Membrane properties in small cutaneous nerve fibers in humans

Hennings, Kristian; Frahm, Ken Steffen; Petrini, Laura; Andersen, Ole Kæseler; Arendt-Nielsen, Lars; Mørch, Carsten Dahl

Published in:
Muscle & Nerve

DOI (link to publication from Publisher):
10.1002/mus.25234

Publication date:
2017

Document Version
Accepted author manuscript, peer reviewed version

Membrane properties in small cutaneous nerve fibers in humans

Kristian Hennings, PhD; Ken Steffen Frahm, PhD; Laura Petrini, PhD; Ole K. Andersen, PhD; Lars Arendt-Nielsen, PhD; Carsten D. Mørch, PhD*

Integrative Neuroscience group, SMI.
Department of Health Science and Technology
Aalborg University
Frederik Bajers Vej 7, Aalborg
Denmark

* Corresponding author:
Carsten Dahl Mørch at the address given above:
Telephone: (+45) 9940 8757
E-mail: cdahl@hst.aau.dk

Keywords: Electrophysiology, Cutaneous Nerve Fibers, Strength-Duration Curves, Selective Electrical Stimulation, Small fiber neuropathy, Nerve fiber excitability

Running title: Human small cutaneous nerve fibers

Abstract

Introduction: Assessment of membrane properties is important for understanding the mechanisms of painful peripheral neuropathy, developing new diagnostic techniques, and for screening/profiling of analgesics that target ion channels.

Methods: Small cutaneous nerves were activated electrically by small diameter (0.2mm) cathodes, and large nerves were activated by ordinary patch electrodes. This new perception threshold tracking (PTT) method combines perception threshold assessment and stimulation paradigms from conventional threshold tracking.

Results: The strength-duration time-constant of large fibers (580μs±160μs) was lower than the time constant of small fibers (1060μs±690μs; $P<0.01$, paired t-test). Threshold electrotonus showed similar threshold reductions to sub-threshold pre-pulses, except for 80ms hyperpolarizing pre-pulses, to which small fibers showed less threshold reduction than large fibers (rmANOVA, Bonferroni, $P=0.006$).

Conclusion: This study is a reliable method to investigate the membrane properties of small cutaneous nerve fibers in humans and may be used in clinical settings as a diagnostic or profiling tool.

List of abbreviations:

τ : strength-duration time constant

σ_S^2 : between subject variability

σ_e^2 :error variance

CI: confidence interval

CV: coefficient of variation

ICC: Intraclass correlation coefficient

I_r: rheobase

I_t : perception threshold

K_v : voltage-gated potassium channels

LoA : limit of agreement

Na_v : voltage-gated sodium channels

PTT: perception threshold tracking

rmANOVA: repeated measures ANOVA

t: stimulus duration

Keywords: Electrophysiology, Cutaneous Nerve Fibers, Strength-Duration Curves, Selective Electrical Stimulation, Small fiber neuropathy, Nerve fiber excitability

Introduction

Axonal ion channels have been proposed as targets for peripherally acting analgesic substances, which act on peripheral nociceptors and their membrane properties. Pharmacological down-regulation of voltage-gated sodium (Na_v) channels and up-regulation of voltage-gated potassium (K_v) channels will reduce the firing of nociceptors and potentially lead to pain relief in subgroups of chronic pain patients where peripheral nociceptor activity can be both the driving and sustaining mechanism behind pain hypersensitivity [1]. Unfortunately, no method for assessing the ion channel properties of cutaneous nociceptors is available,fiber as assessment techniques have only been developed for large myelinated fibers [2].

Threshold tracking is one such assessment technique, established as a tool for studying the biophysical properties large of sensory and motor nerve fiber membranes [3]. Several protocols may be applied during threshold tracking to estimate different properties of the nerve fibers. Among these are the strength-duration and threshold electrotonus protocols [4].

The strength-duration protocol estimates the nerve fiber threshold to square pulses of different durations and describes the relation by a time constant and a rheobase as defined e.g. by Weiss law [5]. This strength-duration relationship is mainly governed by the passive membrane properties of the nodes of Ranvier and the Na_v channels[6].

The threshold electrotonus protocol consists of a single, several milliseconds to a few hundred milliseconds duration conditioning pulse with insufficient amplitude to activate nerve fibers but causing depolarization or hyperpolarization of the nerve membrane potential. The conditioning pulse is immediately followed by a test pulse to estimate the threshold change caused by the

conditioning pulse [5]. The duration and polarity of the conditioning pulse are varied, which provides insight into slow membrane kinetics, primarily as a result of different subtypes of K_v channels [6].

Threshold tracking of the thickest nerve fibers has provided insights into ion channel abnormalities in patients e.g. in diabetic neuropathy [7], and chemotherapy-induced neuropathy [8, 9]. Threshold tracking in patients is traditionally performed by surface stimulation of the nerve trunk and recording of the compound action potential of the sensory nerve or the muscle electromyogram[3]. This has only been done for the largest fibers, as the threshold tracking techniques are based on stimulation with electrodes which primarily excited the largest fibers. However, small nerve fibers can be affected in many peripheral neuropathies, and diagnostic tests are needed for early detection of nerve damage (e.g. diabetes or chemotherapy-induced neuropathies) and possible before clinical manifestations are seen [10-12]. This would allow early intervention and possibly prevent development of painful neuropathy.

