Effects of different vibration frequencies on spinal cord reflex circuits and thermoalgesic perception

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Abstract

Objectives: We studied the effect of different vibration frequencies on spinal cord excitability and heat pain perception. We hypothesized that the effects of vibration on spinal cord reflexes, and, also those on heat pain perception, depend on vibration frequency. Methods: In 9 healthy subjects, we applied vibration over the tibialis anterior muscle at three different frequencies (50, 150, or 250 Hz) on spinal cord reflex excitably, tested with the H reflex and the T wave in the soleus muscle, as well as on sensory and pain perception, tested by measuring warm perception (WT) and heat pain perception thresholds (HPT) in sites rostral and caudal to vibration. Exams were carried out before, during, and after vibration. Results: The amplitude of the H reflex and T wave significantly decreased during vibration in comparison to baseline. Low frequencies (50 and 150Hz) induced greater reflex suppression than high frequency (250Hz). No significant changes were observed on WT and HPT. Conclusions: The effects of vibratory stimulation can be summarized as frequency-related suppression of the spinal cord excitability without an effect on warm and heat pain perception. The present results may help to design vibration-related interventions intended to diminish spinal cord reflex excitability in spastic patients.

Keywords: Heat Pain Perception, Lower Limbs, Spinal Cord Reflex Circuits, Vibration Frequencies, Warm Perception

Introduction

Focal vibratory stimulation, which consists in applying portable vibrators over the tendon or muscle belly, has been employed in healthy individuals to enhance training related muscle strength and neuromuscular adaptation1. In the clinical context, vibratory stimulation is used to prevent muscle weakness due to hypoactivity or long-term immobilization2, as well as to reduce spasticity, promote motor activity and facilitate motor learning in patients with spinal cord injury (SCI), stroke, multiple sclerosis and movement disorders (for details see review by Murillo et al.)3. In rehabilitation, vibratory stimuli effectively reduce spasticity and spinal reflex excitability on soleus muscle in spinal cord injury subjects4,5. This was the case using vibration at 50 Hz over quadriceps muscle4, or 30 Hz vibration over the tibia6, in healthy volunteers and SCI subjects. Achilles or tibialis anterior tendon vibration at 40 and 80 Hz but not 20 or 120 Hz suppresses late spasm-like activity in antagonist muscles in SCI subjects5. A decrease of the H reflex size in healthy subjects has been reported with vibration over the Achilles tendon at 50 Hz frequency7, over the triceps surae muscles at 50 and 100 Hz8, and over the Achilles tendon or the gastrocnemius muscle at 70 Hz9. In spastic upper limbs of hemiplegic patients, the flexed elbow position was reduced with vibration at 100 Hz over the triceps brachii in association with physiotherapy10. Improvement of spasticity

The authors have no conflict of interest.

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Edited by: G. Lyritis
Accepted 23 August 2021
was reported also in post stroke patients with vibration at 300 Hz applied over triceps brachii and extensor carpi radialis longus and brevis muscles during voluntary isometric contraction\textsuperscript{11}. Vibration has also been reported to alleviate pain, both, chronic musculoskeletal pain and central pain\textsuperscript{11-13}. Using vibration at 300 Hz over triceps brachii and extensor carpi radialis longus and brevis muscles during voluntary isometric contraction in post-stroke patients, the authors reported an improvement in pain\textsuperscript{11}. In fibromyalgia, vibration at 100 Hz reduced pain significantly\textsuperscript{13}.

Despite the frequent use of muscle vibration in motor training and rehabilitation, little is known about the physiological mechanisms underlying clinical effects. Current evidence indicates that oscillatory mechanical stimulation induced by vibration activates muscle afferents and cutaneous mechanoreceptors, which in turn modulate spinal cord circuits and areas of sensorimotor integration in the central nervous system. Effects of vibration on spinal cord reflex excitability have been reported, specifically inhibition of the soleus H reflex. This may be due to an increased presynaptic inhibition, the refractory state of la afferent fibers, neuronal transmitter depletion at la terminals, postsynaptic reciprocal and non-reciprocal la inhibition, and effects from cutaneous and other afferent receptors\textsuperscript{6-8,14}. In addition, vibration-induced changes have also been reported at corticospinal level and cortico-cortical inhibitory circuits\textsuperscript{14-16}. The effects of vibration on pain may also be related to the effects on spinal and supraspinal sensory circuits, as inputs carried by sensory Aβ fibers may trigger local segmental inhibition\textsuperscript{17,18} and descending pain inhibition\textsuperscript{13}.

