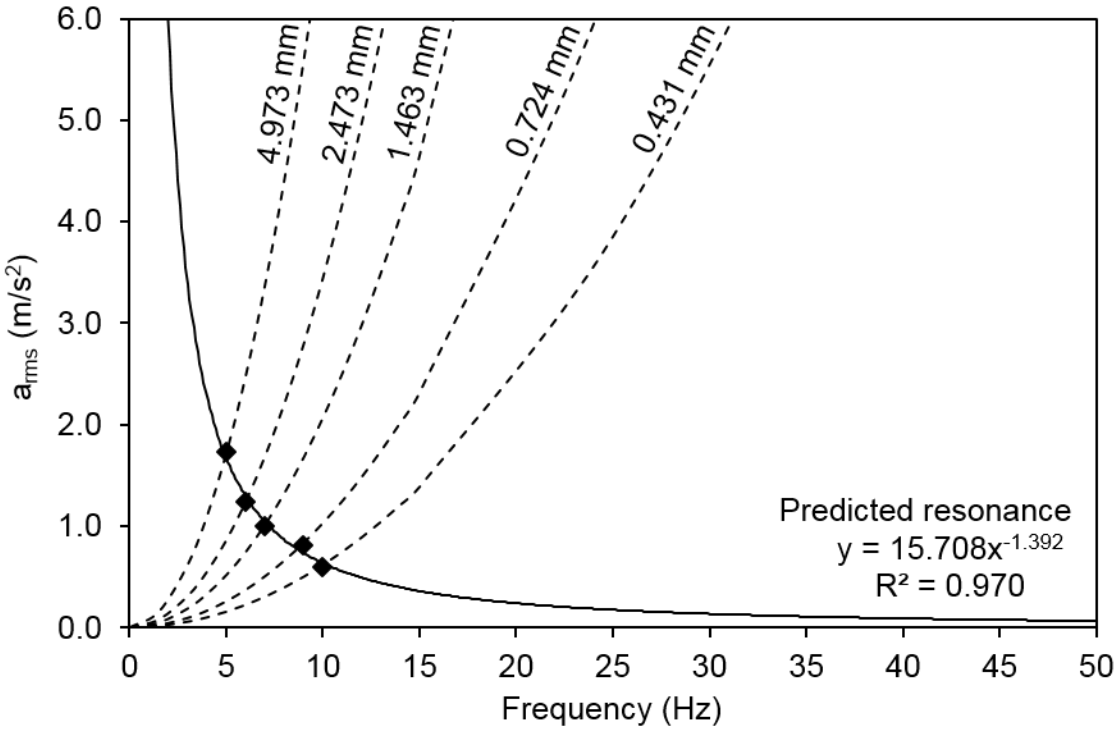

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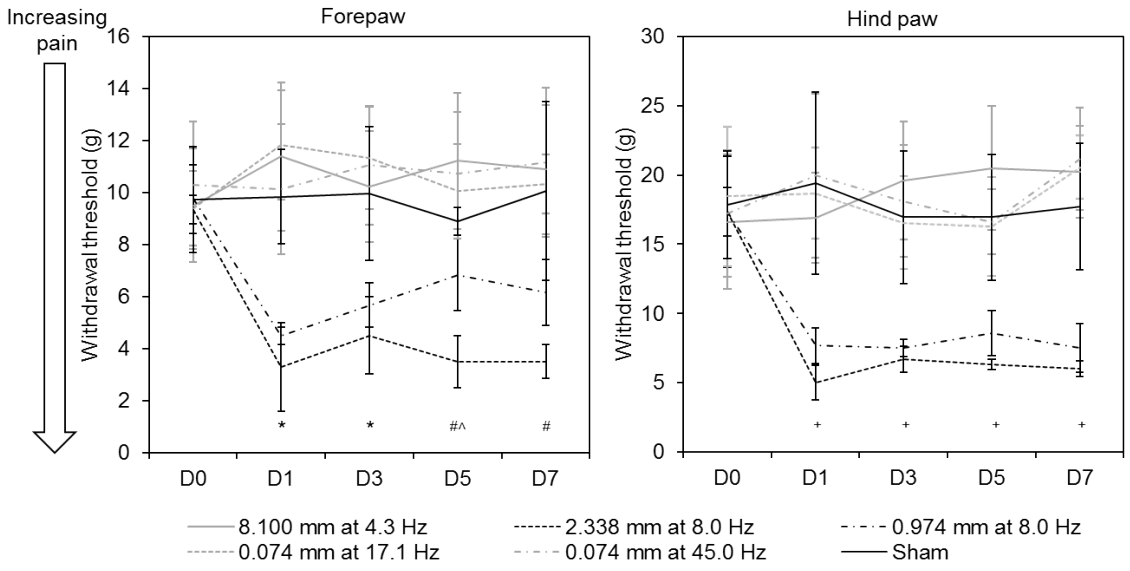

