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Published in:
Muscle & Nerve

DOI (link to publication from Publisher):
10.1002/mus.27967

Publication date:
2023

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Borbjerg, M. K., Antonsson, E., Røikjer, J., Ejskjaer, N., & Mørch, C. D. (2023). The stability of perception threshold tracking for long session evaluation of Aβ- and Aδ-fiber function. *Muscle & Nerve*, *68*(5), 743-749. https://doi.org/10.1002/mus.27967

Received: 20 November 2022 | Revised: 15 August 2023 | Accepted: 20 August 2023

DOI: 10.1002/mus.27967

CLINICAL RESEARCH ARTICLE

MUSCLE&NERVE WILEY

The stability of perception threshold tracking for long session evaluation of Aβ- and Aδ-fiber function

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Funding information
Lundbeck Foundation to the Innovation Centre Denmark, Silicon Valley to fund Danish American Research Exchange for Mette Borbjerg at University of California, San Francisco; Danish National Research Foundation, Grant/Award Number: DNRF121

Abstract

Introduction/Aims: Research has proven that epidermal and transcutaneous stimulation can identify the function of Aβ and Aδ fibers (i.e., in diabetes) individually using different electrodes. In this study we aimed to determine the stability of perception thresholds when using such electrodes.

Methods: Twenty healthy volunteers participated in this study. The perception threshold of Aβ fibers (patch electrode) and Aδ fibers (pin electrode) was estimated 30 times during a period of 60 minutes. A threshold was established every other minute, alternating between the two electrodes. The stimulus duration was 1 millisecond and the interstimulus interval was 1.5 to 2.5 seconds. Linear regressions of the perception threshold as a function of time were performed. The slopes were used as an estimate of habituation and were compared between the electrodes.

Results: The slope was significantly larger when assessed by the pin electrode (median: 0.020 [0.009 to 0.030] mA/trial) than when assessed by the patch electrode (median: 0.005 [0.001 to 0.018] mA/trial) ($P = .017$, paired t test). During the session, total increases in perception threshold of approximately 55% and 1% were seen for the pin and patch electrodes, respectively.

Discussion: The two fiber types assessed showed significant perception threshold increases. The higher slope of the pin electrode indicated that the Aδ fibers were more prone to habituation than the Aβ fibers, and that habituation should be considered during prolonged experiments. This assessment is valuable for future research on nerve fiber function using the technique for long session experiments.

KEYWORDS
habituation, nerve function, neurophysiology, perception threshold, small-fiber neuropathy

1 | INTRODUCTION

Large myelinated Aβ fibers terminate in the dermis, whereas small unmyelinated C fibers and partially myelinated Aδ fibers terminate in the epidermis.[1,2] Small nerve fiber function can be altered in

Abbreviation: SFN, small-fiber neuropathy.

Preliminary data from this study were presented at NeuroDiab in Bergen, Norway, September 2022.

neuropathies of varying etiologies. Small-fiber neuropathy (SFN) is characterized by selective involvement of the Aδ and C fibers.[3]

New diagnostic methods for SFN have emerged, including nociceptive evoked potentials, corneal confocal microscopy, and epidermal electrical stimulation.[3] Many different types of electrodes have been suggested for epidermal electrical stimulation.[3–8] The superficial position of the Aδ and C fibers, along with the high electrical activation threshold, results in difficulty in activating the small Aδ and C fibers selectively.[4,9] Small cathode areas are common for the electrodes designed for Aδ- and C-fiber activation as they generate a high current density at the dermoepidermal junction, thus preferentially activating Aδ or C fibers, depending on pulse shape and duration.[4,6] The pin electrode used by Mørch et al. has been shown to stimulate Aδ fibers preferentially.[4,7–12] The pin electrode has been used together with a conventional patch electrode to stimulate both Aδ and Aβ fibers to determine the perception threshold. One of the benefits of using epidermal electrical stimulation is that it is noninvasive and can evaluate Aδ-fiber function, which could allow for diagnosis of predegenerative functional impairment that may go undetected on a skin biopsy.[3] The ability to detect changes in Aδ-fiber membrane properties and evaluate Aδ-fiber function could be a valuable tool in the early diagnosis of SFN.[7,13,14] Furthermore, assessment of these properties and functions could contribute to a better understanding of the pathogenesis of SFN.[3]

Assessment of Aδ-fiber membrane properties or evoked cortical potentials requires repeated electrical stimulation to obtain perception thresholds or cortical potentials. However, repeated stimuli within the nervous system can lead to a weaker response, that is, habituation.[15] Factors affecting habituation include frequency and intensity of stimuli.[15] One study indicated an increase in the activation threshold for Aδ but not Aβ fibers.[11]

This study aims to determine the stability of the perception threshold for Aβ and Aδ fibers in healthy subjects over 60 minutes when using conventional transcutaneous and epidermal electrical stimulation. This assessment is valuable to research using perception thresholds over a longer course of time.

