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A Two Alternative Forced Choice Method for Assessing Vibrotactile Discrimination Thresholds in The Lower Limb

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Abstract  
The development of an easy to implement, quantitative measure to examine vibration perception would be useful for future application in clinical settings. Vibration sense in the lower limb of younger and older adults was examined using the method of constant stimuli (MCS) and the two-alternative forced choice paradigm. The focus of this experiment was to determine an appropriate stimulation site on the lower limb (tendon versus bone) to assess vibration threshold and to determine if the left and right legs have varying thresholds. Discrimination thresholds obtained at two stimulation sites in the left and right lower limbs showed differences in vibration threshold across the two ages groups, but not across sides of the body nor between stimulation sites within each limb. Overall, the MCS can be implemented simply, reliably, and with minimal time. It can also easily be implemented with low-cost technology. Therefore, it could be a good candidate method to assess the presence of specific deep sensitivity deficits in clinical practice, particularly in populations likely to show the onset of sensory deficits.
Keywords
Constant stimuli; discrimination threshold; vibration sense; vibration perception; high frequency vibrations

Introduction
Somatosensory feedback from mechanoreceptors in the skin, muscles, and joints contributes importantly to motor behaviours including balance, mobility, and motor learning (Umphred et al. [72]). Due to differences in morphology, innervation, and the filtering properties of surrounding tissues, different receptors are sensitive to changes in mechanical stimuli spanning different frequency ranges, with some receptors exhibiting sensitivity to slow changes in pressure or displacement (e.g., <~8 Hz for slowly adapting type I and type II afferent units in the skin (Johansson et al. [34]); <~20 Hz for muscle spindle primary and secondary afferents (Matthews and Stein [43])), while others are sensitive to faster changes (e.g., between 8 and 64 Hz for rapidly adapting (RA) units, >64 Hz for Pacinian corpuscles (Johansson et al. [34])).

Somatosensory deficits in the lower extremities are frequent in older individuals (Lord et al. [42]; Verschueren et al. [77]; Boisgontier et al. [3]) and in people affected by neurological diseases (Winter [78]; Hausdorff et al. [31]; Cameron and Wagner [5]), contributing to an increased incidence of falls and a reduction of independent living (Winward et al. [79]; Uszynski et al. [73]; Carey et al. [6]; Jamali et al. [33]). To minimize the negative consequences of somatosensory deficits, it is imperative that people in the early stages of decline undergo accurate quantitative assessments and rehabilitative treatments that specifically target the sensory impairment (Priplata et al. [50]; Smania et al. [60]; Cattaneo et al. [7]; Brichetto et al. [4]; Gandolfi et al. [16]; Bao et al. [2]; Lin et al. [41]). During neurological inspection, the integrity of somatosensation is commonly inferred by using a 128 Hz tuning fork to assess vibrotactile perception (Pearson [48]; Gilman [23]). The tuning fork is repeatedly placed on a bony prominence (sometimes vibrating and sometimes not) and the subjects are required to indicate whether they feel the vibration or not and, in the former case, to indicate when it starts and stops. The clinician evaluates the ability to perceive the vibration with a score ranging from 0 (very low ability) to 8 (intact ability). Despite its common use, this method is not objective and often not sensitive to subtle abnormalities. Given that even small changes in sensation may impair motor function, a precise, sensitive, and quantitative method is needed to measure sensory deficits. In this study, we focussed on the assessment of the vibration sense using an approach that exploits the precision of computer-controlled stimulus presentation and the sensitivity of gold-standard psychophysical testing techniques.