This study introduces a novel method for estimating the membrane properties of small cutaneous nerve fibers in humans. It is based on a selective small afferent electrical stimulation technique and utilization of the perception threshold tracking technique previously used for assessing large afferent membrane properties. The small cutaneous nerve fibers are activated through an array of small, non-invasive pin electrodes and compared to large nerve fiber activation by a standard patch electrode [13].

Materials and Methods

The study was separated into 2 sessions; a strength-duration session and a threshold electrotonus session.

Subjects

In each session data were acquired from 20 healthy volunteers who gave written informed consent to the experimental procedures that were conducted in accordance with the Helsinki Declaration of 1975 and were approved by the local ethics committee (Den Videnskabsetiske Komité, Region Nordjylland, approval number: N-20120046). Nine women and 11 men between ages 21 and 38 years (mean: 29.4 years) participated in the strength-duration session. Ten women and 10 men aged 21 to 38 years (mean: 27.5 years) participated in the threshold electrotonus session. Fifteen subjects participated in both sessions. Only healthy volunteers were included, and exclusion criteria were addiction or prior addiction to cannabis, opioids, or other drugs, and use of pain relieving medication within the last 48 hours.

Experimental Setup

The subjects were placed in a comfortable inclined position in a hospital bed. The subjects were electrically stimulated through 2 types of surface electrodes, patch and pin electrodes. The patch electrodes were Ag-AgCl surface electrodes; a Neuroline 700 (Ambu A/S, Ballerup, Denmark) electrode was used as the cathode, and a Pals Neurostimulation Electrode (size: 7.5cm x 10cm; Axelgaard, CO., Ltd., Fallbrook, CA, USA) was used as the anode. The pin electrode consisted of a concentric stainless steel ring electrode (area: 8.8 cm^2) that served as the anode surrounding a printed circuit board in which 16 stainless steel pin electrodes were placed in a circle to serve as the cathodes (Figure 1A)[14]. The pins were blunted and had a diameter of 0.2 mm. The cathodes of the patch electrodes and the pin electrodes were placed on the volar forearm 5-8 cm distal to the cubital fossa (Figure 1B). Care was taken to place the cathode of the patch electrodes and the pin electrode in adjacent but not overlapping positions. In order to prevent the position of the pin electrode from shifting during the experiment, it was taped with adhesive tape to the skin, and the arm rested on pillows for comfort.

Electrical stimuli were delivered using a DS5 electrical stimulator (Digitimer Ltd, Letchworth Garden City, UK). The subjects used a custom-made handheld response button to indicate when they felt the electrical stimulations (Center for Sensory Motor-Interaction, Aalborg University). A personal computer and a data acquisition card (National Instruments, Austin, Texas, USA) were used to collect the responses from the subjects and control the electrical stimulator. The electrical stimulations were controlled by a protocol implemented in a custom made LabVIEW program (Aalborg University, Aalborg, Denmark).

Estimation of perception thresholds

Perception thresholds were estimated by the method of limits[15]. Initially, sub-threshold stimulation intensity was found by trial and error for each electrode. Each perception threshold estimation started with an increase of the stimulation intensity in 10% current intensity steps. The computer-controlled stimulation sequence was terminated when the subject indicated perception of the stimulation by pressing the response button. The intensity at which the subject pressed the button then increased by 20%, after which it was decreased in steps of 3% until the subject indicated that the stimulation was no longer felt by pressing the response button. The intensity was then automatically decreased by 20% and increased in steps of 3% until the subject indicated perception of the stimulation again by pressing the response button. This sequence of 3% increments and decrements after 20% increment/decrement was repeated 3 times. The perception threshold was taken as the average of the 6 times the subject had pressed the button. The initial stimulation intensity increase in steps of 10% was not used in the calculation of the perception thresholds. All stimulations were given at an interval of 1s (Aalborg University).

Protocols

Strength-Duration session

The strength-duration curves consisted of determining the perception thresholds to rectangular stimuli of 50μs, 100μs, 200μs, 400μs, 800μs, 2ms, 8ms, and 16ms duration (Figure 2). Both the order of the stimulation durations and the order of the patch and pin electrodes were randomized.

Threshold Electrotonus session

The perception threshold of a 1 ms square wave pulse was initially estimated. A conditioning pulse was applied at an intensity of 20% of the perception threshold of the 1ms pulse. Threshold electrotonus was assessed for both a depolarizing and a hyperpolarizing conditioning pulse. Threshold electrotonus was assessed by estimation of the perception threshold to a 1ms test pulse applied at the end of the conditioning pulse (Figure 3A). The test pulse was applied during the conditioning pulse at 10ms, 20ms, 40ms, and 80ms after the onset of the depolarizing conditioning pulse and at 30ms and 80ms after the onset of the hyperpolarizing conditioning pulse. The order of the time intervals, the polarity of the conditioning pulse, and the order of the patch and pin electrodes was randomized by the controlling computer program (Aalborg University).