2 | METHODS

2.1 | Study design

This was an observational, cross-sectional, experimental study, approved by the North Denmark Region Committee on Health Research Ethics (N-20190055) and conducted in accordance with the Helsinki Declaration of 1975.

2.2 | Participants

Participant eligibility was assessed using a written questionnaire and a verbal discussion with one of the research investigators. The researchers obtained written informed consent. Exclusion criteria were as follows: pregnancy or breastfeeding; drug addiction; previous or current neurological, musculoskeletal, or mental illnesses; a history of conditions possibly leading to neuropathy (e.g., diabetes or autoimmune diseases); current use of medications that may affect the study (e.g., analgesics); a previous traumatic experience of an electrical accident; consumption of alcohol, nicotine or painkillers within the last 24 hours; application of moisturizing lotion over the volar forearm within the last 24 hours; participation in pain studies throughout the study period; and inability to cooperate.

2.3 | Experimental setup

The participants were seated comfortably in a reclining chair in a hospital examination room, and possible distractions were removed. The room temperature was standardized to 20°C. All instructions were standardized. Only two investigators conducted the study, and they received training to standardize the experiment.

Electrical stimulation was given through two types of surface electrodes placed anteriorly on the subject's nondominant forearm (Figure S1).

In what follows, a test round will refer to the determination of the perception threshold using 30 stimuli and their response. Every given stimulus will be referred to as a trial.

2.3.1 | Aβ-fiber activation

The electrical stimulation of Aβ fibers was applied with conventional Ag-AgCl patch electrodes. A cathode measuring 2×1.5 cm (Neuroline 700; Ambu A/S, Ballerup, Denmark) was placed approximately 5 cm proximal to an imaginary line through ulnar and radial styloid processes on the volar forearm. The anode measured 5×9 cm^2 (Pals Neurostimulation Electrode, Axelgaard Company, Fallbrook, CA, USA) and was placed on the dorsal forearm at the same level as the cathode.

2.3.2 | Aδ-fiber activation

Aδ fibers were stimulated using a custom-made cutaneous pin electrode (Aalborg University, Denmark) placed with the center 10 cm distal to the superior border of the cubital fossa and fixed with surgical tape. The pin electrode consists of 16 stainless-steel blunted electrodes (Ø 0.2) serving as cathodes surrounded by a concentric stainless-steel ring (area 8.8 cm^2) serving as the anode. The placement is illustrated in Figure S1.

Electrical stimulation was given using a custom-made program (LabBench; Inventors Way, Denmark) and an isolated bipolar current stimulator (DS5; Digitimer, Letchworth Garden City, UK). Participants indicated a perceived stimulus with a push button (Inventors Way, Denmark) held in their dominant hand.

2.4 | Protocol

The protocol consists of two parts: (1) An introductory test determining the perception threshold using the method of limits for each of the electrodes, which constituted the basis for the 60-minute protocol. The Method of Limits is described in Figure S2. (2) A 60-minute protocol assessing the perception threshold 15 times for each electrode over 60 minutes to evaluate the stability of the threshold. Figure S3 illustrates the study protocol.

2.4.1 | Introductory test

The perception threshold was established for both electrodes using the Psi method. To initiate the maximal range of stimuli in the Psi method, the Method of Limits was used for initial estimation of the perception threshold (Figure S2). The initial threshold was calculated as the average of the maximum and minimum stimulus intensities. The same procedure was followed for the pin electrode. The initial thresholds of the introductory test were used to establish the appropriate intensity of the initial stimuli in the first round of the 60-minute protocol.

2.4.2 | 60-minute protocol

Thirty thresholds were determined with the Psi method; this method is described in Figure S4. A threshold was established every other minute, alternating between the patch and pin electrodes (Figure S3). The electrode order (pin or patch) was randomized. The perception threshold was determined based on 30 rectangular stimuli. A stimulus duration of 1 millisecond was chosen, and the stimuli were given at intervals of 1.5 to 2.5 seconds. The Psi method estimates the psychometric function often called the Psi function and the perception threshold is defined as the intensity that has 50% probability of being perceived. The psychometric function was estimated as the cumulative distribution function of the posterior function.[16,17] The "Quick" distribution from the Weibull distribution function was used and implemented using LabBench software (Inventor's Way, Denmark) based on the paper of Kontsevich and Tyler.[16] [Correction added on 15 September 2023, after first online publication: In the preceding sentence, the citation to reference 18 and 'Palamedes Toolbox' has been changed to reference 16 and the 'paper of Kontsevich and Tyler'.] The intensity of the first stimulus was based on the initial Method of Limits estimation. The initial stimulus intensity in the following 14 threshold estimations was based on the threshold from the previous estimation. The psychometric function's lapse and guess rates were fixed at 0.02% and 0%, respectively.[16,18] The psychometric function and an overview of a test round are illustrated in Figure S4.