For instance, low-frequency vibratory stimulation (50-100 Hz) may induce reduction of muscle tone during the stimulation and, therefore, has a predominant anti-spastic effect\textsuperscript{4,6,10,19}. In contrast, stimulation at 200-300 Hz may reduce sarcopenia and increase muscle strength and motor performance after repeated applications\textsuperscript{20,21}.

We studied the physiological effects of different frequencies of vibration applied over the tibialis anterior (TA) muscle on the Soleus H reflex and T wave, as surrogate measures of spinal cord reflex excitability, and on warm and heat pain perception thresholds in dermatomes rostral and caudal to the level of vibration application, as measures of sensory integration systems. Focal vibration to the TA has been used before to examine the after-hyperpolarization time course of motoneuron firing\textsuperscript{22} and to investigate the inhibitory effects over cutaneous muscle reflexes\textsuperscript{5}. Here, we hypothesize that the effects of vibration over TA muscle on spinal cord reflexes, and, possibly, also those on heat pain perception, depend on vibration frequency.

**Methods**

The study protocol was approved by the Research Ethics Committee of the Institute Guttmann and was carried out in accordance with the Declaration of Helsinki.

Nine healthy subjects (mean age: 29.0±5.7 years; 5 males (age range: 20-35 years) and 4 females (age range: 20-49 years) were enrolled after signing a written informed consent for participation in this research study.

Inclusion criteria were: male or female between 18 and 75 years old without any neurological disorder and uncontrolled disorder which could limit the experiment and had written informed consent. Exclusion criteria were: any implanted metallic or electrical devices, or pregnancy.

**Neurophysiological evaluation**

We used routine electrodiagnostic equipment and accessories (Synergy, Oxford Instruments; Surrey, England). Ag-AgCl surface recording electrodes were attached to the skin overlying the right soleus muscle on a belly-tendon arrangement. The amplifier’s gain was predetermined at 1 mV/division and the band-pass filters were 3 Hz to 10 kHz. Stimulation electrodes (0.8 cm diameter and 2.0 cm fixed interelectrode distance) were fixed at the site over the tibialis posterior nerve in the popliteal fossa where a stable H wave could be obtained with repeated single stimuli given with an interstimulus interval of 10 s. Stimulus duration was set at 1 ms. During the preliminary procedure, once the position of the recording and stimulation electrodes was set, we obtained the supramaximal soleus M wave.

Spinal cord reflex excitability changes were assessed by measuring the following responses:

1. H reflex of maximal amplitude (Hmax). This was obtained after applying single stimuli of gradually increasing intensity in steps of 0.6 mA from the one eliciting the smallest H wave to the one eliciting maximal H reflex.

2. H10%. We adjusted stimulus intensity to obtain an H wave with a peak-to-peak amplitude of about 10% of the baseline Mmax response (before vibration). The same intensity was used to elicit H10% in the examinations during and after vibration. At each time of evaluation, we measured the amplitude of the averaged H10% wave out of 5 consecutive single stimuli separated by 10 s.

3. T wave amplitude (Twave). The T wave was recorded with the same setup while tapping with a sweep triggering hammer (Kawe reflex hammer Trömner, Germany) over the Achilles tendon applied by the same researcher (HK) trying to use similar strength and precision during the whole experiment. We measured the amplitude of the averaged response of five recordings obtained before, during and after vibration.