Finally, the perception threshold to a 1ms square wave pulse was reassessed to estimate within-session reproducibility.

Data Analysis

Strength-Duration curves

The strength-duration time constant (τ) and rheobase (I_r) was estimated using the Weiss law, which states that the charge ($I_t * t$) required to just excite the nerve fiber is linearly related to the stimulus duration (t) [16]:

$$I_t * t = I_r(t + \tau)$$

This gives a hyperbolic relationship between the stimulus duration (t) and the perception threshold (I_t). τ and I_r were found by least squares estimation of the strength-duration curve. τ and I_r were compared using paired *t*-tests.

Threshold Electrotonus

Threshold electrotonus was expressed as the reduction in threshold caused by the conditioning pulse [5], and the thresholds were normalized to the average of the 1ms unconditioned pulse assessed at the beginning and end of the session. A 2-way repeated measures ANOVA (rmANOVA) was performed, with conditioning (6 levels) and electrode type as repeated factors, and a Bonferroni corrected *post-hoc* analysis was performed between the electrodes for each conditioning pulse and between conditions for each electrode (SPSS 23, IBM).

Reproducibility

The within-session reproducibility of the perception threshold was assessed for the 1ms pulses assessed as the first and last threshold estimations in the threshold electrotonus sessions. The mean difference and the 95% confidence intervals (CI) between the test and retest assessment of the perception thresholds were used to assess a possible systematic bias. The intraclass correlation coefficient (ICC) model 2,1[17] was used to estimate the relative reliability. The ICC is the between-subjects variation divided by the total variation:

$$ICC = \frac{\sigma_S^2}{\sigma_S^2 + \sigma_e^2}$$

where σ_S^2 is the between-subject variability, and σ_e^2 is the error variance. ICC relates to the consistency of the subject's rank or position in the test relative to the retest [18]. The absolute reliability was estimated by calculating the coefficient of variation (CV):

$$CV = \frac{SD}{\mu}$$

where *SD* is the standard deviation, and μ is the mean of the measurements for each individual. CV is therefore an absolute measure of reliability describing the typical error divided by the mean [19]. The 95% limit of agreement (LoA) and Bland-Altman plots were established to estimate the precision of a single assessment of the perception threshold. The reliability measures were calculated by a custom made script (MatLab, MathWorks, R2015b)

All data are presented as mean ± standard deviation. *P*-values less than 0.05 were considered significant.

Results

All 20 subjects completed each session, and no data were excluded.

Strength-duration relationship

The average τ was significantly lower when assessed by the patch electrode (580µs ± 160µs) as compared to the pin electrode (1060µs ± 690µs; $P = 0.01$, paired *t*-test), while the average I_r was significantly larger when assessed by the patch electrode (0.43mA ± 0.10mA) compared to the pin electrode (0.070mA ± 0.041mA; $P < 0.001$, paired *t*-test; Figure 2).

Threshold Electrotonus

The perception threshold was reduced by depolarizing conditioning pulses and increased by hyperpolarizing conditioning pulses (Figure 3). There were statistically significant main effects between conditions ($P < 0.001$) and interaction between conditions and electrodes ($P < 0.018$), but no main effect between electrodes ($P = 0.343$) was observed (rmANOVA). For the patch electrode, the threshold reduction accumulated during the initial part of the conditioning pulse but returned

towards the 20% reduction of the threshold at 80ms (23.2% ± 2.2%) and was significantly less reduced than the threshold at 10ms inter-stimulus interval (29.8% ± 1.9%; Bonferroni corrected pairwise comparison, $P = 0.044$). A similar curve was observed for the pin electrode, but there were no significant differences between depolarizing conditioning pulses. For the patch electrode, the threshold was increased more at 30ms (35.0% ± 2.5%) than at 80ms (27.1% ± 2.3%; Bonferroni, $P = 0.008$). No differences between hyperpolarizing pulses were observed for the pin electrode. For the 80ms hyperpolarizing conditioning pulse, the threshold increase was higher for the pin electrode (61.2% ± 11.2%) than the patch electrode (27.1% ± 2.3%; Bonferroni, $P = 0.006$).

Reproducibility

The test-retest reproducibility measures of single 1 ms square pulse assessment are shown in table 1, and the Bland-Altman plots are shown in figure 4.

Discussion

This study was based on the assumption that electrical stimulation at the perception threshold through predominantly large surface electrodes would activate large myelinated cutaneous nerve fibers whereas pin electrodes would activate predominantly small cutaneous nerve fibers. Hence, the study showed that the strength-duration relationship and threshold electrotonus properties can be investigated for both small and large cutaneous nerve fibers using perception threshold tracking (PTT). The study further showed different strength-duration relationship and threshold electrotonus properties between small and large sensory fibers, indicating that different membrane properties can be assessed. This is most likely caused by different ion channel composition for small and large nerve fibers and may be a technique with clinical implications.

