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RESEARCH ARTICLE

Sensory Processing

Transcutaneous conditioning electrical stimulation alters the cold detection threshold

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Abstract

Many patients with small fiber neuropathy experience elevated pain sensation for cold stimuli, and the pathophysiology is highly unknown. Therefore, the aim of this study was to evaluate a new method for probing the peripheral cold-sending fibers by combining electrical and thermal stimulation. The cold and warm detection thresholds (CDT and WDT) were measured in 17 healthy participants under the conditions with and without electrical conditioning stimulation. The electrical stimulation was tested using both 4 and 250 Hz at 50% of the electrical perception threshold. A small area cathode electrode was used for the electrical stimulation, and a thermode was placed on top of the electrode to estimate the thermal thresholds. Two-way RMANOVA was performed to analyze the results. The CDT decreased from 26.8°C (SD −4.1, +2.5, log-transformed) to 25.8°C (SD −4.9, +2.9, log-transformed) by the conditioning electrical stimulation ($P = 0.006$). The mean WDT was 41.6°C (SD −3.0, +4.1, log-transformed) without and 40.7°C (SD −3.1, +4.6, $\log_{10}$-transformed) with conditioning electrical stimulation ($P = 0.12$). No significant main effect was found for the frequency of electrical stimulation for the two thermal thresholds. Conditioning electrical stimulation significantly altered the CDT but not the WDT, which can be explained by the small cathode's preferential activation of Aδ fibers to a greater extent than C fibers since the cold, innoxious sensation is mainly mediated by Aδ fibers and the warm sensation by C fibers. Combining thermal and electrical stimulation may, in the future, be used for probing cold-sensing fiber excitability, but further studies are necessary to validate the results.

NEW & NOTEWORTHY A novel approach is evaluated for probing peripheral cold-sensing fiber by using electrical stimulation's large variety of protocols in combination with activation of the fiber by thermal stimulation to ensure selective activation of the cold-sensing fibers. The results showed that the cold detection threshold could be altered by electrical stimulation, but no significant differences were found for warm temperatures or different frequencies of conditioning electrical stimulation. The result was promising, but further studies are needed.

cold allodynia; cold detection threshold; peripheral nerve excitability; thermal thresholds; transcutaneous electrical stimulation

INTRODUCTION

Patients with small fiber neuropathy experience a wide range of symptoms, such as burning pain (69%), sharp pain (31%), and "sunburn-like" pain (24%) (1). One symptom that puzzles researchers is abnormal pain sensations in response to cold stimuli, manifesting either as a heightened response to painful stimuli or as pain caused by normally nonpainful stimuli (1). Three percent of people with small fiber neuropathy have spontaneously evoked cold pain, whereas 27% have altered responses to evoked cold stimulation (1). A warm cutaneous sensation is mediated primarily by C fibers, whereas cold detection is primarily a function of Aδ fibers (2, 3). Because knowledge of cold sensory nerve fibers is limited, additional methods must be developed to obtain excitability measurements of cold-sensing fibers. Quantitative sensory testing is the conventional method for probing thermal sensitive peripheral nerve fibers (4–6). During quantitative sensory testing, the cold sensory fibers are probed by measuring the cold

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detection and pain thresholds, thereby limiting the information that can be obtained through this method to a few measurements. Therefore, this study evaluates a novel method for probing cold-sensitive nerve fibers. Electrical stimulation to activate nerve fibers has frequently been used to probe nerve fibers in the skin (7–11). This method has the advantage of high temporal resolution, and since it bypasses sensory transduction, it can be used to study membrane excitability to a greater extent than the thermal stimulus (7, 9). The major disadvantage of electrical stimulation is its lack of selectivity for small nerve fibers. Fibers of large diameter have a lower activation threshold than fibers of small diameter with transcutaneous electrical stimulation (12, 13); this phenomenon is usually referred to as the recruitment order of nerve fibers. A small area cathode can be used to reverse the recruitment order of nerve fibers (11, 14–17) by generating high voltage potentials in the upper layer of the skin, where the small fibers terminate, unlike the large fibers, which terminate deeper in the skin (18–20). The sensation from small area cathode stimulation is usually described as stabbing, shooting, and sharp (10, 21), thus indicating primary activation of Aδ fibers; however, coactivation of C fibers may also occur (22). Because electrical stimulation by small-area cathodes activates all subtypes of Aδ fibers, it cannot be used for studying cold-sensing fibers in isolation. The aim of this study was to evaluate an approach for probing cold-sensing fibers by conditioning nerve fibers with subthreshold electrical stimulation simultaneously as the cold threshold is estimated. The intent of the electrical stimulation was not to activate the nerve fibers but to condition the fibers with different stimulation paradigms. In contrast to electrical stimulation, cold stimulation was used to selectively activate cold-sensing fibers. By combining the two stimulation modalities, the advantages of both modalities can be harnessed to probe the excitability of cold-sensing fibers. Hence, different high-resolution conditioning electrical stimulation paradigms can be used, whereas cold stimulation ensures selective activation of the cold-sensing fibers.