**Quantitative thermal testing**

A computer-controlled thermofoil heating system (Pain and Sensory Evaluation system, Pathway System, Medoc Ltd, Ramat Yishai, Israel) was employed. We used the method of limits to assess warm and heat pain perception thresholds (WT; HPT). The thermode was applied over two skin areas: at a middle site of the anterior thigh, corresponding to the dermatome L3, cranial to the site of vibratory stimulation, and the dorsum of the foot, corresponding to the dermatome...
L5, caudal to the site of vibratory stimulation. The two sites were examined in pseudo-random alternating order in the three separate sessions but always in the same order in the examinations before, during and after vibration. From each time of evaluation, we calculated the WT and HPT values after averaging the results of 4 individual stimuli.

**Study design and Vibratory stimulation protocol**

The study included 3 sessions. In each one of them, we used a different vibratory stimulation frequency (either 50 Hz, 150 Hz or 250 Hz), applied over the belly of the right tibialis anterior (TA) muscle, because vibration over muscle belly may provide more effective muscle tone reduction as compared to the application over tendon. According to literature and our experience, we suggested that the lowest frequencies (50 Hz/150 Hz) could reduce spinal cord excitability more than higher frequencies (250 Hz) and pain perception could be reduced in higher than lower frequencies. And we decided on one low frequency (50 Hz), one intermediate frequency (150 Hz) and high frequency (250 Hz). The order of sessions was randomized. The sessions were conducted in different days, separated by at least 1 week.

Subjects were sitting barefoot, reclined on a comfortable chair with supported feet stands and exposed knee and calf stimulation and recording sites. Vibration was applied over the belly of the right tibialis anterior (TA) muscle by a stimulator Vibra 3.0 (AD Swiss MedTech SA), attached with ribbons over the belly of the right TA muscle. The device was set to deliver mechano-sound square wave vibrations at an intensity of >350 mbar.

At each session, we first obtained the Hmax, M response, H10% and Twave, and determined WT and HPT (examination time ‘before’). Then, neurophysiological and psychophysical tests were carried out during vibration (examination time ‘during’), starting 5 minutes after initiation of stimulation when spinal cord reflexes are reduced in healthy and spinal cord injury subjects, as shown by Murillo et al. Then, neurophysiological and psychophysical testing was repeated immediately after finalizing the stimulation (examination time ‘after’). The total duration of each study session was around 2 hours and included: preparation of the equipment (EMG, vibratory machine and thermal stimulation) and the subject (application of recording electrodes for spinal reflexes), vibratory stimulation, and determination of the H reflex-related parameters and thermal perception thresholds before, during and after vibration (20-25 min for each evaluation).

**Data reduction and statistical analyses**

We measured peak-to-peak amplitude in the Mmax, Hmax, H10% and Twave and calculated their mean and standard deviation (SD) per each individual and examination time. We expected no effects of vibration on the Mmax response (even if we ran statistical analyses on Mmax amplitude, as described below) and, therefore, we normalized inter-individual’s data by expressing Hmax, H10% and Twave as percentages of the individual’s Mmax amplitude for each examination time.

No data reduction was necessary for WT and HPT, which values were entered in the statistical analysis once grouped per examination time and session. In all variables, we obtained data in the examination times before, during and after vibration.

The Shapiro-Wilk’s test was used to examine normality of the data. A two-way repeated measures ANOVA was used to analyse the effects of factors ‘vibration frequency’ (50, 150, 250 Hz) and “examination time” (before, during and after stimulation) on Mmax, Hmax, H10%, Twave, WT and HPT (the Mmax amplitude was included in the analyses in absolute values). A one-way repeated measures ANOVA was used to study separately the simple main effects of each vibration frequency on the same parameters. The Mauchly test was used to evaluate whether the sphericity assumption was violated, in which case the Greenhouse-Geisser correction was applied to correct the degrees of freedom of the F-distribution. Partial $\eta^2$ was calculated to measure the effect size. We considered effect sizes of 0.01 small, 0.09 medium, and 0.25 large. A Bonferroni correction was used for multiple comparisons.

A two-tailed test with an alpha level of 0.05 was used for all analyses.

All statistical analyses were performed with a commercial software package (IBM SPSS, version 13.0, SPSS Inc., Chicago, IL, USA).

**Results**

All subjects tolerated well the tests, without any dropout nor incompletion of the study. For the evaluation during vibration, the vibratory stimuli were applied for as long as the time the test needed to be completed, roughly 30 minutes.