Activation of small cutaneous nerve fibers

Preferential activation of small intact cutaneous nerve fibers by electrical stimulation was shown by Bromm and Meier [20]. They showed that by drilling a hole in the epidermis, nociceptors could be activated to a greater extent than non-nociceptors based on the quality of the sensation evoked by the stimulation and the recorded brain responses. Several non-invasive electrodes have been developed to generate high epidermal current density using an electrode contact or an array of small area pin cathodes similar to the electrode used in this study [13, 21, 22]. The hypothesized mechanism of preferential nociceptor activation is a combination of high current density in the epidermis and the fact that most nociceptors terminate there [23, 24], whereas non-nociceptors terminate deeper in the dermis. We have previously shown by a mathematical model that the high epidermal current density is enough to overcome the higher activation threshold of the nociceptors [13].

It has been demonstrated that the morphology of cortical potentials evoked by a pin electrode resemble those evoked by radiant laser heat that selectively activates nociceptors [21, 22]. It has further been demonstrated that the brain areas activated by pin electrode stimulation are different from those evoked by non-noxious stimulation through ordinary patch electrodes [14]. It must be noted, however, that Perchet et al. [25] did not find similarities between small area electrical and laser evoked cortical potentials and, moreover, the small area electrodes still induced evoked potentials in patients with spinothalamic lesions, whereas laser stimulation did not. It is therefore likely that preferential activation of small nerve fibers through small area electrodes is only possible at low intensities close to the perception threshold, and hence contamination from activation of larger diameter afferents is possible at higher intensities.

In a series of studies the Inui and Kakigi groups showed that by using a particular configuration of small area electrodes and triangular stimulation pulses, C-fibers rather than Aδ-fibers can be

activated [26, 27]. In our study we use square wave pulses, therefore, the most probable fibers to be activated are Aδ-fibers, but coactivation of C-fibers cannot be excluded.

Topical application of lidocaine blocks small fibers before large fibers and alters the perception and cortical potentials evoked by pin but not patch electrodes [26]. In the same way, topical lidocaine application similarly altered peception and cortical evoked potential of pin electrode stimulation [22].

Strength-duration curves

The Weiss law was used to estimate rheobase and time constant, as Mogyoros et al [28] have shown that it provides a better fit than other theoretical approximations and even an accurate estimation with as few as 2 stimulation duration assessments. The strength-duration time constants found in this study (580μs ± 160μs) by applying electrical stimulation through a patch electrode corresponds to the time constant observed for peripheral sensory fibers by conventional threshold tracking [28]. These fibers are most likely large diameter cutaneous nerve fiber (Aβ-fibers). The significantly larger time constants (1060μs ± 690μs) found by applying electrical stimulation through pin electrodes indicate that a different subset of sensory nerves was activated, most likely small cutaneous nerve fibers (Aδ-fibers). The larger time constant may be explained by different passive properties of the nodes of Ranvier and/or different composition of subtypes of Na_v channels between the fiber types. This corresponds well to properties of $Na_v1.8$ and $Na_v1.9$ mainly being expressed in nociceptors, whereas it appears that $Na_v1.7$ are expressed in most dorsal ganglion neurons [29].

Threshold electrotonus

The threshold electrotonus experiments showed a threshold reduction caused by depolarizing conditioning pulses and a threshold increase (negative threshold reduction) caused by hyperpolarizing conditioning pulses. This general excitability change is similar to the threshold

electrotonus assessments using classical threshold tracking [3, 4]. The initial increased threshold reduction to short (10ms – 40ms) depolarizing conditioning is most likely caused by passive current spread along the axon and activation of nodal K_v^+ (delayed rectifying) channels [30]. The initial threshold reduction was then followed by a decreased threshold reduction (i.e. a threshold increase) at longer depolarizing conditioning pulses (80ms). This counteraction was probably caused by activation of slow K_v channels located in the node and the internode [4]. The slow K^+ currents are mediated by K_v7.2/KCNQ2 channels and are activated around the resting membrane potential, thus regulating the resting membrane potential [1]. Hyperpolarizing conditioning currents close the internodal slow K_v, which will increase internodal resistance [3, 4] and in turn cause a decreased threshold reduction by 30ms pulses (Figure 3). Increasing the hyperpolarizing conditioning pulses to 80ms returned the threshold reduction when the current was applied through patch electrodes. This is in concordance with studies which have shown that for large sensory fibers, K_{ir} channels are activated by long hyperpolarizing currents [3, 4]. This increase was not seen when the pin electrode was used (Figure 3), indicating different densities or properties of K_{ir} channels in small cutaneous nerve fibers.

Perception threshold as a measure of nerve fiber activation

The reproducibility assessments revealed acceptable repeatability when the patch electrode was used to assess the excitability of large cutaneous nerve fibers (Table 1 and Figure 4). The reproducibility of the perception threshold assessment was less acceptable when the pin electrode was used to assess the excitability of small cutaneous nerve fibers.