Numerous computer-controlled devices have been developed to deliver precise vibrotactile stimuli (e.g., Arezzo et al. [1]; Mortimer et al. [44]; Tannan et al. [68]; Temlett [70]; Choi et al. [9]; Suh et al. [66]; Chinello et al. [8]; Sonar and Paik [61]; Georgarakis et al. [17]) and several different experimental protocols have been designed to use these devices to assess vibrotactile sensation (e.g., Gerr and Letz [18]; Frenette et al. [14]; Collins et al. [10]; Newsome et al. [47]; Enders et al. [13]; Ribot-Ciscar et al. [53]; Stronks et al. [63]; Shah et al. [59]). Such techniques have been used to determine the smallest detectable stimulus intensity (i.e., the absolute threshold) and/or the smallest difference in a vibrotactile stimulus needed to reliably discriminate between two different stimuli (i.e., the
discrimination threshold). One well-known psychophysical method that can be used to assess the discrimination threshold is the two-alternative forced choice method of constant stimuli (MCS; see Gescheider ([19]) for a review). Here, an individual is exposed to a pre-determined sequence of paired stimuli and must indicate after each pair, which of the two was more intense. The MCS is popular because it yields unbiased threshold estimates if a sufficient number of stimulus pairs is used, while also requiring a fixed amount of time to perform. By contrast, other methods such as the adaptive staircase method and methods using Bayesian algorithms (e.g., QUEST) use responses from early stimuli to determine the intensity of stimuli later in assessment. These methods can introduce response bias (Treutwein [71]; Leek [40]), and more importantly, the number of stimulus presentations required to converge to an accurate discrimination threshold (i.e., testing time) can be unpredictable, which is problematic in the clinical setting. MCS has been already employed in previous studies to determine vibrotactile discrimination thresholds (Post et al. [49]; Tegenthoff et al. [69]; Shah et al. [59]). In one example, Shah et al. ([59]) found that discrimination thresholds vary across dermatomes in the upper limb and that subjects are better able to discriminate pairs of stimuli presented sequentially rather than simultaneously.

Here, we used the MCS and vibrotactile stimuli with frequencies in the sensitivity range of Pacinian corpuscles to identify vibrotactile discrimination thresholds at stimulation sites in the lower extremity. We compared discrimination thresholds between sites within and across the two legs in younger and older adults. Differences in vibrotactile discrimination thresholds across stimulation sites within the same limb may be expected due to variations in the mechanical filtering properties of tissues underlying the stimulation site. For example, vibrotactile stimuli delivered on a bony prominence will elicit a response predominantly from Pacinian corpuscles (Goble et al. [26]), whereas the same stimuli applied on the skin over a tendon may additionally elicit responses from muscle spindle receptors as well (Goble et al. [26]). Differences in vibrotactile discrimination thresholds across the two sides of the body may be expected because the processing of other mechanoreceptor signals (i.e., proprioception) also differs between the right and left sides for the upper (Goble et al. [28]; Goble and Brown [24]) and lower extremities (Symes et al. [67]; Han et al. [29]; Galamb et al. [15]). Finally, differences in vibrotactile discrimination thresholds across the lifespan may be expected due to the numerous physiological changes that occur with aging. These include: morphological changes in vibration sense receptors (Daly and Odland [11]), including the Pacinian corpuscle, which loses the integrity of its mechanical structure leading to a decreased sensitivity to vibrotactile stimuli (Verrillo [75]; Kenshalo [36]); a degradation of the skin’s mechanical properties (Daly and Odland [11]), which can alter the propagation of vibration within the skin; and changes in both axons and Schwann cells occurring with age, causing an altered nerve conduction in older people (Rivner et al. [54]).

We sought to test the hypothesis that the MCS is suitable to detect differences in vibrotactile perception in the lower limbs between younger and older groups, as well as to assess differences in vibration discrimination threshold that may depend on where the stimulation is applied.
Materials and methods

Participants

Nineteen younger subjects (10 females; 27.3 ± 2.2 years, mean ± SD) and seven older subjects (5 females, 81.4 ± 3.9 years, mean ± SD) provided written consent to participate in this experiment. Inclusion criteria were: 1) no previous history of psychiatric/neurological illness or musculoskeletal disorders; 2) right-footed (Waterloo inventory score >6) (Elias et al. [12]); and 3) age <35 years for the younger group or >75 years for the older group. The study conformed to the Declaration of Helsinki and was approved by the local ethical committee (Comitato etico regione liguria, n. 222REG2017).