**Modulation of spinal cord reflex excitability**

Raw data on H10%, Hmax, Hmax/Mmax and Twave are given in Table 1 while the same data represented as percentages of Mmax are shown in Figure 1. The results of statistical comparison are given in Tables 2A (two-way ANOVA) and 2B (one-way ANOVA). Figure 2 shows the EMG recording of H10% before, during and post vibration at frequency of 50 Hz, 150 Hz and 250 Hz.

The two way repeated measures ANOVA revealed a statistically significant effect of time of evaluation and time x frequency interaction on H10%, Hmax and Twave (p<0.05). Simple main effects analyses showed that H10%, Hmax/Mmax ratio and Twave significantly decreased as compared to baseline during stimulation at 50 and 150 Hz (p<0.05) and recovered when vibration was stopped (Figure 1, A, C, D). Focal muscle vibration at 250 Hz did not induce any significant changes in spinal cord excitability measures at any time of evaluation.
Table 1. Raw data of H10%, Hmax, Mmax, Hmax/Mmax ratio and T wave.

| Frequency | H10% (μV) | Hmax (μV) | Mmax (μV) | Hmax/Mmax | T wave (μV) |
|-----------|-----------|-----------|-----------|-----------|-------------|
| 50 Hz     | Before    | During    | After     |           |             |
|           | 1680.0 (311.3) | 2.2 (2.2) | 372.7 (122.9) |
|           | 6318.4 (1332.1) | 2243.2 (814.0) | 6581.1 (1298.4) |
|           | 17424.1 (3764.8) | 17431 (3764.8) | 17594.9 (3180.0) |
|           | 0.4 (0.1) | 0.2 (0.1) | 0.4 (0.1) |
|           | 2973.6 (592.9) | 762.4 (264.5) | 2721.9 (643.6) |
| 100 Hz    | Before    | During    | After     |           |             |
|           | 1616.6 (279.7) | 825.9 (496.4) | 1734.4 (489.2) |
|           | 6436.3 (993.3) | 3598.0 (1048.0) | 6947.1 (989.7) |
|           | 13233.2 (1626.5) | 13646.3 (1690.7) | 14515.2 (1560.6) |
|           | 0.5 (0.1) | 0.3 (0.1) | 0.5 (0.1) |
|           | 2712.9 (652.1) | 1673.9 (547.1) | 2563.5 (403.8) |
| 150 Hz    | Before    | During    | After     |           |             |
|           | 1272.9 (148.6) | 1566.2 (899.1) | 1833.1 (869.1) |
|           | 8643.8 (1404.0) | 6603.5 (1314.1) | 8160.0 (1128.6) |
|           | 18474.8 (2198.9) | 19295.4 (2317.9) | 17486.8 (1344.5) |
|           | 0.5 (0.1) | 0.4 (0.1) | 0.5 (0.1) |
|           | 2765.8 (479.9) | 2189.3 (473.1) | 2848.3 (505.7) |

The figures are the mean and one standard deviation (within parenthesis), given in μV for H10%, Hmax, Mmax and T wave, and as a ratio for Hmax/Mmax, for the three evaluation times and the three frequencies of vibratory stimuli.

Figure 1. A) H10%; B) Mmax; C) Hmax; D) T wave. H10%, Hmax and T wave are represented as percentages of Mmax.
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Quantitative thermal testing

Data of the two-way repeated measures ANOVA are summarized in Table 2C. Muscle vibration did not cause significant changes in WT at any stimulation site (dermatome L3, above the knee and dermatome L5, dorsum of foot), any evaluation time or any stimulation frequency (p>0.05 for all comparisons) (Figure 3 A, B).

There was a significant effect of evaluation time on HPT, when tested at the dermatome L5, dorsum of the foot (p=0.001). Further simple main effects analyses revealed an increase in HPT following stimulation at 50 Hz compared to baseline (F[2,16]=4.73; p=0.024) but the post hoc analysis did not reach significance (Figure 3D).

