REFERÊNCIA
Current perception threshold and reaction time in the assessment of sensory peripheral nerve fibers through sinusoidal electrical stimulation at different frequencies

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Abstract  Introduction: The Perception Sensory Threshold (ST) for sinusoidal current stimuli at 5, 250, and 2,000 Hz is commonly used in the assessment of peripheral nerve fibers (C, Aδ, and Aβ, respectively). However, the neuroselectivity of these frequencies is far from consensus. In addition, Reaction Time (RT) measurements suggest that 2,000 Hz stimuli excite Aβ-fibers, 250 Hz Aβ- or Aδ-fibers, as well as 5 Hz Aβ-, Aδ- or C-fibers. Therefore, we suppose that the sinusoidal current neuroselectivity may be better observed if ST and RT parameters are jointly evaluated. In addition, we have investigated whether there are other sets of frequencies that could be used. Methods: Thus this work investigates ST and RT for stimuli with frequency ranging from 1 to 3,000 Hz, on 28 healthy subjects aged from 19 to 44 years old (27.1 ± 5.49). ST and RT dissimilarity among different frequencies was evaluated applying bi-dimensional Fisher Quadratic Discriminant. Results: The lowest classification error (3.6%) was obtained for 1, 250, and 3,000 Hz. Error for 5, 250, and 2,000Hz was 16.7%. Stimulation frequency at 1 Hz evoked more sensations related to C-fibers (53% of reports) than to Aβ-fibers (36%). However, this behavior did not repeat itself at 5 Hz (only 21% of perceptions were related to C-fibers against 64% to Aβ-fibers). Sensations related to Aβ-fibers prevailed for the highest frequencies presented to the subjects (2,000 Hz – 82% and 3,000 Hz – 93%). Mean RT values showed a decreasing trend with frequency. Conclusion: These results suggest that frequencies 1, 250, and 3,000 Hz are more neuroselective than 5, 250, and 2,000 Hz for the evaluation of peripheral sensitive fibers. Furthermore, they show RT usefulness.

Keywords  Sensory threshold, Reaction Time, Current Perception Threshold (CPT), Nervous fibers evaluation, peripheral neuropathies.
Introduction

The skin is widely innervated by axons that are distributed through a vast network of peripheral nerves that carry sensory information from the somatosensory receptors to the central nervous system. These axons are known as primary afferents (Gardner and Kandel, 2000; Gardner et al., 2000; Purves, 2004). The axons have a variety of diameters correlated with the type of sensory receptor to which they are linked, making it possible to classify them in three large groups: Aβ, Aδ, and C (Manzano et al., 2008). Type C axons are unmyelinated and present a diameter lower than 1.5 µm, showing the lowest speed of conduction (between 0.5 and 2 m/s). Such fibers are related to pain, temperature, and itching sensations (Gardner and Martin, 2000; Purves, 2004). Thin myelinated Aδ-fibers conduct pain and temperature sensations, but at higher speeds that can achieve 30 m/s. On the other hand, tactile sensations are transmitted by Aβ-fibers, which are thick myelinated axons that can show a conduction speed as high as 75 m/s.

Some neuropathies affect peripheral fibers selectively, such as diabetes (Matsutomo et al., 2005; Richerson et al., 2005), or even gradually, such as the carpal tunnel syndrome (Nishimura et al., 2004) and the leprosy neuronal disease (Van Brakel et al., 2005; Villarroel et al., 2007). This makes the evaluation of each type of fiber an important tool for the diagnosis of diseases or even a progressive indicator of these neuropathies (Matsutomo et al., 2005).