2.5 | Statistical analysis

Statistical analysis was performed using R statistical software version x64 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

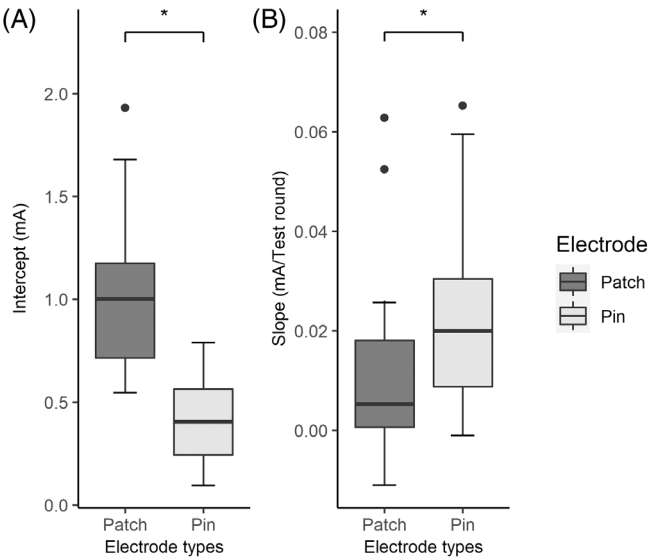

FIGURE 1 Intercept and slope for the pin and patch electrode. Boxplot illustrating intercept of the linear regression between perception threshold and time. (A) There was a significant difference in intercept between the pin electrode (0.403 mA, interquartile range [IQR] = 0.243 to 0.563) and patch electrode (1.002 mA, IQR = 0.715 to 1.175; $P < .001$; paired Wilcoxon test). (B) The slopes were significantly different from zero indicating habituation for both the pin (0.020 mA/trial, IQR = 0.009 to 0.030; $P < .001$; one-sample Wilcoxon test) and patch electrode (0.005 mA/trial, IQR = 0.001 to 0.018; $P = .007$, one-sample Wilcoxon test), and a significant difference was seen between the pin and patch electrodes ($P = .017$; paired Wilcoxon test).

Simple linear regression of perception threshold as the function of trial number was performed for each participant. Slopes and intercepts were estimated for each participant. Slopes were interpreted as the increase in perception threshold as the function of trial number (milli-Amps per trial), also referred to as neural habituation, that is, nerve adjustment in response to repeated exposure to the same stimulus type. This adjustment leads to a higher threshold. The intercept was interpreted as the habituation-free perception threshold (milli-Amps).

To test the significance of the habituation of the perception threshold, a Wilcoxon test was performed on the linear regression slope for each electrode. A paired Wilcoxon signed-rank test was performed on the linear regression slopes to test for possible differences in habituation between the nerve fiber types. Similarly, a paired Wilcoxon signed-rank test was performed on the linear regression intercepts. For the Wilcoxon test results, a significance level of $\alpha = 0.05$ was chosen.

Percentage increase in perception threshold was calculated as follows:

$$\frac{\text{Finale value} - \text{Starting value}}{\text{Starting value}} \times 100$$

The starting value was the median of the perception thresholds for all participants at trial 1 and the final value was the median of perception thresholds for all participants at trial 15.

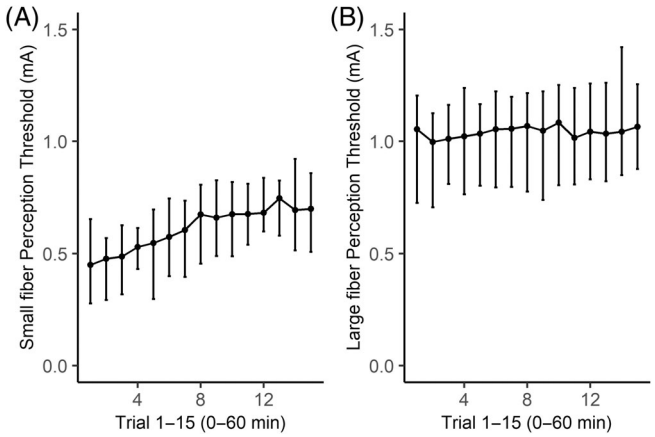

FIGURE 2 Perception threshold for Aβ and Aδ fibers. Median perception threshold for participants for the patch electrode (on the left) and the patch electrode (on the right). For each test round interquartile range is calculated and shown as error bars.