Discussion

Our results show that changes in spinal cord excitability with focal vibration depend on the vibratory stimulation frequency: i.e., low frequencies (50 and 150 Hz) cause greater suppression of H reflex and T wave than high frequencies (250 Hz). Furthermore, we have shown no effect of vibration at any of the frequencies tested on warm and heat pain perception thresholds, examined above and below the dermatome where vibratory stimulation was applied.

Modulation of spinal cord reflex circuits

We found a significant effect of 50 Hz and 150 Hz on the amplitude of H 10%, Hmax, and T wave and 150 Hz on the Hmax and the amplitude of Twave, while the highest frequency vibration (250 Hz) did not induce changes on the spinal cord reflex circuits.

Importantly, changes in H reflex and T wave were observed during the application of vibration, returning to baseline values after the procedure ended. Our results are in line with previous reports in healthy individuals\(^2^3\) and in patients with motor impairment due to spinal cord injury\(^4\). One of the

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**Table 2A.** Two-way repeated measures ANOVA on the effects of different frequencies of vibration on spinal cord reflexes.

| Variable | Source | Df/Error | F    | p   | \(\eta\) | \(\eta^2\) |
|----------|--------|-----------|------|-----|---------|----------|
| Mmax     | Time   | 1.07/25.60| 0.23 | 0.66| 0.01    | 0.01     |
|          | Time x Frequency | 2.13/25.60 | 0.98 | 0.40| 0.08    | 0.08     |
| Hmax*    | Time   | 2/48      | 48.82| 0.001| 0.67    | 0.67     |
|          | Time x Frequency | 4/48 | 2.87 | 0.033| 0.19    | 0.19     |
| H10%*    | Time   | 2/48      | 41.07| 0.001| 0.63    | 0.63     |
|          | Time x Frequency | 2/48 | 8.66 | 0.001| 0.42    | 0.42     |
| Twave*   | Time   | 1.47/35.30| 31.82| 0.001| 1.0     | 1.0      |
|          | Time x Frequency | 2.94/35.30 | 3.94 | 0.016| 0.25    | 0.25     |

* Statistical analysis was done with normalized data by expressing Hmax, H10% and Twave as percentages of the individual's Mmax amplitude.

**Table 2B.** One way repeated measures ANOVA on the effects of different frequencies of vibration on spinal cord reflexes.

| Frequency | Variable | Df/Error | F    | p   | \(\eta\) | \(\eta^2\) | PostHoc (P value) |
|-----------|----------|-----------|------|-----|---------|----------|-------------------|
| 50 Hz     | Mmax     | 1.2/9.8   | 0.02 | 0.98| 0.01    | -        | -                 |
|           | Hmax*    | 2/16      | 28.13| 0.001| 0.84    | 0.001    | 0.001 0.04        |
|           | H10%*    | 2/16      | 43.13| 0.001| 0.75    | 0.001    | 0.001 0.39        |
|           | Twave*   | 2/16      | 23.57| 0.001| 0.77    | 0.001    | 0.001 0.99        |
| 150 Hz    | Mmax     | 1.1/8.7   | 3.62 | 0.09| 0.31    | -        | -                 |
|           | Hmax*    | 2/16      | 26.36| 0.001| 0.77    | 0.001    | 0.006 1.0         |
|           | H10%*    | 2/16      | 12.86| 0.001| 0.62    | 0.001    | 0.006 1.0         |
|           | Twave*   | 2/16      | 6.46 | 0.009| 0.45    | 0.001    | 0.047 1.0         |
| 250 Hz    | Mmax     | 1.0/8.2   | 0.67 | 0.44| 0.08    | -        | -                 |
|           | Hmax*    | 2/16      | 4.22 | 0.03| 0.35    | 0.001    | 0.087 1.0         |
|           | H10%*    | 2.16      | 1.42 | 0.27| 0.15    | -        | -                 |
|           | Twave*   | 2/16      | 4.38 | 0.03| 0.36    | 0.001    | 0.047 1.0         |

* Statistical analysis was done with normalized data by expressing Hmax, H10% and Twave as percentages of the individual's Mmax amplitude.
Figure 2. EMG recording of H10% before, during and post vibration at frequency of 50Hz, 150Hz and 250Hz.