In the 80’s, a psycho-physical procedure for assessing the sensitivity to sinusoidal electrical stimulation was proposed (Katims et al., 1986a; Masson et al., 1989; Neurotron Inc., 2012). This method was based on studies suggesting that sinusoidal stimulation at different frequencies would excite sensory systems related to fibers of different diameters, thus increasing the stimulation selectivity (Katims et al., 1986a). The technique used in this evaluation, called Current Perception Threshold (CPT), determines the Sensory Threshold (ST) to the sinusoidal current, which corresponds to the lowest current intensity capable of eliciting perception. Many studies have suggested that a 5 Hz frequency would stimulate unmyelinated fibers (C-fibers), a 250 Hz one, thin myelinated fibers (Aδ-fibers), and a 2,000 Hz one, thick myelinated fibers (Aβ-fibers) (Chado, 1995; Katims et al., 1986b; Katims et al., 1987). However, frequency-based neuroselectivity is far from consensus. In the literature there are several studies that agree with the existence of such phenomenon (Lowenstein et al., 2008; Masson et al., 1989; Matsutomo et al., 2005; Nishimura et al., 2004; Tierra-Criollo et al., 2006), and others that disagree (Tack et al., 1994; Vinik et al., 1995).

Recently, Félix et al. (2009) have associated the Reaction Time (RT), which corresponds to the time elapsed between the application of a stimulus and the indication of perception by the subject, with different stimulation frequencies whenever stimuli with intensity equal to 1.1 times ST are applied, at the frequencies of 5, 250, and 2,000 Hz, and twice ST, at the frequency of 5 Hz. Findings suggest that a 2,000 Hz stimulus would excite Aβ-fibers, a 250 Hz one, Aβ- or Aδ-fibers, and a 5 Hz one, Aβ-, Aδ- or C-fibers, according to Pimentel et al. (2006) and Liu et al. (1996). Therefore, we suppose that the sinusoidal current neuroselectivity may be better observed if ST and RT parameters are jointly evaluated. In addition, we have investigated whether there are other sets of frequencies which could be used. This work aims to analyze whether ST and RT parameters, jointly evaluated at different frequencies (between 1 Hz to 3,000 Hz), contribute to better characterize nervous fibers in order to assist the early diagnosis of neuropathies.

Methods

The experiment assessed ST and RT at different frequencies of sinusoidal current stimulation applied to the skin.

This project was approved by the Ethics Committee of the Federal University of Minas Gerais (UFMG) and registered under N° 0722.0.203.000-11. The volunteers were instructed about the procedures to be performed and were included in the study only after signing up the “Free and Clarified” Consent Term.

The procedures were performed in a controlled environment at the Biomedical Engineering Laboratory in the Electrical Engineering Department – Federal University of Minas Gerais (UFMG).

The NeuroStim System (Martins, 2008) was used for the electrical stimulation, with frequency ranging from 1 to 3,000 Hz. The system is capable of generating programmable electrical current stimuli as high as 8 mA with resolution of 8 µA. The waveforms may range from 1 Hz to 5,000 Hz at steps of 0.1 Hz, with total harmonic distortion (THD) below 1.5%.

The study included 28 male subjects, aged from 19 to 44 years old (27.1 ± 5.49), without cognitive impairment, after a minimal mental health examination (Bertolucci et al., 1994). All experiments were conducted under controlled room temperature (25 ± 2 °C). Volunteers were positioned in a comfortable armchair with upper limbs supported by the chair arms.
For stimulation, the dorsal region of the non-dominant hand was first cleaned with alcohol-embedded cotton. Stimulation was applied with the use of two 10 mm diameter gold electrodes with a thin amount of conductive gel, separated by a distance of 2 cm between centers. Subjects were instructed to remain relaxed, with eyes open, during all the procedure, and to press a button positioned in their dominant hand whenever they felt any somatosensory perception. Such information was used to determine both ST and RT, the latter defined as the elapsed time (ms) between stimulation and motor response to the stimulus perception.