Variations in pain perception have been observed in several studies [31, 32]. Age, gender, and temporal variations seem to influence pain perception thresholds [33, 34]. It has been shown that more psychological variables can contribute to inter-session variations during the experience of pain [35, 35,

[36]. Fluctuating anxiety over experimental stimuli or changing expectations over the experimental design are more likely to influence pain detection thresholds. Furthermore, reproducibility variations in pain detection could be related in the way people interpret or produce decision making on what a painful stimulus is rather than differences in the actual pain [37]. We applied a pin electrode to assess the activation of small cutaneous nerve fibers rather than using the pain perception threshold. The differences in reproducibility between pin and patch electrodes may be due skin irritation caused by the protruding pins and the more diffuse sensation caused by small fiber nerve activation.

Conclusion

PTT provides an indirect method for assessing the functionality of ion channels in small and large diameter sensory peripheral nerve fibers. The technique of utilizing a pin electrode in combination with the PPT may be used as a new diagnostic method to investigate small fiber neuropathies.

Acknowledgements

The project was partly funded by Eir - Empowering Industry and Research.

References

1. Moldovan M, Alvarez S, Romer Rosberg M, Krarup C. Axonal voltage-gated ion channels as pharmacological targets for pain. European Journal of Pharmacology 2013; 708:105-112.

2. Z'Graggen WJ, Bostock H. Nerve membrane excitability testing. European Journal of Anaesthesiology (EJA) 2008; 25.

3. Bostock H, Cikurel K, Burke D. Threshold tracking techniques in the study of human peripheral nerve. Muscle & Nerve 1998; 21:137-158.

4. Burke D, Kiernan MC, Bostock H. Excitability of human axons. Clinical Neurophysiology 2001; 112:1575-1585.

5. Kiernan MC, Burke D, Andersen KV, Bostock H. Multiple measures of axonal excitability: A new approach in clinical testing. Muscle and Nerve 2000; 23:399-409.

6. Bostock H, Baker M. Evidence for two types of potassium channel in human motor axons in vivo. Brain Research 1988; 462:354-358.

7. Horn S, Quasthoff S, Grafe P, Bostock H, Renner R, Schrank B. Abnormal axonal inward rectification in diabetic neuropathy. Muscle and Nerve 1996; 19:1268-1275.

8. MC Hugh JC, Tryfonopoulos D, Fennelly D, Crown J, Connolly S. Electroclinical biomarkers of early peripheral neurotoxicity from oxaliplatin. European Journal of Cancer Care 2012; 21:782-789.

9. Park SB, Lin CSY, Krishnan AV, Goldstein D, Friedlander ML, Kiernan MC. Oxaliplatin-induced neurotoxicity: Changes in axonal excitability precede development of neuropathy. Brain 2009; 132:2712-2723.

10. Haanpää M, Attal N, Backonja M, Baron R, Bennett M, Bouhassira D, et al. NeuPSIG guidelines on neuropathic pain assessment. Pain 2011; 152:14-27.

11. Lauria G, Merkies ISJ, Faber CG. Small fibre neuropathy. Current Opinion in Neurology 2012; 25:542-549.

12. Brismar T, Sima AAF, Greene DA. Reversible and irreversible nodal dysfunction in diabetic neuropathy. Ann Neurol 1987; 21:504-507.

13. Mørch CD, Hennings K, Andersen OK. Estimating nerve excitation thresholds to cutaneous electrical stimulation by finite element modeling combined with a stochastic branching nerve fiber model. Medical and Biological Engineering and Computing 2011; 49:385-395.

14. Lelic D, Mørch CD, Hennings K, Andersen OK, Drewes AM. Differences in perception and brain activation following stimulation by large versus small area cutaneous surface electrodes. EJP 2011. p827-837.

15. Gracely RH. Studies of pain in normal man. In: Melzack R, Wall PD, editors. Textbook of pain. London: Churchill Livingstone; 1994. p 315-336.

16. Weiss G. Sur la possibility de rendre comparables entre eux les appareils servant à l'excitation électrique. Arch Ital Biol 1901; 35:413-446.

17. Shrout PE, Fleiss JL. Intraclass correlations: Uses in assessing rater reliability. Psychological Bulletin;Psychological Bulletin 1979; 86:420-428.

18. Weir JP. Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. Journal of Strength and Conditioning Research 2005; 19:231-240.

19. Atkinson G, Nevill AM. Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. Sports Medicine 1998; 26:217-238.

20. Bromm B, Meier W. The intracutaneous stimulus - A new pain model for algesimetric studies. Methods and Findings in Experimental and Clinical Pharmacology 1984; 6:405-410.

21. Inui K, Tran TD, Hoshiyama M, Kakigi R. Preferential stimulation of A-delta fibers by intra-epidermal needle electrode in humans. Pain 2002; 96:247-252.

22. Mouraux A, Iannetti GD, Plaghki L. Low intensity intra-epidermal electrical stimulation can activate Ad-nociceptors selectively. Pain 2010; 150:199-207.

