Investigating stimulation parameters for preferential small fiber activation using exponentially rising electrical currents

Hugosdottir, Rosa; Mørch, Carsten Dahl; Andersen, Ole K; Arendt-Nielsen, Lars

Published in:
Journal of Neurophysiology

DOI (link to publication from Publisher):
10.1152/jn.00390.2019

Publication date:
2019

Document Version
Accepted author manuscript, peer reviewed version

Investigating stimulation parameters for preferential small fiber activation using exponentially rising electrical currents.

Authors: Rosa Hugosdottir, Carsten Dahl Mørch, Ole Kæseler Andersen, and Lars Arendt-Nielsen

Center of Neuroplasticity and Pain, SMI, Department of Health Science and Technology, Aalborg University

Corresponding author information: Carsten Dahl Mørch, email address: cdahl@hst.aau.dk

Abstract

Electrical stimulation is widely used in pain research and profiling but current technologies lack selectivity towards small sensory fibers. Pin electrodes deliver high current density in upper skin layers and it has been proposed that slowly rising exponential pulses can elevate large fiber activation threshold and thereby increase preferential small fiber activation. Optimal stimulation parameters for the combined pin electrode and exponential pulse stimulation have so far not been established as is the aim of this study. Perception thresholds were compared between pin- and patch electrodes using single 1-100 ms exponential- and rectangular pulses. Stimulus-response functions were evaluated for both pulse shapes delivered as single pulses and pulse trains of 10 Hz using intensities from 0.1-20 times perception threshold. Perception thresholds (mA) decreased when duration was increased for both electrodes with rectangular pulses and also for the pin electrode with exponential pulses. For the patch electrode, perception thresholds for exponential pulses decreased for durations ≤10 ms, but increased for durations ≥15 ms, indicating accommodation of large fibers. Stimulus-response curves for single pulses were similar for the two pulse shapes. For pulse trains, the slope of the curve was higher for rectangular pulses. Maximal large fiber accommodation to exponential pulses was observed for 100 ms pulses, indicating that 100 ms exponential pulses should be applied for preferential small fiber activation. Intensity of 10 times

perception threshold was sufficient to cause maximal pain ratings. The developed methodology may open new opportunities for using electrical stimulation paradigms for small fiber stimulation and diagnostics.

New and noteworthy

Selective activation of small cutaneous nerve fibers is pivotal for investigations of the pain system. The present study demonstrated that patch electrode perception thresholds increase with increased duration of exponential currents from 20- to 100 ms. This is likely caused by large fiber accommodation, which can be utilized to activate small fibers preferentially through small-diameter pin electrodes. This finding may be utilized in studies of fundamental pain mechanisms and e.g. in small fiber neuropathy.

1. Introduction

Selective activation of small fibers could be useful in various applications such as bladder function restoration (Brindley and Craggs 1980), and in pain research (Inui and Kakigi 2012) and pain diagnostics (Hennings et al. 2017). However, selective activation of small nerve fibers is difficult since the pain sensing primary afferents have smaller diameters then tactile afferent skin fibers. Electrical stimulation with standard surface patch electrodes activates large diameter fibers at lower intensities than small fibers (Grill and Mortimer 1995). With electrical stimulation, the fiber recruitment order is based on two principles: 1) distance from electrode to the fibers and 2) fiber diameter (Grill and Mortimer 1995). Both of these likely affect the transmembrane potential. Special intra-epidermal (Inui et al. 2002) and cutaneous pin electrodes (Klein et al. 2004; Lelic et al. 2012; Mørch et al. 2011) have been developed to activate the small Aδ- and C-fibers preferentially by "isolating" the electrical field in the upper skin layers where primarily small fibers terminate (Ebenezer et al. 2007; Hilliges et al. 1995; Provitera et al. 2007). With short rectangular pulses at intensities close to perception threshold, evoked potential latencies in the Aδ-fiber range have been reported for intra-epidermal and pin electrode stimulation (Lelic et al. 2012; Mouraux et al. 2010). At higher intensities, co-activation of larger diameter fibers cannot be excluded (Mouraux et al. 2010). Another approach for targeting small fibers is to utilize the membrane kinetics of the voltage sensitive ion channels. The rate of fiber inactivation is slower than the rate of activation (Grill and