ST was assessed at 1, 3, 5, 7, 10, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 400, 500, 1,000, 2,000, and 3,000 Hz, performing two measurements for each frequency, on two different days, in order to evaluate the test’s reproducibility and reliability. The frequency values were randomly presented during the procedure, in order to avoid electrical stimulus adaptation and distribute the effects of tiredness, mental fatigue, decrease of motivation and attention at RT among tested frequencies. The total time for the experiment ranged from 90 min to 150 min for each volunteer, depending on the number of failures for ST (CPT); the latter defined as the elapsed time for each frequency, comparing both measurements. Each valid stimulus identification and RT was calculated as their mean value.

At the end of each frequency tested, volunteers were asked to describe the sensation perceived during stimulation. Sensory perceptions, for the arrangement with the lowest classification error (explained later) and for the one commonly employed in literature (5, 250, and 2,000 Hz), were grouped according to the following criteria:

- All reports having at least one word such as “prick”, “pang”, “twinge”, and “burn” (Pimentel et al., 2006) or related, such as “heating” or “pinching” were classified as “Group X”;
- All reports that showed at least a word such as “squeeze”, “pressure”, “movement”, and “vibration” (Pimentel et al., 2006) or related, such as “tingling sensation”, “contraction”, “touch” or “numbness”, were classified as “Group Y”;
- Reports that showed words related to both groups above were classified as “Group XY”.

Thus it was possible to check, through the sensory perceptions reported, whether there was any correlation between the stimulus frequency and the sensory perception reported.

The statistical analysis was performed using the software package SPSS 13.0 and MATLAB and differences were considered significant whenever the probability associated was lower than 5%.

The test reproducibility was assessed using the scattering plot and boxplot of ST and RT values for each frequency, comparing both measurements.

The dissimilarity of the parameters ST and RT for different frequencies was evaluated applying bi-dimensional Fisher Quadratic Discriminant (Khemchandani et al., 2010).

**Results**

Stimulations at 1 Hz showed (Figure 1 and Figure 2) the lowest ST mean (240 µA ± 96 µA) followed by 3, 5, 7 Hz (around 360 µA ± 130 µA), and 10 Hz (388 µA ± 120 µA), but there were no statistical differences (p > 0.05, ANOVA with Tukey’s post hoc test). Frequencies from 30 to 300 Hz showed mean values around 486 µA ± 130 µA, but there were no statistical differences among them (Figure 2). On the other hand, frequencies from 500 to 3,000 Hz presented statistical differences (p < 0.05). ST mean values in ranges of 1-10 Hz, 30-300 Hz, and 500-3,000 Hz were statistically different. Thus, stimuli were divided into three frequency classes: low ($F_L$ – 1 to 10 Hz), medium ($F_M$ – 30 to 300 Hz), and high ($F_H$ – 500 to 3,000 Hz).
RT mean values showed decreasing trend with stimulation frequency, although they had high variability (Figure 3).

The Fisher Quadratic Classifier (FQC) error was calculated for the 220 different possible arrangements created taking one frequency from each class – 5 possible values for \( F_L \), 11 for \( F_M \) and 4 for \( F_H \) (Figure 4). The arrangements for \( F_L = 1 \) Hz (solid lines) presented the lowest FQC error, whereas arrangements using \( F_L = 5 \) Hz (dotted lines) had the highest classification errors (Figure 4). The lowest FQC error was 3.6%, occurring with \( F_L = 1 \) Hz, \( F_M = 250 \) Hz, and \( F_H = 3,000 \) Hz (named Arrangement 2). For \( F_L = 5 \) Hz, \( F_M = 250 \) Hz, and \( F_H = 2,000 \) Hz (Arrangement 1), the FQC error (16.7%) was 4.6 times greater.

The ST vs RT scatter plot showed a better class separation among \( F_L \), \( F_M \), and \( F_H \) frequencies in Arrangement 2 (Figure 5) compared to Arrangement 1 (Figure 6), resulting in a lower FQC error for the former.

Figure 1. Boxplots generated from Sensory Threshold (ST) data at each stimulus frequency (28 subjects).