Experimental setup and protocol

Subjects reclined supine on a physiotherapy table. A vibration motor (tactor) was secured to one of four testing locations on the lower extremities using medical tape. We used commercially available 10 mm coin disc tactors (Precision Micro-drives Inc., London, UK Model 310–117). These inexpensive devices are energy-efficient, easy to use (Stronks et al. [64], [65]), and have been successfully used for similar purposes in recent works (Krueger et al. [38]; Shah et al. [59]). Vibration is produced by an eccentric rotating mass, the speed of which is controlled by an applied voltage within the range 0–3 V. Changing the applied voltage corresponds to changing both the frequency and the amplitude of the vibration. The relation between the applied voltage (V) and the frequency of the vibrotactile stimulus (f) has been described previously (Krueger et al. [38]):

\[
 f \text{vibe} = [-17.36 \cdot (V^2) + 135.89 \cdot (V) - 14.57]
\]
Figure 1. Stimulus locations: mechanoreceptors in the dark-grey area are innervated by the fifth Lumbar spinal nerve (L5). The tactor was affixed by medical tape onto one of the two stimulus sites (black circles): the crest of the tibia bone and the metatarsal tendon. These locations are tested separately and in both legs.

We evaluated the ability of the subjects to discriminate two sequential vibrotactile stimuli provided in the same location. For each leg, we tested two different anatomical locations within the same dermatome (L5) (Lee et al. [39]) one was over the foot metatarsal tendon and the other was over the distal portion of the tibialis crest. We refer to these stimulation sites as tendon and bone (Goble et al. [26]), respectively (see Figure 1). Vibratory stimuli applied at these locations differ in terms of both their representation in brain areas and stimulated peripheral receptors (Gilman [23]; Goble et al. [26]; Proske and Gandevia [52]). The order of four testing conditions (2 sites per leg) was counterbalanced across subjects to avoid potential order effects.

We used the MCS to identify the smallest discriminable difference (i.e., the discrimination threshold, or just-noticeable difference JND) between pairs of vibrotactile stimuli. On every trial, subjects were presented with two sequential stimuli in the same location: a standard stimulus and a probe stimulus. The frequency of the standard was always 186 Hz, while the probe could assume 11 different values spanning the range from 102 to 235 Hz (see Shah et al. [59]). Each of the 11 stimuli was presented 10 times in pseudo-randomized order for a total of 110 trials per testing site. The number of times the stimuli were presented was optimized using the simulation described in Appendix A. The order of probe and standard stimuli was randomized across trials.

It is known that the detectability of vibrotactile stimuli improves with increased stimulus duration (Verrillo [74]; Gescheider et al. [21]). In preliminary testing, we found that stimulus and inter-stimulus interval durations of 750 ms suffice to allow subjects to store the information in working memory (Romo et al. [57], [56]), avoiding the performance decrements observed with short observation intervals, particularly in older adults (Hasher and Zacks [30]; Klingberg [37]). We, therefore, defined the probe and standard stimuli durations and the inter-stimulus interval to be 750 ms. For each trial, subjects were to verbalize if the first stimulus had greater intensity than the second (regardless of whether the difference was perceived in the frequency or amplitude of vibration). Subjects wore headphones playing white noise to minimize auditory cues from the tactors.

Evaluating the perceptual discrimination threshold
Subject responses were used to calculate the probability of identifying each probe stimulus as greater than the standard stimulus for each pair of stimuli. Probability values for each of the probe stimuli (of frequency x) were fitted with a psychometric Gaussian cumulative distribution function (CDF, Equation (2):

\[
\Phi = \frac{1}{\sqrt{2\pi} \sigma} \int_{-\infty}^{+x} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \, dx
\]

where \(\mu\) (the average value of the distribution) estimates the perceptual 'bias' of the subject. As an estimate of the participant's perceptual acuity, we defined the discrimination threshold as one standard deviation \(\sigma\) of the CDF. This is a choice because one standard deviation also corresponds to
the probability of correctly discriminating stimuli of different intensity 84% of the time (Gescheider [19]; Wong et al. [80]). The greater the threshold, the lower the vibrotactile discrimination ability (i.e., higher difference in vibrotactile intensity required to reliably detect a difference). Equation (2) was fitted to each subject's responses using non-linear least square estimation (Matlab function fminsearch).