We hypothesized that the conditioning electrical stimulation would depolarize the membrane potential of fibers, thereby altering the thermal thresholds. Since small area cathode electrode preferentially activates Aδ fibers (11, 15, 16), which primarily mediate cold rather than warm sensations (3). It was expected that the conditioning electrical stimulation would have a larger effect on the cold detection threshold (CDT) than on the warm detection threshold (WDT).

MATERIALS AND METHODS

Participants

Seventeen healthy participants (10 males) with an average age of 23.9 ± 4.4 yr were recruited to participate in this experiment. The exclusion criteria were neurological diseases, previous involvement in a traumatic accident involving electrical shock, nonintact skin, consumption of alcohol, drugs, or analgesic substances within 24 h before participating in the experiment, heart disease, pacemaker implantation, or chronic pain disorder. The participants were given detailed written and verbal information regarding the study and provided signed informed consent before participation. The study was approved by the local ethics committee (Den Videnskabsetiske Komité, Region Nordjylland; Approval Number N-20200014) and was conducted in accordance with the Declaration of Helsinki.

Experimental Setup

The participants were placed in a comfortable position in an upright bed. The participants were offered blankets to ensure that they were not cold. To ensure that the temperature was as even as possible in the room, the windows were shaded and closed. To condition the small nerve fibers, we used a small area cathode (23). The electrode was a custom-made planer array electrode (size: 40 × 34) which comprised 12 small quadratic cathodes (size: 0.6 × 0.6 mm) and two anodes (size: 5 × 24 mm) located on both sides of the cathode array (see Fig. 1). The electrode was placed on the left leg 10 cm proximal to the talus and fastened with tape. On top of the electrode, a thermode (3 × 3 cm thermode (Pathway, Medoc Ltd., Ramat Yishai, IL) was placed and fastened to the leg with a Velcro band. The electrical stimuli were administered with a DS5 electrical stimulator (Digitimer Ltd., Welwyn Garden City, UK), and the voltage of the stimulation was controlled manually.

Estimation of the Conditioning Electrical Perception Threshold

The method of limits was used to determine the electrical perception threshold (see Fig. 2). It took ∼10–15 min to estimate the electrical perception threshold. Participants were instructed to respond when they experienced any sudden transient change in skin sensation. Initially, the intensity was set to 10 µA; if the participant did not feel the stimulation, the intensity was increased by 20 µA. The intensity increase was increased by 50% every time the participant did not feel the stimulation. The stimulus intensity was increased until the participants responded three times to the same stimulation intensity. This intensity was defined as the upper limit. Thereafter, the stimulus intensity decreased by 50% of the increase in intensity previously used. The stimulus intensity continued to be decreased until the participants did not respond to the stimulation intensity three times. This intensity was defined as the lower limit. The

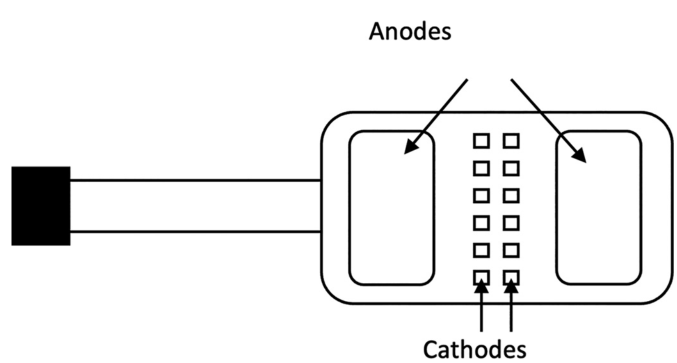

Figure 1. Electrode design. The planner surface electrode comprises 12 small-area cathodes in arrays. Two large anodes are located on both sides of the cathode.