Figure 3. A) Warm perception threshold -L3 B) Warm perception threshold-L5 C) Heat pain perception threshold -L3 D) Heat pain perception threshold -L5. L3: the dermatome L3 (at a middle site of the anterior thigh), cranial to the site of vibratory stimulation. L5: the dermatome L5 (at the dorsum of the foot), caudal to the site of vibratory stimulation.
Table 2C. Two-way repeated measures ANOVA on the effects of different frequencies of vibration on QST measures.

| Variable | Source          | Two-way RM ANOVA |
|----------|-----------------|------------------|
|          |                 | Df/Error | F    | p    | np2  |
| WT-L3    | Time            | 1.30/31.29 | 0.82 | 0.40 | 0.03 |
|          | Time x Frequency| 2.61/31.29 | 0.55 | 0.63 | 0.04 |
| HPT-L3   | Time            | 1.51/36.16 | 3.16 | 0.07 | 0.12 |
|          | Time x Frequency| 3.01/36.16 | 0.97 | 0.42 | 0.08 |
| WT-L5    | Time            | 1.55/37.22 | 2.03 | 0.16 | 0.08 |
|          | Time x Frequency| 3.10/37.22 | 1.45 | 0.25 | 0.11 |
| HPT-L5   | Time            | 2/48      | 9.71 | 0.001| 0.29 |
|          | Time x Frequency| 4/48      | 0.88 | 0.48 | 0.07 |

Novelties of our study is the examination of the effects of more than one frequency. Using vibration at 50 Hz over quadriceps muscle, spasticity and spinal cord excitability reduced in the ipsilateral knee and H reflex and T wave on soleus muscle in spinal cord injury patients. Achilles or tibial anterior tendon vibration at 40 and 80 Hz but not 20 or 120 Hz suppresses late spasm-like activity in antagonist muscles. Vibration at 100 Hz applied to the triceps brachii of a spastic upper limb in association with physiotherapy reduced the spasticity of the flexor agonist, biceps brachii in hemiplegic patients. Improvement in spasticity was reported also with vibration at 300 Hz in post stroke patients.

It is known that vibratory stimulation at frequencies between 80-120 Hz produce an involuntary contraction in the targeted muscle and a relaxation of the antagonist muscle. However, the effects of different frequencies on the H reflex or T wave have not been examined before.

The H reflex and T wave are reliable indirect measures of spinal cord reflex circuits excitability. The H reflex amplitude depends not only on alpha motoneuron excitability but also on the degree of synchronization of the Ia afferent input and the level of presynaptic inhibition. The Achilles T reflex has the same afferent and efferent pathways as the H-reflex, except for the site where the Ia fibers are activated: the H-reflex stimulates directly the Ia fibers, bypassing the muscle spindle organs, while the T-reflex activates the muscle spindle organs. The inhibition induced in spinal cord reflex excitability, specifically in the soleus H and reflexes, may be due, among other causes, to: (1) increased discharge threshold by group Ia afferents, (2) enhanced presynaptic inhibition at Ia terminals, (3) transmitter depletion at Ia synapses, and/or (4) postsynaptic changes leading to a decrease in motoneuron excitability.

Vibration at 250 Hz was not able to induce changes in spinal cord excitability. It is possible that the lack of inhibitory effect of high frequency vibration may be due to the fact that the discharges of Ia afferents become disharmonious with frequencies of vibration above 150 Hz. Therefore, the Ia volley might be unable to activate the intraspinal inhibitory circuits, which might explain the minimal or absent modulatory effects of vibration at 250 Hz in our study. Furthermore, the lack of significant modulation of M wave at any of the explored frequencies suggests the absence of any effect of local vibration on distal axonal excitability.

In our study, the effects seen with 5 minutes vibration at 50 and 150 Hz were not maintained after the intervention. On the contrary, Miyara et al. reported effects on F-wave parameters and spasticity that remained even 20 minutes after 5 minutes of whole body vibration at 30 Hz in stroke. These differences may be explained by the different stimulation characteristics, study subjects and outcome measures between the two studies. We believe that the effects of focal vibration on spinal cord reflexes is limited to the time that vibration is being applied.