Statistical analysis
We used mixed model, repeated measures ANOVA with two within-subject factors related to the location of the stimuli, i.e., 'side' (left vs. right leg) and 'site' (tendon vs. bone) and one between-subjects factor related to, 'group' (younger vs. older subjects). We tested the hypothesis that discrimination thresholds vary across stimulus locations by evaluating the statistical significance of the two within-subject factors and their interaction. The results related to the between-subjects factor 'group' were used to test the hypothesis that this method is sensitive to age-related differences in vibrotactile acuity. Significant differences across the 'group' factor would provide a first proof of concept that the proposed method is able to identify subtle age-related changes in vibrotactile perception. Prior to statistical testing, the normality and sphericity of the data were verified with Kolmogorov–Smirnov and Mauchly's test, respectively. Significance was accepted at $p < .05$.

To further understand the pattern of variability induced by each of the independent factors of our experimental design (side, site, and age group), we analysed the Pearson correlation coefficient between discrimination thresholds obtained for the two sides (right vs. left; averaging the values obtained for the bone and the tendon locations) and between the values of the two sites (tendon vs. bone; averaging the estimated thresholds obtained for the right and left leg). For the correlation analyses, we considered the two age groups separately.

Results
Vibration discrimination threshold was examined in the distal lower limb of two populations using an eccentric rotating mass vibration motor (tactor) and the MCS to determine if stimulus site (location on the lower leg and the side of the body) and/or the age of the participant would contribute to systematic variations in vibration discrimination threshold. All participants remained attentive to the experiment and provided a timely response after each trial.

Lower leg vibration discrimination thresholds
Across all testing conditions, subjects responded reliably and accurately when the probe stimulus frequency was either much greater than or much lower than the standard stimulus frequency. Figure 2 presents results from a selected subject from the younger group. Discrimination thresholds were similar across all four testing locations for this individual.
Figure 2. Psychometric function fits to single-subject response data. Top row: bone locations; bottom row: tendon locations. Left column: left limb; Right column: right limb. Black squares: probability of response indicating that the probe stimulus was greater than the standard across all ten trials at each stimulus magnitude. Blue line: the best-fit psychometric curve. Red lines and grey shading: discrimination thresholds (σ) corresponding to one standard deviation of the underlying Gaussian normal distribution (see Equation (2)). Grey dashed horizontal line: chance probability. Black dotted horizontal line: 0.84 probability to identify the probe stimulus as greater than the standard stimulus.

Mixed model repeated measures ANOVA revealed that vibrotactile discrimination thresholds were significantly different between the younger (22.20 ± 5.51, mean ± SD Hz) and older (38.55 ± 10.56, mean ± SD Hz) populations (group main effect $F(1,24) = 26.98; p < .0001$, Figure 3). By contrast, no significant differences were found between stimulation sites ($F(1,24) = 0.072; p = .79$) or between the two sides of the body ($F(1,24) = 0.186; p = .67$). These results indicate that for healthy individuals, the testing procedures we describe can result in similar discrimination thresholds across tendon and bone on the left and the right lower extremities.

Figure 3. Discrimination thresholds (MEAN ± SE) in the four tested locations for both age groups. Left panel: younger group; right panel: older group. White and black bars indicate bone and tendon discrimination thresholds, respectively. The side of the body tested is indicated along the horizontal axis.