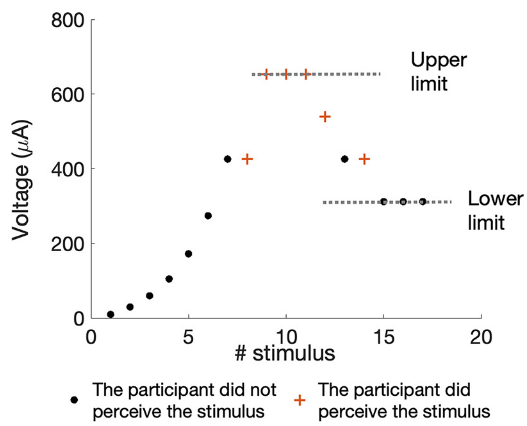

Figure 2. Estimation of the electrical perception threshold. The *x*-axis represents the order of the stimulus, and the *y*-axis represents the intensity of the electrical stimulation. The dots and crosses indicate the stimulation intensity not perceived and perceived, respectively. The electrical intensity started low and was increased until the participant perceived the specific intensity three times. Thereafter, the intensity was decreased until the participant could not perceive the stimulus three times.

electrical perception threshold was defined as the average of the upper and lower limits. The intensity of the conditioning electrical stimulation was set to 50% of the perception threshold. The electrical conditioning stimulation was estimated for two frequencies (4 Hz and 250 Hz). The order of the frequencies was randomized.

Psychophysical Questionnaire

After the electrical perception threshold was estimated, each participant was asked to rate their sensation of the electrical stimulation on a scale of 0–10, where 0 was no sensation, and 10 was the worst sensation imaginable. The participants were asked to rate the sensations: itching, tapping touch, aching, hot/burning, cold/freezing, sharp, stinging, and pinching.

Thermal Threshold Estimation

After estimating the conditioning electrical stimulation perception threshold, each participant was familiarized with the thermal stimulation protocol. The familiarized procedure took ~5 min. The results from the familiarization session were not analyzed. The conditioning electrical stimulation was set to 50% of the intensity of the perception threshold, which is below the intensity for the participants to perceive the stimulus. The temperature was initially set to 32°C, and was subsequently either increased or decreased (1 C/s) until the participant perceived the stimulus. The participants were instructed to push a button when they felt the thermal stimulus. This procedure was repeated four times for the thermal detection threshold. To estimate the CDT and WDT took ~5 min. The cold thermal threshold was assessed before the warm threshold in seven participants, and the opposite order was used in ten participants. The thermal threshold was assessed for two conditions: with and without the conditioning electrical stimulation. The order of the two conditions was randomized (see Fig. 3 for a flowchart of the experiment). The participants were blinded to the condition performed. To control the blinding, the participants were asked whether they perceived the sensations of the two conditions differently and, if so, how and for which condition.

Statistical Analysis

A two-way repeated measures analysis of variance (RMANOVA) was performed with the thermal threshold as the dependent variable, and frequency (4 Hz and 250 Hz) and electrical stimulation (control and electrical stimulation) as independent within-subject variables (SPSS 28, IBM). For seven participants, the warm thresholds were estimated first, and for ten participants, the cold threshold was estimated first. To investigate the influence of the order of thermal thresholds, we used a between-subject variable representing the two conditions of warm thresholds first or cold thresholds first. A separate RMANOVA was conducted for the CDT and WDT. A two-way repeated measure ANOVA was performed with psychophysical sensations as the dependent variable and frequency (4 Hz and 250 Hz) as an independent within-subject variable. We examined the skewness and kurtosis to investigate whether the data were normally distributed. Because the thermal threshold was right-skewed, the thresholds were $\log_{10}$-transformed.