Mortimer 1995; Hill 1936; Kugelberg 1944) and by slow depolarization of the membrane, inactivation can be initiated, and thus increasing the activation threshold (accommodation) (Baker and Bostock 1989; Bostock et al. 1998; Hennings et al. 2017; Hill 1936; Kugelberg 1944; Li and Bak 1976; Sassen and Zimmermann 1973). The distribution of the different ion channel subtypes, with varying inactivation rates differs between small- and large fibers (Amaya et al. 2000; Blair and Bean 2002; Djouhri et al. 2003; Fang et al. 2002). Extensive depolarization can be obtained with linearly increasing currents (Hennings et al. 2005a; Lucas 1907) and the shape of the depolarizing pulse can be even further improved by applying a slowly rising pulse with an increasing form of an exponential decay (exponential), since the accommodation depends on the current gradient (Hill 1936; Hugosdottir et al. 2017; Lucas 1907). Perception thresholds of sensory cutaneous nerve fibers were recently compared between different slowly rising pulse shapes of 50 ms duration and the above mentioned exponential pulse showed largest ratio between activation of large- and small fibers indicating preferential small fiber activation (Hugosdottir et al. 2017).

The combined application of high current density delivered through a pin electrode and slowly raising pulses has not been studied in details and optimal stimulation duration and -intensity remain unknown. In a previous study, at least 2-fold increase in thresholds was found when pin electrode stimulation was applied to skin exposed to lidocaine, which partially blocks the small fibers (Hoberg et al. 2019). This indicated that stimulus intensity above the activation threshold of small fibers is likely needed to depolarize- and accommodate large fibers with slowly increasing pulses. The requirement of using low intensity for selective activation of superficial small fibers may therefore only apply for short duration stimuli (Mouraux et al. 2010), making this electrode-parameter combination highly appealing for use in experimental pain studies where usually higher intensity is needed (spatial recruitment). However, the intensity should be examined carefully, since there might be a narrow range of large fiber accommodation without activation.

In this study, perception thresholds to patch- and pin electrodes were assumed to indicate activation of large- and small fibers, respectively. Perception thresholds were obtained for both electrodes to create strength-duration curves for exponential- and rectangular pulses. The aim was to investigate the pulse duration of a single

exponential pulse that caused greatest large fiber accommodation while concurrently activated small fibers. Furthermore, the pain response was compared between short rectangular and long duration exponential pulses delivered with the pin electrode at varying stimulus intensities for single pulse and repetitive stimulation.

2. Methods

This study consisted of two experimental sessions performed with approximately two months in between.

Subjects

25 healthy individuals (12 females; 19-30 year; mean age 23.6 years) participated in both experiments (A and B) but one was excluded from Experiment B due to early state pregnancy. All subjects gave their written informed consent to participation in the experiments which was approved by the local ethical committee (N-20160076). The experiment was performed according the declaration of Helsinki.

Experimental setup

In both experiments, subjects were seated in an inclined hospital bed with their arms resting on pillows. A voltage controlled electrical current stimulator (DS5; Digitimer Ltd; Welwyn Garden City, UK) was used to deliver electrical pulses to the volar forearm of the participants. Stimulation was controlled by the experimenter via a custom-made program (LabVIEW, National Instruments) on a personal computer and a data acquisition card (USB-6351; National instruments). In experiment A, two types of electrodes were used (Fig. 1). A pin electrode was used to activate small Aδ- and C-fibers (Klein et al. 2004; Lelic et al. 2012). 15 short circuited small diameter (0.2 mm) stainless steel pins were placed in a circle with a diameter of 10 mm and used as cathodes. The anode was a concentric stainless-steel ring, with an inner diameter of 22 mm and an outer diameter of 36 mm. A standard patch electrode was used to activate mainly the large Aβ-fibers. The cathode was a 15 mm x 20 mm Ag-AgCl electrode (Neuroline 700, Ambu A/S, Ballerup, Denmark) and the anode was a large 5 cm x 9 cm patch (Pals Neurostimulation electrode, Axelgaard, Co., Ltd., Fallbrook, CA, USA).

Figure 1. The electrodes used in the experiment. Left) Pin electrode is composed of 15 small cathodal pins and a concentric anode. Right) The patch electrode cathode.

Pulse shapes

Two pulse shapes were applied: 1) rectangular- and 2) exponential pulse (i.e. an increasing form of exponential decaying current which was bounded to a maximal stimulation current, Fig. 2, Eq. 1).

$$i(t) = \begin{cases} \dfrac{I_s}{1 - e^{\frac{-T_S}{\tau}}}\left(1 - e^{\frac{-t}{\tau}}\right), & 0 \le t < T_s \\ I_s \cdot e^{\frac{-t}{\tau_{tr}}}, & T_s \le t \le T_{tr} \end{cases}$$

Equation 1. Mathematical expression of the stimulation current i(t) for the exponential pulse is shown. I_s = stimulation current intensity, T_S = stimulation duration, τ = time constant of current increase = T_S/2. Trailing phase: T_{tr} = T_S ∗1.4 and τ_{tr} = τ/6.6.