Whereas individuals in the younger group had consistent performances in all testing locations (Figure 4), the older group had variable individual performances across locations (Table 1 and Figure 4). This conclusion was supported by a set of analyses that found robust correlations for the younger group when comparing thresholds obtained for the left and the right legs (averaging across site: $r = 0.62, p = .0047$; Figure 4, right) and when comparing the two stimulation sites (bone vs. tendon, averaging across side: $r = 0.79, p < .0001$; Figure 4, left). A line with unity slope fell within the 95% confidence bounds of the regression lines comparing the performance between sides and between sites. This fits the younger participants' data in both cases. By contrast, the older population presented a markedly different pattern of behaviour, with no evident correlation between discrimination thresholds for the analysis that averaged sites within each side ($r = -0.20, p = .66$) and
for the analysis averaging across sides at each site \((r = -0.40, p = .37)\). This was evidently due to idiosyncratic differences between discrimination values obtained across all sites (i.e., high inter-subject variability; Table 1), and was not an artefact of a lower sample size of the older population. These results support the lack of systematic significant differences between conditions reported by the ANOVA.

Figure 4. Left panel. Discrimination thresholds obtained by averaging the right and left side data on the same anatomical location (tendon and bone). Right panel: discrimination thresholds obtained by averaging the data of the tendon and bone on the same leg (right and left). The blue bold line is the linear fit to the younger healthy data while the dotted blue lines represent the confidence intervals at \(\alpha = 0.05\) for the regression line. Red squares and black circles indicated the older and younger subjects' data, respectively.

Table 1. Perceptual discrimination thresholds for the seven older subjects.

<table>
<thead>
<tr>
<th>Older subjects</th>
<th>Gender</th>
<th>Age</th>
<th>Bone left (Hz)</th>
<th>Bone right (Hz)</th>
<th>Tendon left (Hz)</th>
<th>Tendon right (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old 1</td>
<td>M</td>
<td>84</td>
<td>13.7</td>
<td>27.8</td>
<td>44.9</td>
<td>122.0</td>
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<tr>
<td>Old 2</td>
<td>F</td>
<td>77</td>
<td>61.5</td>
<td>28.5</td>
<td>34.4</td>
<td>21.1</td>
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<tr>
<td>Old 3</td>
<td>F</td>
<td>87</td>
<td>25.1</td>
<td>39.3</td>
<td>62.0</td>
<td>45.6</td>
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<tr>
<td>Old 4</td>
<td>F</td>
<td>85</td>
<td>99.7</td>
<td>27.0</td>
<td>27.0</td>
<td>34.6</td>
</tr>
<tr>
<td>Old 5</td>
<td>F</td>
<td>80</td>
<td>42.4</td>
<td>36.4</td>
<td>42.5</td>
<td>35.5</td>
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<tr>
<td>Old 6</td>
<td>F</td>
<td>79</td>
<td>4.92</td>
<td>26.1</td>
<td>29.4</td>
<td>19.2</td>
</tr>
<tr>
<td>Old 7</td>
<td>M</td>
<td>78</td>
<td>23.6</td>
<td>56.9</td>
<td>23.7</td>
<td>24.4</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td>81.43 ± 3.87</td>
<td>38.7 ± 32.7</td>
<td>34.6 ± 11.1</td>
<td>37.70 ± 13.3</td>
</tr>
</tbody>
</table>

Discussion

The capability to objectively quantify subtle changes in somatosensory perception over time is clinically important for the clinical management of people who have a neurodegenerative disease in contrast to those who may be experiencing sensorimotor changes due to normal aging. Unfortunately, the current standard for assessing vibration sensation (using a tuning fork) is neither objective nor able to assess subtle differences in sensation. In this experiment, we used computer controlled vibrotactile simulators and the MCS to test vibration discrimination thresholds in two locations on each lower limb. We implemented this test in younger and older adults. We sought to test for differences in vibrotactile acuity related to age differences (younger vs. older) and to stimulation site (i.e., whether it was located over bone or tendon, and which side of the body was stimulated). We demonstrated that the method provides results that show small decreases in the acuity of vibrotactile sensation deficits in older
subjects relative to younger subjects. We found that for younger subjects without proprioceptive deficits, there was no difference in vibration discrimination across the two sides of the body, or between the bony vs. tendinous sites within dermatome L5 on each leg. For the older adults, however, there were individual differences across locations, but no systematic differences were detected when investigating the population behaviour. Overall, the described approach was demonstrated as a robust and objective method for determining vibrotactile discrimination thresholds. With continued validation, the approach could be developed into a reliable clinical test.