23. Frahm KS, Mørch CD, Grill WM, Lubock NB, Hennings K, Andersen OK. Activation of peripheral nerve fibers by electrical stimulation in the sole of the foot. BMC Neuroscience 2013; 14:116.

24. Hilliges M, Wang LX, Johansson O. Ultrastructural evidence for nerve-fibers within all vital layers of the human epidermis. Journal of Investigative Dermatology 1995; 104:134-137.

25. Perchet C, Frot M, Charmarty A, Flores C, Mazza S, Magnin M, et al. Do we activate specifically somatosensory thin fibres with the concentric planar electrode? A scalp and intracranial EEG study. Pain 2012; 153:1244-1252.

26. Otsuru N, Inui K, Yamashiro K, Miyazaki T, Takeshima Y, Kakigi R. Assessing Ad Fiber Function With Lidocaine Using Intraepidermal Electrical Stimulation. The Journal of Pain 2010; 11:621-627.

27. Motogi J, Kodaira M, Muragaki Y, Inui K, Kakigi R. Cortical responses to C-fiber stimulation by intra-epidermal electrical stimulation: An MEG study. Neuroscience Letters 2014; 570:69-74.

28. Mogyoros I, Kiernan MC, Burke D. Strength-duration properties of human peripheral nerve. Brain 1996; 119:439-447.

29. Dib-Hajj SD, Cummins TR, Black JA, Waxman SG. Sodium Channels in Normal and Pathological Pain. Annu Rev Neurosci 2010; 33:325-347.

30. Baker M, Bostock H, Grafe P, Martius P. Function and distribution of three types of rectifying channel in rat spinal root myelinated axons. Journal of Physiology 1987; Vol. 383:45-67.

31. Strian F, Lautenbacher S, Galfe G, Hölzl R. Diurnal variations in pain perception and thermal sensitivity. Pain 1989; 36:125-131.

32. Procacci P, Buzzelli G, Passeri I, Sassi R, Voegelin MR, Zoppi M. Studies on the cutaneous pricking pain threshold in man. Circadian and circatrigintan changes. Res Clin Stud Headeache 1970; 3:260-276.

33. Nielsen CS, Stubhaug A, Price DD, Vassend O, Czajkowski N, Harris JR. Individual differences in pain sensitivity: Genetic and environmental contributions. Pain 2008; 136:21-29.

34. Chung SC, Um BY, Kim HS. Evaluation of pressure pain threshold in head and neck muscles by electronic algometer: intrarater and interrater reliability. Cranio : the journal of craniomandibular practice 1992; 10:28-34.

35. Rosier EM, Iadarola MJ, Coghill RC. Reproducibility of pain measurement and pain perception. Pain 2002; 98:205-216.

36. Price DD. Psychological and neural mechanisms of the affective dimension of pain. Science 2000; 288:1769-1772.

37. Nielsen CS, Staud R, Price DD. Individual differences in pain sensitivity: Measurement, causation, and consequences. Journal of Pain 2009; 10:231-237.

Figure legends

Figure 1. Experimental setup. The subjects were electrically stimulated with a pin electrode (A) to preferentially activate small cutaneous fibers and a patch electrode (B) to preferentially activate large cutaneous fibers. A computer controlled the pulse shapes of the electrical stimulation, which were applied through a constant current stimulator (C). The subjects indicated perception of the individual stimulation by pressing a handheld response button.

Figure 2. Strength-duration curves for perception thresholds estimated with a pin electrode (solid line) and a patch electrode (dashed line). The x-axis is the logarithm to the stimulus duration, and the perception thresholds were normalized to the rheobase. Error bars, SEM.