Figure 2. Multiple comparison test among balanced two-way ANOVA stats for ST values (28 subjects). Highlighted, the multiple comparison with respect to 250 Hz (dark gray), which does not show significant statistical difference for the frequencies of 30 to 300 Hz (light gray).

Figure 3. Boxplots generated from Reaction Time (RT) data at each stimulus frequency (28 subjects).

Figure 4. FQC errors for the 220 arrangements tested. Different lines represent low frequencies (\( F_L \)): 1 Hz – solid line, 3 Hz – dashed line, 5 Hz – dotted line, 7 Hz – dash-dot line, and 10 Hz – thick solid line. Symbols represent high frequencies (\( F_H \)): 500 Hz – circle, 1000 Hz – triangle, 2000 Hz – square, and 3000 Hz – inverted triangle.
Reports having at least one word from each sensation group were prevalent at 250 Hz (Table 3).

Finally, RT and ST measurements reliability and reproducibility were analyzed intra-subject with Pearson correlation ($p < 0.05$) between sections, leading to $r = 0.97$ and $r = 0.95$, respectively. These results indicate an almost perfect agreement, according to Landis and Koch (1977).

### Discussion

ST values found for the frequencies of 5 Hz ($370 \pm 140 \mu A$), 250 Hz ($520 \pm 150 \mu A$), and 2,000 Hz ($1575 \pm 350 \mu A$) are similar to the results reported by the normative data obtained with Neurometer (Neurotron Inc., 2012) for sinusoidal electrical current stimulation, often used in the literature (Galvão et al., 2005).
In this study, we were able to separate ST behavior for three frequency bands: low (1 to 10 Hz), medium (30 to 300 Hz), and high (500 to 3,000 Hz).

Mean RT values for 5 Hz (1,200 ms), 250 Hz (850 ms), and 2,000 Hz (650 ms) showed a decreasing trend with respect to the frequencies. The same behavior was observed by Félix et al. (2009), who reported lower RT values. This difference might be due to the distinct methodologies adopted. While Félix et al. (2009) determined the RT value as the subject shortest response time in a series of 10 stimuli at the intensity of 1.1 × ST, in this study, this parameter was determined as mean RT during the validation stage of ST at 1.0 × ST intensity level.

The joint dissimilarity of ST and RT among all tested frequencies was analyzed by using the Fisher Quadratic Discriminant Classifier for the three distinct classes (low, medium, and high frequencies), taking into consideration all possible arrangements of frequencies, taking a single frequency from each band. The classification errors led to a better class separation for Arrangement 2 (1, 250, and 3,000 Hz) than Arrangement 1 (5, 250, and 2,000 Hz), the latter widely used in literature for the evaluation of peripheral nervous fibers (Hedman and Sullivan, 2011, Lowenstein et al., 2008; Matsutomo et al., 2005; Nishimura et al., 2004; Pimentel et al., 2006).

Additionally, it was possible to verify that classification errors occur mainly at low and medium frequencies, for both arrangements, suggesting that such stimuli can activate similar fiber groups. On the other hand, high frequencies (2,000 Hz or 3,000 Hz) showed a well-defined separation compared to the other classes, which could mean stimulation of another fiber group.

It is noteworthy that the classification was based on two variables (ST and RT). If the ST value were considered alone, as it is usually done in literature, the classification error would increase from 16.7% to 19.1% for Arrangement 1 and from 3.6% to 7.1% for Arrangement 2. When RT alone is taken into account, the error would be even higher (47.6% for Arrangement 1 and 41.7% for Arrangement 2), which may explain the poor neuroselectivity for such parameter. These findings agree with Félix et al. (2009), who found poor neuroselectivity for low and medium frequencies.

Evaluating the RT parameter, we observed that, at high frequencies (2,000 and 3,000 Hz), only 3,000 Hz was statistically different (p < 0.05) from 250 Hz (Table 2), suggesting that the classification between 250 and 3,000 Hz would be more efficient than 250 and 2,000 Hz.