3 | RESULTS

Twenty healthy volunteers, 10 men and 10 women, aged 20 to 32 years (mean 24.6 ± 2.7 years), participated in the study. Four participants (20%) held the button in the right hand. Linear regression results for the individual participants had an adjusted R^2 range from 0.02 to 0.87 and a range of 0.00 to 0.86 for pin and patch electrode results, respectively.

The intercept of the pin electrode was significantly lower than that of the patch electrode Figure 1A. The slopes were significantly different from zero, indicating habituation for both the pin and patch electrodes, and a significant difference was seen between the pin and patch electrodes ($P = .017$) (Figure 1B).

The increase in perception threshold of 0.02 mA/trial in Aδ fibers led to a 55.4% increase in perception threshold between trials 1 and 15. The increase in perception threshold for Aβ fibers was 1% when assessing the perception threshold median over time (Figure 2).

4 | DISCUSSION

This study has shown that the perception threshold increased for both Aβ (patch) and Aδ (pin) fibers, indicating habituation to repeated stimuli. A significantly larger increase was observed for the Aδ fibers than the Aβ fibers. This suggests a greater habituation in the activation threshold for Aδ fibers, consistent with that found by Hugosdottir et al.[11] Furthermore, the study showed a significant difference between intercept for the pin and patch electrodes; this is not surprising, as electrode size and design are different between the two electrodes. Small cathodes were used to stimulate Aδ-nerve fibers selectively, which has been shown to reduce the stimuli intensity needed to activate the nerves.[4]

4.1 | Habituation and dishabituation

The significant increase in perception threshold may be indicative of habituation. The greatest habituation was seen for Aδ fibers. This is similar to what has been shown in studies of withdrawal reflexes, in which the habituation was seen mainly for the RIII component, which is primarily mediated by Aδ fibers.[19,20] The stimulus intensity was very low as the purpose was to find the perception threshold. A weak stimulus is known to lead to a higher degree of habituation.[20] Considering the difference in stimulus intensity between electrodes, the difference in perception threshold increase could be explained as increased habituation.

In contrast to the apparent habituation of Aδ fibers observed in this study and in RIII reflexes, temporal summation of pain is a well-established phenomenon. Temporal summation of pain has been shown for repeated heat pain[21] and pressure pain[22] stimuli. When exposed to tonic heat at a constant temperature, the pain perception tends to decrease in healthy young participants (e.g., see Yelle et al.[23] for control condition). Temporal summation of pain may also be observed for high-intensity (10× perception threshold) and high-frequency (100 Hz) electrical stimulation,[24] which was explained by an long-term potentiation-like increase of synaptic strength. The similar wind-up phenomenon is mainly seen in animal studies of ∼1-Hz activation of C fibers and is mainly driven by the *N*-methyl-D-aspartic acid receptor. In contrast, similar activation of small cutaneous nerve fibers in human studies tends to exhibit a decreased response over time, often referred to as long-term depression.[24] Therefore, the temporal properties of activation of Aδ fibers and the temporal properties of the pain system seem to be rather influenced by stimulation intensity and modality.

In this study, multiple factors could have helped prevent habituation. These are the changes of stimuli intensity and frequency throughout the study as well as the long pause between threshold estimations. Interstimulus intervals of more than 25 seconds have been shown to remove habituation altogether.[25] In this study, a pause of 60 seconds was used between electrode types.

The habituation in this study was relatively linear, unlike the typical habituation, which is a negative exponential function.[15,20] This study should have revealed a positive exponential regression in the perception threshold to follow this pattern, presumably with a flattening of the curve, as increase in stimuli intensity will decrease habituation.[20] Is not entirely clear why the pattern that was found is not an exponential curve. One possibility is partial spontaneous recovery between each threshold estimation. It is commonly understood that part of habituation is recovery of the response if the habituating stimulus is withheld.[15] The recovery time varies between stimulus types. Partial recovery could explain why threshold tracking over 60 minutes was not enough to observe the typical exponential habituation.[15] A second possibility may be the presence of some degree of dishabituation. This dishabituation is an exposure to a different stimulus, which will lead to a recovery of the habituated response.[15]