Similarity of vibrotactile discrimination thresholds across side and site

As highlighted in Figure 4, we found no systematic differences in vibrotactile discrimination thresholds between testing sites within the same dermatome of the leg for the younger group of participants. This was true even though the tested locations differed in their underlying tissue properties (i.e., over muscle tendon vs. boney prominence). Previous research has found that the application of vibratory stimuli over tendon vs. bone differs in terms of stimulated receptors, cortical representation (Goble et al. [26]; Goble et al. [27]), and the distribution of receptors themselves between sites (Proske and Gandevia [51]). Therefore, one might reasonably expect a quantitative difference in vibrotactile perception between testing sites. Instead, we found no differences in discrimination thresholds between testing sites on the two sides of the body. A possible reason for the lack of difference across sites might be related to the specific mechanoreceptors preferentially stimulated by the tactors used in this study. Previous studies that have used tendon vibration to elicit limb movement illusions have used tactors with a larger contact area and/or controlled displacements to ensure stimulation of muscle spindles (Romaiguère et al. [55]; Naito et al. [46], [45]; Kavounoudias et al. [35]; Goble et al. [26]). By contrast, the tactors used in this study provided vibrations in a frequency range that preferentially stimulates Pacinian Corpuscles. The coin-shaped tactors also have limited contact area (10 mm diameter) that may not be able to elicit a robust response in muscle spindles, which should be preferentially excited by tendon vs. bone vibration. And whereas previous studies has demonstrated a left side advantage in the discrimination of limb position, the perception of which relies mostly on muscle spindle activity (Symes et al. [67]; Han et al. [29]; Iandolo et al. [32]), we found no corresponding left side advantage for vibrotactile discrimination thresholds. Our results, therefore, support the idea that the central processing of somatosensory stimuli related to vibrotactile perception differs from the central processing of somatosensory stimuli related to limb position and movement.

Age-related difference in vibrotactile perception

This study extends previous findings of age-related differences in vibrotactile perception during stimulus detection tasks (Schmidt and Wahren [58]; Gescheider et al. [20]; Gescheider et al. [22]; Goble et al. [25]; Stevens et al. [62]; Verrillo et al. [76]), demonstrating age-related decrements also in a vibrotactile discrimination task. Moreover, the older adult group demonstrated discrimination thresholds with much higher intra- and inter-subject variability than the younger group. While older subjects did not perform as well as younger subjects in most tested locations, we did not observe a systematic increase of error in one specific location with respect to the others, but rather higher individual variability overall. That is, most of older subjects' discrimination thresholds fell far outside the variability of the values obtained for the younger subjects (Figure 4). Individual site-specific declines in discrimination threshold in the older population could be the result of the degenerative
processes involving sensory fibres and/or changes in the mechanical properties of skin that normally occur with age (Daly and Odland [11]; Kenshalo [36]). If so, morphological changes in mechanoreceptors and age-related changes in skin mechanical properties may not arise uniformly within the lower limb, but may rather vary within and across limbs in an individual-specific manner. Future efforts should test this idea directly with a larger sample of subjects across the lifespan.

Disclosure statement
The authors report no conflict of interest.