RESULTS

Psychophysical Characteristics of Electrical Stimulation

The psychophysical characteristics of electrical stimulation are presented in Fig. 4. A two-way repeated measures ANOVA was conducted. In the analysis of psychophysical sensation, a main effect of the sensations ($F = 9.971$, $P <$

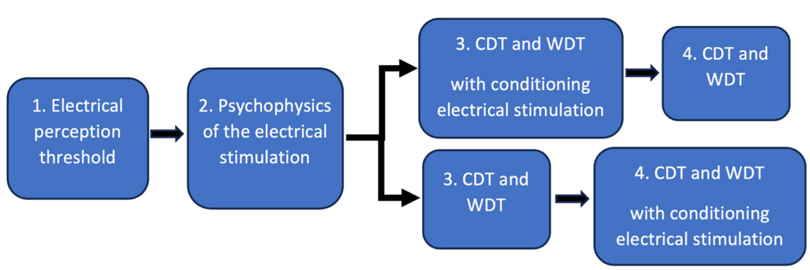

Figure 3. Experimental procedure flowchart. The procedure illustrated in the figure was performed for the two conditioning electrical stimulations, 4 Hz and 250 Hz. *1*) The electrical perception threshold was estimated using the limits method. Two different electrical stimulations were used (4 or 250 Hz), and the order of the conditions was randomized. *2*) The participant was asked to rate the sensation of the electrical stimulus they experienced when the electrical perception threshold was estimated. *3*) and *4*) The participants were randomly divided into two groups. In the first group, the CDT and WDT were estimated with conditioning electrical stimulation first and without after (*top* path in the flowchart). In the second group, the order was switched (*bottom* path in the flowchart). CDT, cold detection threshold; WDT, warm detection threshold.

0.001) was observed. Pairwise comparisons between sensations are shown in Fig. 4 and Table 1. Adjustment for multiple comparisons was performed with Bonferroni correction. No significant main effect was found for frequency (P = 0.437, F = 0.638), or the interaction between sensation and frequency (P = 0.397, F = 0.760).

The Influence of Conditioning Electrical Stimulation on Thermal Thresholds

The electrical perception thresholds were estimated with the method of limits for the two frequencies (4 Hz: 103.2 µA ± SD 80.0 µA, and 250 Hz: 142.4 µA ± SD 77.9 µA). The CDT and WDT were estimated for the conditions with and without conditioning electrical stimulation (see Fig. 5). A two-way repeated measures analysis of variance (RMANOVA) was performed with the thermal threshold as the dependent variable, and frequency (4 Hz and 250 Hz) and electrical stimulation (control and electrical stimulation) as independent within-subject variables. Analysis of the thermal thresholds revealed a main effect of conditioning electrical stimulation for the CDT (F = 10.018, P = 0.006) but not for the WDT (P = 0.12, F = 2.612). No significant main effect was found for frequency for the two thermal thresholds (P = 0.885, F = 0.022 for CDT; P = 0.768, F = 0.097 for WDT). In addition, no significant effect was found for the order of thermal stimulation (P = 0.721, F = 0.132 for CDT; P = 0.325, F = 1.036 for WDT).

The mean CDT was 26.8°C (SD −4.1, +2.5 log-transformed) without and 25.8°C (SD −4.9, +2.9, $\log_{10}$- transformed) with conditioning electrical stimulation. For the CDT, the coefficient of variation was 15% and 9% without the electrical stimulation, and with the electrical stimulation, 19% and 11%. The mean WDT was 41.6°C (SD −3.0, +4.1, log-transformed) without and 40.7°C (SD −3.1, +4.6, $\log_{10}$-transformed) with conditioning electrical stimulation. For the WDT, the coefficient of variation was 7% and 10% without the electrical stimulation and with the electrical stimulation, 8% and 11%.

The blinding of the participants was successful since only 4 of 17 participants perceived a difference between the presence and absence of conditioning electrical stimulation.

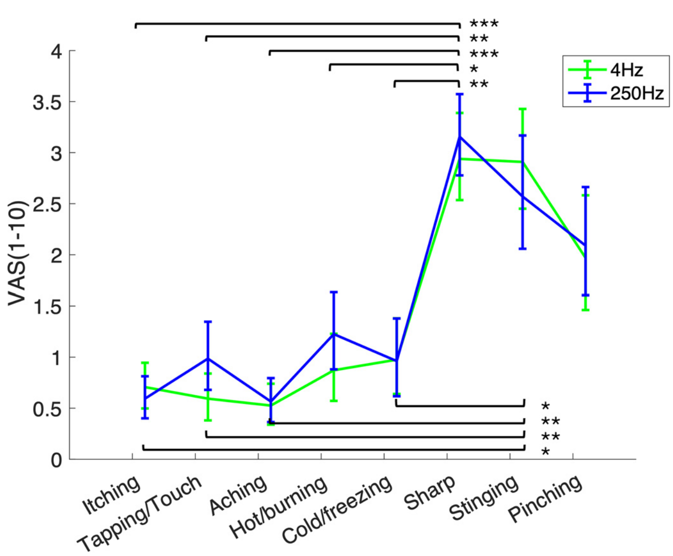

Figure 4. Psychophysical characteristics of electrical stimulation. After the electrical stimulation, the participants were asked to rate their sensation on a visual analog scale (VAS) of 0–10, where 0 indicated no sensation and 10 indicated the highest intensity imaginable. The error bars represent the standard error, and the means were calculated on $\log_{10}$-transformed data (n = 17). The statistics are conducted on data when both frequencies are pooled together. *P < 0.05; **P < 0.01; and ***P < 0.001.