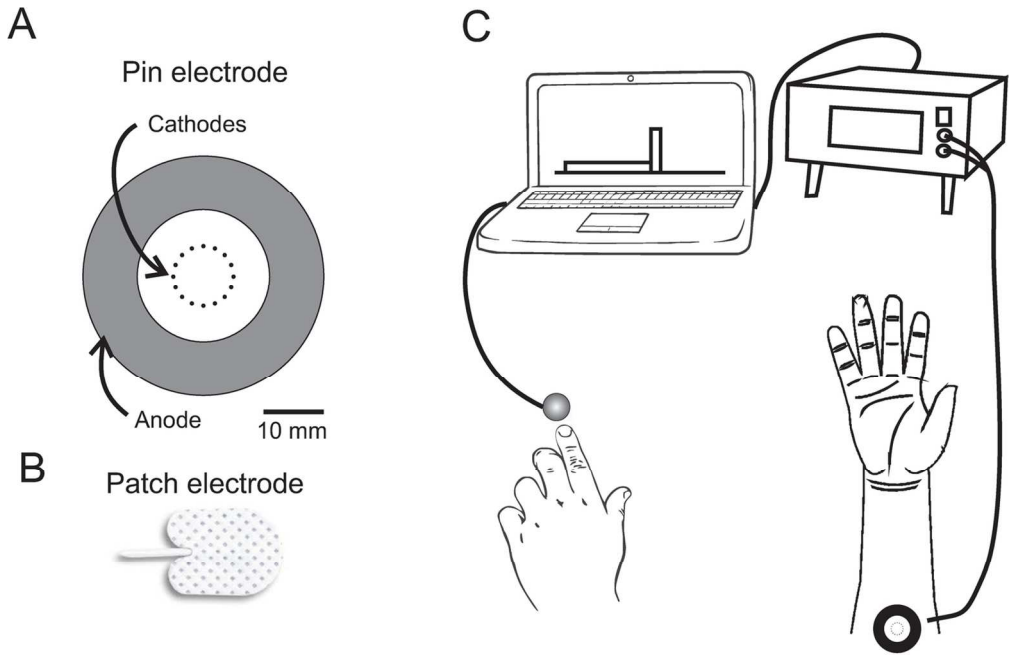
Figure 3. Threshold electrotonus. A) Threshold electrotonus stimulation consisted of depolarizing (illustrated) or hyperpolarizing (not illustrated) conditioning stimuli followed by a test pulse assessing the perception threshold. A series of stimulations with different conditioning pulse durations was performed. B) The threshold reduction to depolarizing (upper 2 lines) and to hyperpolarizing (lower 2 lines) square wave pulses. The inter-stimulus interval indicates the duration between onset of the conditioning pulse and the test pulse. The threshold was reduced by depolarizing conditioning currents and increased (negative reduction) by hyperpolarizing conditioning currents. The nerve fibers excited by the pin electrode showed a larger threshold increase than nerve fibers activated by the patch electrode when conditioned by an 80ms hyperpolarizing current (* rmANOVA, Bonferroni, $P = 0.006$). Error bars, SEM.

Figure 4. Bland-Altman plots show the absolute test-retest reproducibility of perception threshold estimation by (A) the pin and (B) the patch electrodes.

Table

Table 1. Test-retest reproducibility measures of the perception threshold estimation of a 1 ms square pulse. Intraclass correlation coefficient (ICC) indicates the relative reproducibility. The coefficient of variation (CV) and the 95 % limits of agreement (LoA) indicate the absolute reproducibility. The bias indicates a significant increase in perception threshold for both pin and patch electrode.

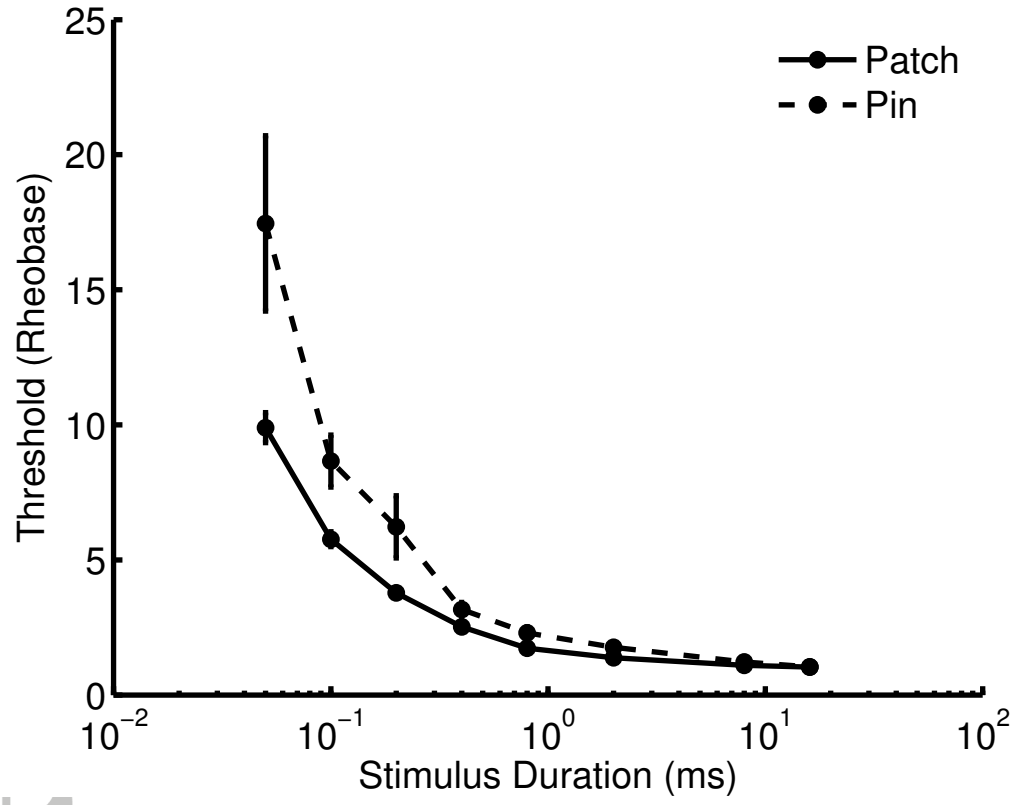
	ICC	CV (%)	Bias (mA), [95% CI]	95% LoA (mA)
Pin electrode	0.64	26.7	0.064, [0.035 to 0.093]	-0.055 to 0.184
Patch electrode	0.94	6.46	0.055, [0.012 to 0.098]	-0.125 to 0.235