Abbreviations
• RA Rapidly adapting
• MCS method of constant stimuli
• JND just noticeable difference
• CDF cumulative distribution function

Appendix A. Optimization of the constant stimuli method parameters
We performed a set of simulations to identify a combination of task parameters that would allow a reliable and stable estimate of vibrotactile discrimination threshold within the shortest possible testing time. We estimated how the perceptual threshold varies in relation to (i) the number of different stimuli provided as probes and (ii) the number of repetitions of each of those stimuli. Decreasing these two values would shorten the total testing time because fewer trials would need to be collected. In the simulation, the number of probe stimuli varied from 3 to 39 and the number of repetitions for each stimulus varied between 5 and 25. We simulated 500 different experiments using this approach. For each simulated experiment, the algorithm selected a pair of experimental parameters (i.e., number of probe stimuli and number of repetitions). For example, for the simulated experiment wherein 3 probe stimuli were to be simulated 5 times each, 15 total trials were simulated; for the case where 39 probe stimuli were to be simulated 25 times each, 975 total trials were simulated. For each trial, the correct response for the selected stimulus was computed by substituting the frequency of the stimulus (normalized within the $[-1, +1]$ interval of the psychometric CDF) into Equation (2) with $\mu$ set to 0 and $\sigma$ to 0.5. Simulated subject responses were determined by selecting a random value from a standard uniform distribution in the interval $[0, 1]$. If the randomly-generated response fell below the value defined by the sigmoid curve, the response was marked as correct, otherwise, it was considered as incorrect.

For each simulated experiment, the simulated responses obtained for each probe stimulus were averaged across repetitions prior to fitting the CDF of Equation (2). This process yielded estimated mean and standard deviation values ($\mu$ and $\sigma$) for a specific pair of experimental parameters (number of probe stimuli and number of repetitions).

Simulation results
We sought to identify a combination of experimental parameters that would provide a reasonable trade-off between the precision of estimated discrimination threshold and the duration of assessment.
Trivially, either increasing the number of probe stimuli or the number of repetitions per probe stimulus increases the duration of the experimental session. Also as expected, the variability of the threshold estimate (\(\text{var}\)), reported as a percentage of the pre-defined threshold \(\sigma\); Equation (3) decreases when either the number of stimuli presented as probe or the number of trial repetitions increases.

\[
\text{var}[\%] = \sqrt{\frac{\sum_{i=1}^{N} (\bar{\sigma}_i - \frac{1}{N} \sum_{i=1}^{N} \bar{\sigma}_i)^2}{N}} \times \frac{100}{\sigma}
\]

Here, the number of simulated experiments \(N\) is 500, is the estimated value of \(\sigma\) per each pair of experimental variables (number of probe stimuli and repetitions), and \(\sigma = 0.5\) (i.e., the pre-defined sigmoid curve \(\sigma\) value). Figure 5 shows how the variability of the estimated discrimination threshold changes as a function of the number of probe stimuli and the number of repetitions at each probe stimulus. In the yellow/orange portions of the heat map, it is unlikely that the estimated discrimination threshold will be accurate. As the colour map tends toward blue in the right upper corner of the plot, the likelihood of obtaining a threshold estimate close to the actual threshold increases (i.e., the variability of the threshold estimate decreases).

Pilot testing in healthy adult subjects found that experimental trials last about 7.1 s on average. This period is comprised 2250 ms fixed duration stimuli (750 ms first stimulus + 750 ms inter-stimulus interval + 750 ms second stimulus) plus the time needed to record the subject’s response. Thus, it would require about 48 min to obtain a single threshold estimate with 10% or less variability with respect to the real threshold (e.g., 25 probe stimuli repeated 20 times each, or 35 probe stimuli repeated 15 times each). Because we sought to assess discrimination thresholds at four different locations, the experimental session would have to last over 3 h to obtain threshold estimates with less than 10% variability. Therefore, we elected to trade-off the variability of the estimated threshold and the duration of the experiment. Selecting 11 different probe stimuli repeated 10 times each would yield an estimated threshold variability of about 20% and an estimation time of about 13 min per testing (≈ 1 h total for four testing sites). Further reduction of the assessment time is probably not worth considering, as the estimated threshold variability increases sharply with decreasing numbers of
probe stimuli and/or trial repetitions at each stimulus. Conversely, increasing the number of probe stimuli and/or repetitions would decrease the estimated threshold variability, but only at a slow rate. Therefore, an increase in the number of stimuli and/or trials would be time-consuming and yield only small improvements in the estimated threshold variability.

Footnotes

1 These authors contributed equally to this work.

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