DISCUSSION

This study evaluated the effects of conditioning electrical stimulus on thermal detection thresholds to develop a novel method for probing cold-sensing fibers. The conditioning electrical stimulus significantly reduced the CDT, but it did not significantly increase the WDT. Although the change in the WDT was not significant, it might be due to the small number of participants included in the study. There was no significant difference between the stimulus frequencies used for conditioning (4 Hz and 250 Hz). Both the CDT and WDT differed substantially from those in previous literature, in which a lower CDT [26.8°C vs. 30.3°C (24)] and higher WDT [41.6°C vs. 36.6°C (24)] have been reported. A possible explanation for these differences might be the insulating effect of the electrode.

Sensation of the Electrical Stimulus

Participants reported the strongest psychometric sensations for sharp, stinging, and pinching, and mild sensations for itching, tapping touch, hot/burning, and cold/freezing. These findings were similar to those in previous studies using electrical stimulation with small-area cathodes, in which the psychophysical characteristics comprised stabbing, shooting, and sharp sensations (10, 17).

Effects of the Conditioning Electrical Stimulus on Thermal Thresholds

The primary hypothesis in this study was that the conditioning electrical stimulation would depolarize the membrane potential of Aδ nerve fibers and thereby increase the cold thermal thresholds. The conditioning electrical stimulation unexpectedly decreased rather than increased the CDT. One possible explanation for this finding might be that the conditioning electrical stimulation distracted the participants from sensing the thermal stimulus, and the threshold consequently decreased. However, the blinding to electrical stimulation was successful, and only the CDT, but not the WDT, was affected. Another possible explanation might be habituation, involving a decreased threshold due to repetitive activation arising from either central processing, such as the descending pain pathway (25), or peripheral mechanisms (26, 27). Increased electrical threshold during transcutaneous electrical stimulation has been shown to be particularly elevated for small fibers (10). In addition, microneurography has shown that activity-dependent slowing, an indication of decreased excitability, is pronounced in C fibers (26, 27).

Different frequencies or shapes of electrical stimulation have been shown to preferentially activate subpopulations of small fibers. For instance, 4 Hz sinus stimulation preferentially activates mechanosensitive C fibers (28). The results of this study did not show any difference between

Table 1. *Pairwise comparisons*

	Itching	Tapping Touch	Aching	Hot/Burning	Cold/Freezing	Sharp	Stinging	Pinching
Itching		1	1	1	1	**<0.001**	**0.014**	0.348
Tapping touch	1		1	1	1	**0.003**	**0.005**	0.344
Aching	1	1		1	1	**<0.001**	**0.002**	0.078
Hot/burning	1	1	1		1	**0.02**	0.199	1
Cold/freezing	1	1	1	1		**0.003**	**0.013**	0.341
Sharp	**<0.001**	**0.003**	**<0.001**	**0.02**	**0.003**		1	1
Stinging	**0.014**	**0.005**	**0.002**	0.199	**0.013**	1		1
Pinching	0.348	0.344	0.078	1	0.341	1	1	

Statistical test of the pairwise comparison of the psychophysical characteristics of electrical stimulation. A two-way repeated measures ANOVA was conducted. In the analysis of psychophysical sensation, a main effect of the sensations ($F = 9.971$, $P < 0.001$) was observed. Pairwise comparisons between sensations are shown in the table. P values < 0.05 are shown in boldface.

frequencies in either the sensation or the effect of the conditioning electrical stimulation on thermal thresholds. Because small area cathodes activate preferentially Aδ fibers (10, 17, 22), the pulse shapes' preferential activation ability might not have been sufficient to overcome the recruitment order of the nerve fibers; that is, fibers of larger diameter had a lower electrical activation threshold than the ones of smaller diameter.