4.2 | Contributing factors

Several factors, including room temperature, skin temperature, distracting noises, and the participants' attention, have been shown to cause an increased perception threshold during quantitative sensory testing.[26,27] As the method used in this study was subjective measures of Aβ- and Aδ-fiber function, similar mechanisms may apply. In this study we have attempted to control these external factors by standardizing the room temperature and avoiding potential distractions during testing.[27] Hugosdottir et al. saw a significant decrease in perception threshold when the skin temperature was 20°C compared with 32°C. This decrease was observed for long (50 and 100 milliseconds) but not short pulse durations.[28] This study had a pulse duration of 1 millisecond and was therefore not at risk of a decrease in perception threshold. We do not suspect that the increase in perception threshold in this study was due to the cooling of participants' skin temperature.[28]

4.3 | Future research

Perception threshold tracking using pin and patch electrodes as used in this study can be used to estimate Aβ- and Aδ-fiber function.[4,7,9–13,29] Our study has shown that the method does have a statistically significant increase in perception threshold, most likely due to habituation brought on by low stimuli intensity. These findings will not change the reliability of using perception threshold tracking for diagnosing neuropathy.

The knowledge is valuable when designing future research investigating the pathogenesis of neuropathy. In designing long session studies, one should bear in mind methods to prevent habituation or evaluate the habituation's impact on the findings. This study could not prevent habituation or achieve dishabituation even when using long pauses between threshold estimations, changing frequency and stimulation intensity, and those methods may therefore not be an appropriate solution.

4.4 | Limitations

The participants were all healthy young individuals, which makes it difficult to generalize these findings to different patient groups.

In the present study we assume that pin electrode preferentially activated Aδ fibers, whereas the patch electrode preferentially activated Aβ fibers. This assumption was based on earlier studies showing differences between electrodes with large and small cathodes, including differences in latencies of evoked cortical potentials,[5,30] source localization,[10] accommodation to slowly increasing stimulation pulses,[31] quality of the perceived sensations,[32] and computational modeling of the electrical current generated by the electrodes and the consequent nerve fiber activation.[4,8] We did not attempt to confirm these differences in the present study, but the finding of different habituation between the electrodes further indicated activation of different fiber groups.

The pin electrode was located proximal to the patch electrode in all sessions. Any potential difference in habituation between proximal and distal receptive areas of the volar forearm may therefore have biased the findings of more habituation in Aδ fibers than Aβ fibers. Differences in perception thresholds appears to be marginal, and could not be observed in heat pain and heat tolerance thresholds,[33,34] or vibrotactile perception thresholds.[35] However, higher order perception processing may be related to stimulation site, such as the phenomenon of distal inhibition of pain perception[34] or even to the position of the stimulated limb, such as increased defensive reflex responses in the peripersonal space.[36]

4.5 | Conclusion

In conclusion, both large-diameter myelinated Aβ fibers (patch electrode) and small-diameter myelinated Aδ fibers (pin electrode) showed a significant perception threshold increase. The increase was more pronounced for Aδ fibers presumed to be caused by unequal habituation. Taking habituation's effect into account when studying nerve perception threshold is necessary to distinguish habituation from the impact of short-term interventions on nerve function.

AUTHOR CONTRIBUTIONS

Mette Krabsmark Borbjerg: Formal analysis; visualization; writing – original draft; methodology; investigation; project administration; writing – review and editing. **Elin Antonsson:** Investigation; writing – original draft. **Johan Røikjer:** Formal analysis; writing – review and editing; supervision. **Niels Ejskjaer:** Funding acquisition; resources; writing – review and editing; supervision. **Carsten Dahl Mørch:** Conceptualization; methodology; supervision; project administration; writing – review and editing; software; validation; funding acquisition; resources.

ACKNOWLEDGMENTS

M.K.B. was supported in part by a grant from the Lundbeck Foundation to the Innovation Centre Denmark, Silicon Valley to fund Danish American Research Exchange for Mette Borbjerg at University of California, San Francisco. The Center for Neuroplasticity and Pain is supported by the Danish National Research Foundation (DNRF121).

CONFLICT OF INTEREST STATEMENT

Aalborg University holds a patent for the perception threshold method used in the study. C.D.M. has company holdings in Inventors Way ApS, which licenses the perception threshold method. The sponsors had no role in any part of the research process during the conduct of the study. The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICAL PUBLICATION STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Borbjerg MK, Antonsson E, Røikjer J, Ejskjaer N, Mørch CD. The stability of perception threshold tracking for long session evaluation of Aβ- and Aδ-fiber function. *Muscle & Nerve*. 2023;68(5):743-749. doi:10.1002/mus.27967