The sensory perceptions reported in Table 3 suggest that Arrangement 2 showed higher neuroselectivity than Arrangement 1. The C-fibers activation was more evident at 1 Hz than 5 Hz. On the other hand, taking into consideration high frequencies for Arrangement 1 ($F_1 = 2,000$ Hz) and Arrangement 2 ($F_2 = 3,000$ Hz), the sensory perception reports associated with Aβ-fibers were higher (93% at 3,000 Hz and 82% at 2,000 Hz) than reports concerning C-fibers (4% at 3,000 Hz and 2,000 Hz). These findings might explain the lower FQC error for Arrangement 2.

Several studies confirming the outcomes of Arrangement 1 are found in literature. For example, Tack et al. (1994) compared standardized clinical examination scores with measurements of vibratory and current perception threshold in diabetic polyneuropathy. Correlations between CPT and neurological examination scores (reflecting C-fibers and Aβ-fibers functions) were the highest ones at 2,000 Hz ($r = 0.88$) and no advantage for lower frequency CPT could be identified.

Results related to sensory perception (Table 3) are similar to those found in works of Tierra-Criollo et al. (2006) and Pimentel et al. (2006), which suggested that sinusoidal electrical currents at 5 and 2,000 Hz evoke different sensations. According to Félix et al. (2009), stimuli at 2,000 Hz would activate Aβ-fibers while stimuli at 5 Hz would activate Aβ-, Aδ-, and C-fibers. Furthermore, Liu et al. (1996) reported that ST values obtained during analgesic administration (Fentanyl) were affected only for stimuli at 5 and 250 Hz. This might indicate that 2,000 Hz stimuli have no connection with pain-conductive fibers.

Such phenomena may be justified by the sensory receptors anatomic layout. According to Grimnes and Martinsen (2000), low-frequency currents are expected to penetrate the skin less than high-frequency ones, having, therefore, a higher effect on free electrical terminations laid more superficially than thick fibers. In addition, since thin fibers have a higher capacitance (Koester and Sielgelbaum, 2000), one expects that currents with a slower ascension would, initially, favor these fibers depolarization. Moreover, low frequencies stimulus at a lower ST value might also hamper the development of action potentials in thick fibers due to the sodium channels inactivation rate and could explain why low frequencies stimuli with increased amplitude active thick fiber (Félix et al., 2009).

Some limitations of this work should be kept in mind. First, we assumed that the central processing and efferent response time were similar for Aβ-, Aδ-, and C-fibers (Félix et al., 2009). According to Yamitsky and Ochoa (1991) this time is around 200 ms for the Aδ- and C-fibers. However, it is not proven by laser studies that RT measurements produced similar results (Mouraux et al., 2003).

Another limitation is that an increase in RT could occur for low frequencies, being associated with time for waves reach its peak value. However, the
difference between mean RT measurements for 1 Hz and 5 Hz frequencies was 500 ms, greater than the delay between theirs peaks (200ms), being insufficient to explain this difference.

Our findings suggest that the evaluation of peripheral nervous fibers by electrical stimulation with sinusoidal currents might have better results if the CPT technique also used the RT parameter in its classification. It also indicates that the joint evaluation of ST and RT parameters showed greater dissimilarity for stimuli at 1, 250, and 3,000 Hz than at 5, 250, and 2,000 Hz - the former, therefore, more suitable for neuroselectivity evaluations by sinusoidal electrical current.

Sinusoidal electrical currents at different frequencies evoke different sensations. Stimuli at 3,000 Hz are more associated with sensations related to thick myelinated fibers and, at 1 Hz, with sensations related to Aβ- and C-fibers, although sensations associated with the latter are more often reported.

The more neuroselective characteristic of the frequencies of Arrangement 2, associated with the better description of the joint evaluation for parameters ST and RT, may produce significant benefits to the diagnosis of neuropathies affecting peripheral fibers either selectively or gradually, allowing their early identification, or even indicating their degree of evolution.

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