The Habituation in Peripheral Sensory Neurons

The perception threshold of transcutaneous stimulation is altered with repeated stimulation for both small and large fibers (29); however, the habituation is much more pronounced for small fibers (55%) versus large fibers (1%) (29). The origin of the habituation has not been fully understood, but long-term depression of synaptic strength has been proposed, as low-frequency stimulation reduced perceived pain by 27% in humans and correlates with animal experiments showing altered synaptic strength in the spinal cord (16).

Another pronounced mechanism that generates reduced excitability of small fibers upon repetitive electrical stimulation is activity-dependent slowing (ADS). During microneurography, the latency of C fibers can be assessed, and for frequencies between 0.25 and 2 Hz, the latency can increase by 30% (26). ADS is occurring in the peripheral nerve endings, and accumulation of intracellular sodium and slow inactivation of sodium channels have been proposed as possible mechanisms (30).

All of these three studies presented above use electrical stimulation intensities that are above the perception threshold of the participants. This is not the case for this study; however, the stimulus may be generating action potentials that the participants do not perceive, and thereby, synaptic depression or ADS could play a role in the altered thermal threshold reported in this study.

Limitations of the Study

Combining electrical and cold stimuli is a new approach that has not been rigorously studied; therefore, several experimental procedures could be improved. The electrical stimulation was continuously applied when the thermal threshold was measured. An improvement would be to apply the electrical stimulus only when thermal stimulation is applied to decrease the amount of electrical current in the skin and thereby limit the habituation effect.

Applying electrical stimulation at the exact location as thermal stimulation is difficult because conventional small area cathodes are pin electrodes, and the thermode cannot be placed on top of the electrode. Our research group has developed a novel planar electrode for preferential activation of small fibers (21, 23), thus enabling the thermode to be placed on top of the electrode.

Future Perspectives

Our group has previously used temperature to condition the electrical test pulse to obtain more information about the excitability properties of nerve fibers (23). This study demonstrates that various stimulation modalities, including electrical and thermal modalities, can be combined, potentially providing additional insights into the excitability of peripheral nerves.

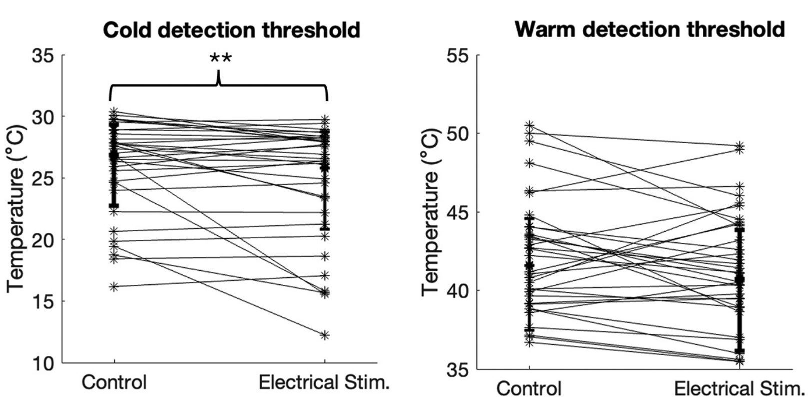

Figure 5. Influence of conditioning electrical stimulation on thermal detection thresholds. The error bars represent the standard deviation, and the means are calculated on log_{10}-transformed data ($n = 17$). The mean for both frequencies for the conditioning electrical simulation is illustrated. There was no difference between the frequencies; therefore, the data were pooled together. The statistics are conducted on data when both frequencies are pooled together. $^{**}P < 0.01$.

Future studies may test a broader range of electrical stimulation pulse shapes and use more sophisticated algorithms for measuring both thermal and electrical perception thresholds, such as the adaptive Psi method (31).

DATA AVAILABILITY

Data will be made available upon reasonable request.

GRANTS

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DISCLOSURES

J.T. and C.D.M. have a noncontrolling interest in the company Inventors Way, which supplies research equipment and software. None of the other authors has any conflicts of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

K.S.F., C.D.M., and J.T. conceived and designed research; C.C. and J.T. performed experiments; J.T. analyzed data; C.C., K.S.F., C.D.M., and J.T. interpreted results of experiments; J.T. prepared figures; C.C. drafted manuscript; K.S.F., C.D.M., and J.T. edited and revised manuscript; C.C., K.S.F., C.D.M., and J.T. approved final version of manuscript.

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