1

2 **Figure 3.** Behavioral sensitivity was measured by withdrawal threshold at times
3 immediately before (day 0; D0) and for 7 days after WBV in the forepaw and hind paw.
4 Exposures of 2.388mm or 0.974mm at 8Hz significantly lower withdrawal thresholds from
5 responses before exposure (D0) and relative to sham exposure at all days in the hind paw
6 ($^{+}p<0.039$), and at day 1 (D1) and day 3 (D3) in the forepaw (*$p<0.033$). Only the exposure
7 of 2.338mm at 8Hz induces sensitivity in the forepaw at day 5 (D5) and day 7 (D7)
8 ($^{\#}p<0.010$). The 8Hz groups are only different at day 5 (D5) in the forepaw (^$p=0.049$).

9

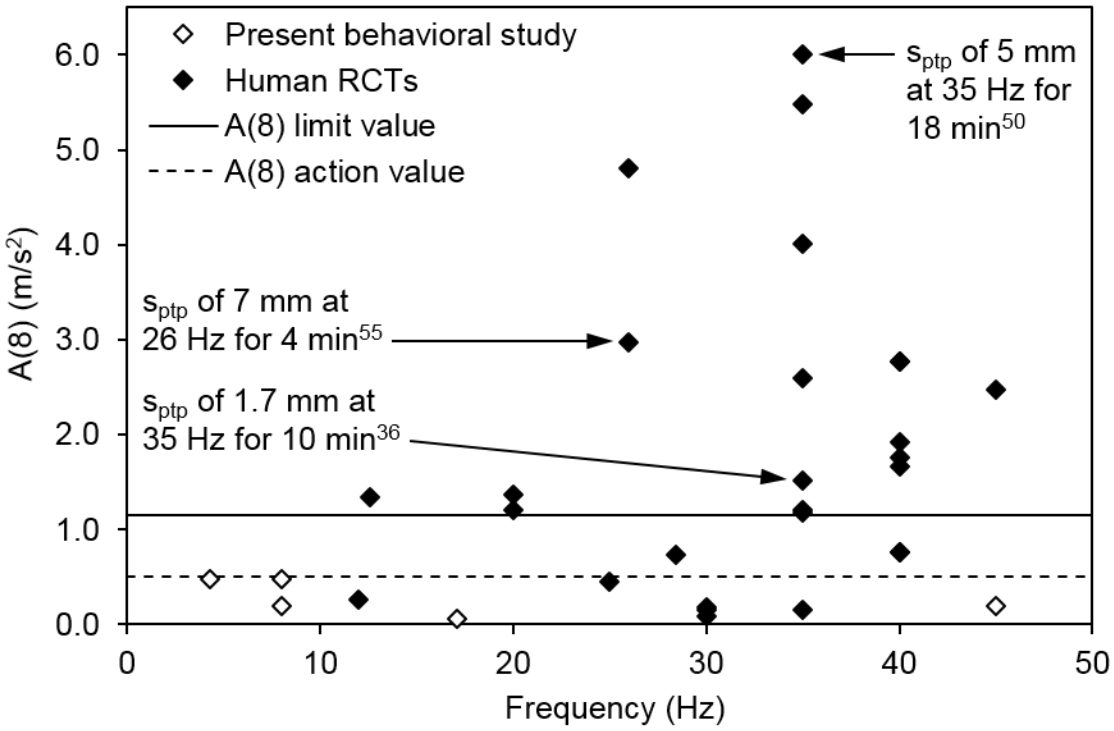

Figure 4. WBV exposures of both the current behavioral study using an in vivo rat model, and random controlled in-vivo human studies of therapeutic WBV converted to A(8) exposures are shown together. Over half (18/27) of the human studies used an exposure greater than the A(8) limit. Studies include a wide variety of displacements, frequencies, and durations.