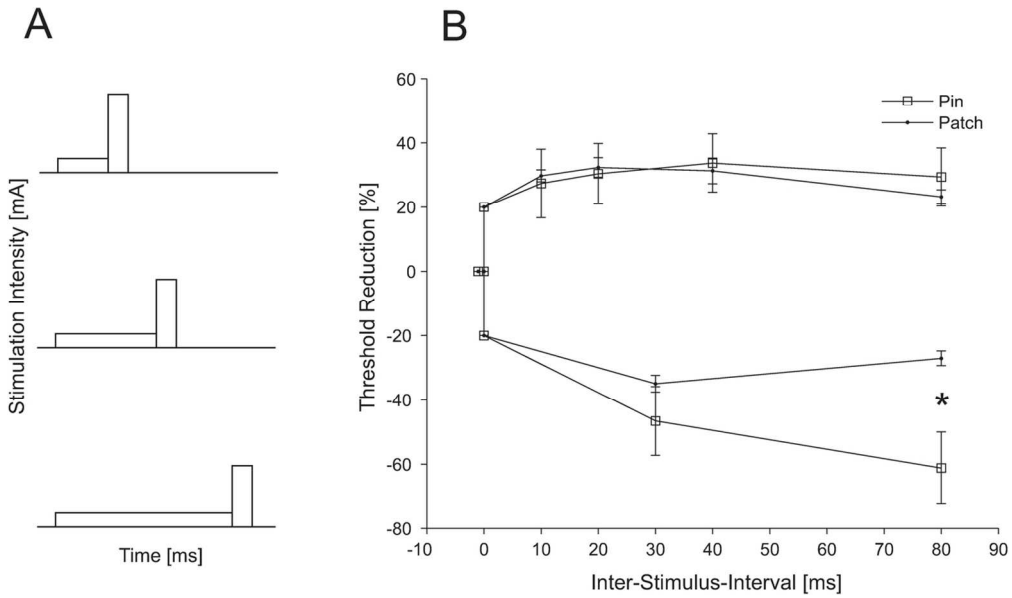


Experimental setup. The subjects were electrically stimulated with a pin electrode (A) to preferentially activate small cutaneous fibers and a patch electrode (B) to preferentially activate large cutaneous fibers. A computer controlled the pulse shapes of the electrical stimulation, which were applied through a constant current stimulator (C). The subjects indicated perception of the individual stimulation by pressing a handheld response button.

Figure 1

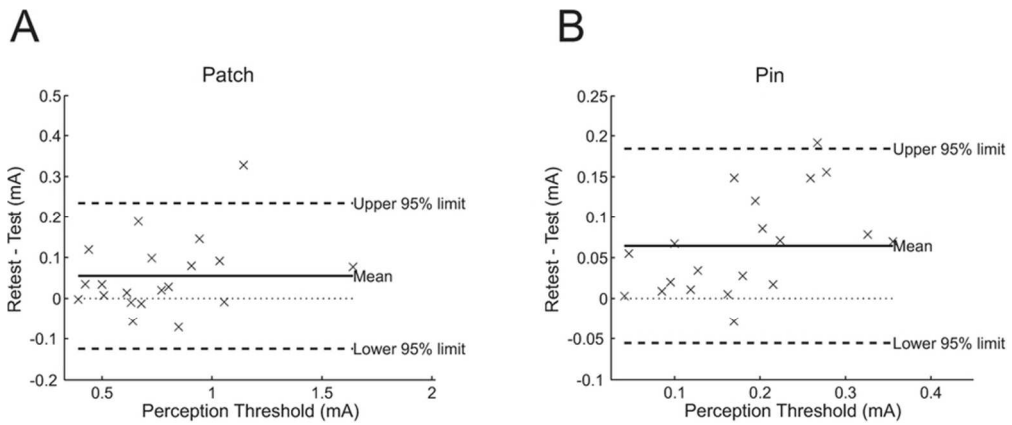
128x83mm (300 x 300 DPI)





Threshold electrotonus. A) Threshold electrotonus stimulation consisted of depolarizing (illustrated) or hyperpolarizing (not illustrated) conditioning stimuli followed by a test pulse assessing the perception threshold. A series of stimulations with different conditioning pulse durations was performed. B) The threshold reduction to depolarizing (upper 2 lines) and to hyperpolarizing (lower 2 lines) square wave pulses. The inter-stimulus interval indicates the duration between onset of the conditioning pulse and the test pulse. The threshold was reduced by depolarizing conditioning currents and increased (negative reduction) by hyperpolarizing conditioning currents. The nerve fibers excited by the pin electrode showed a larger threshold increase than nerve fibers activated by the patch electrode when conditioned by an 80ms hyperpolarizing current (* rmANOVA, Bonferroni, P = 0.006). Error bars, SEM.

Figure 3
118x69mm (300 x 300 DPI)



Bland-Altman plots show the absolute test-retest reproducibility of perception threshold estimation by (A) the pin and (B) the patch electrodes.
Figure 4
79x32mm (300 x 300 DPI)