Modulation of thermoalgesic perception by focal muscle vibration

Our results show only a small effect of focal 50 Hz vibration on heat pain threshold, with no effect on warm perception threshold. The effect, which was not robust enough to sustain significance in the post-hoc analysis, was found in the dermatomes below the site of stimulus application. Previous studies investigating the effects of focal vibration on thermoalgesic perception have reported different findings. Using a hand grip vibrating device applied over the finger of healthy individuals, Hirokawa et al. reported increased threshold for warm perception, but only a small effect on perception of cold, with vibration frequencies of 125 and 250 Hz. On the contrary, Burnstrom et al. used frequencies of 31.5 and 12 Hz to find a significant effect of vibration on cold perception and no effects on perception of warm sensation. The contradictory results were interpreted by the authors as due to different vibration frequencies and magnitude of the vibrators. However, both studies measured only perception of warm and cold sensation, but not of heat pain. Also, they measured the effect in the area where vibration was applied, exploring mostly in such a way, the local effects of vibration. In the study by Yarniskky et al., subjects reported reduced pain intensity to suprathreshold heat when vibration was given to the same dermatome on
the contralateral side or to the dermatome adjacent to that receiving heat stimulation, but had no effects on perception of temperature sensation two or more dermatomes above or below the vibration site. A relevant methodological difference between our study and that of Yarnitsky et al. is in the way to assess pain. While Yarnitsky et al. used a visual analogue scale, we utilized a quantitative measure of pain threshold. Other methodological differences related to duration of stimulation and frequency of vibration could also explain the different results.

On the other side, using high-frequency TENS induced a significant attenuation of both the acute pain and the laser evoked potentials induced by stimuli applied on the same dermatome. However, the stimulation technique and methodology used in Vassal et al.’s study is different from ours, which makes difficult to compare the results between studies.

In spite of methodological differences, our results go in the same line as the cited previous studies to indicate that there can be a small effect of vibration on perception of thermoalgesic stimuli. According to the gate control theory an inhibitory interaction exists between large and small fiber mediated messages arising from the same dermatome, so that the first inhibits the second. Therefore, some form of inhibition of pain perception can be expected by vibratory stimuli, known to activate large afferent fibers. On the other side, reduced input transmission in the cuneate nucleus is likely to be responsible for perceptual alterations induced by vibrations.

**Limitations**

We acknowledge several limitations of the current study: (i) Our study sample was relatively small and limited to healthy individuals. (ii) Obviously, the results could be different in patients with lesions of the descending tracts for control of alpha motoneurons and spinal inhibitory interneurons. (iii) We have not found any significant change induced by vibration on Mmax. However, there is at least one study indicating that, in rats, the M wave can increase in size with high frequency vibration. This has not been reported in humans and we did not apply vibration during sufficient duration or repeated sessions as to trigger possible mechanisms for structural changes in the muscle. (iv) The other possible limitation of the study was tendon tapping which was an inconsistent stimulus. However, the effects observed on the T wave data were similar in all respects to those observed on the H reflex data. (v) Spread of vibration from the TA to the soleus muscle known as crosstalk effect is possible and we cannot rule it out. We have limited the evaluation of thermoalgesic sensation to the determination of thresholds. Considering that analgesic effects of focal vibration are mediated by spinal descending inhibition mechanisms, future studies may include specific measures of nociceptive flexion reflex and pain-related brain evoked potentials.

**Conclusion**

The effects of vibratory stimulation can be summarized as frequency-related suppression of the spinal cord excitability without effect on heat pain perception. Low frequencies (50 and 150 Hz) determine greater suppression of spinal cord excitability measured by H reflex and T wave but not high frequencies (250 Hz) without an effect on warm perception and heat pain perception, above and below the dermatome where vibratory stimulation was applied. The data presented here may help designing vibration-related interventions intended to decrease spinal cord reflex excitability in patients with spasticity. In accordance with the results of our study, this would be better accomplished with low frequency vibration (50-150 Hz) than with high frequency vibration (250 Hz).

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