Figure 2. Examples of the shape of the exponential pulse are shown for durations of 1, 5, 50, and 100 ms. The illustrations show the proportion of the maximal stimulation current intensity.

At short durations, the rate of current increase of the exponential pulse was high and thus similar for both pulse shapes, but as the duration increased, the time constant of current increase (τ) for the exponential pulse increased according to $2\tau = T_s$. The exponential pulse had a decaying phase to avoid anodal break excitation, see Fig. 2 (Burke and Ginsborg 1956; Hennings et al. 2005c). All pulses were followed with no inter-phase delay by a pulse phase of opposite polarity of same duration and shape, but with amplitude scaled to half the amplitude of the stimulating phase (not shown in illustration of pulses in Fig. 2 and Eq. 1). The pulses were therefore charge imbalanced, but this configuration limited the charge delivered in one direction to avoid skin damage (Merrill et

al. 2005). Prior to the experiment, the shapes of the pulses when stimulating a resistor and a capacitor in parallel were verified on an oscilloscope.

Experimental protocol

Experiment A

Experiment A consisted of two randomized crossover sessions with a short break in between. First, either a pin- or a patch electrode (see Fig. 1) was randomized to be placed on either the right- or left volar forearm. The cathodes of the pin- and patch electrodes were placed 5 cm distal to the cubital fossa. The anode for the patch electrode was placed on the dorsal forearm. The arm and the electrode were then switched in the second session.

Strength-duration curves: In each session, perception thresholds (see sec: Perception thresholds) were obtained for single rectangular pulses with durations of 1, 5, 50, and 100 ms and single exponential pulses with durations of 1, 5, 10, 15, 20, 30, 40, 50, 60, 80, 100 ms for both electrodes to create strength-duration curves. The order of all pulse forms and durations were randomized.

Experiment B

In experiment B, electrical stimulation was only delivered with the pin electrode, which was placed 5 cm distal to the cubital fossa on the same arm as the subjects had received stimulation with the pin electrode in experiment A.

Perception thresholds were initially identified (see sec: Perception thresholds) for a single exponential pulse of 40 ms, and a rectangular pulse of 1 ms.

Stimulus response curves: Both pulse shapes were delivered as single pulses and pulse trains of 10 Hz for 1 s at intensities of 0.1, 0.5, 1, 2, 3, 4, 6, 8, 10, 15, 20 times perception threshold. The stimulation paradigms delivered in experiment B will be referred to as follows: 1) Exponential single pulse, 2) rectangular single pulse, 3)

exponential pulse train, and 4) rectangular pulse train. For each paradigm and intensity, the subjects were asked to rate the pain on a numerical rating scale (NRS) from 0-10. The order of the paradigm was first randomized and the order of the delivered intensity was randomized within each paradigm. The long duration pulse of 40 ms was selected for being able to deliver pulse trains of 10 Hz as previously has been applied in experimental pain studies with the pin electrode (Xia et al. 2016a, 2016b).

Perception thresholds

The perception threshold was found using the following staircase procedure (method of limits): The intensity was initially set to 20 μA for the pin electrode and 200 μA for the patch electrode. Single pulses were delivered at 0.5 Hz and the intensity of each pulse increased by 5% compared to previous pulse until the subject perceived the stimulation and pressed a handheld custom-made button. Single pulses were again delivered from 110% of the perceived intensity, which then decreased by 5% until the perception dissipated and the subject pressed the button again. Stimulation intensity was thereafter decreased to 90 % of intensity when perception dissipated and the procedure was repeated. The perception threshold was calculated as the average of the three intensities where perception was indicated and three intensities when perception dissipated. A training sequence of this staircase procedure was initially carried out in order to familiarize the subjects with the procedure and the sensation of electrical stimulation.

Data analysis

Experiment A

Data analyses was performed using MATLAB (R2017b) and SPSS 25, (IBM). The perception threshold representing the current strength in the strength-duration curves were normalized to the rheobase value and then log transformed prior to the statistical analysis.

Strength-duration curves: The strength-duration relations between pulse duration, electrode, and pulse shape were analyzed by a three-way linear mixed model (LMM). The model had the electrode (pin- and patch

electrode), pulse shape (rectangular and exponential), and duration (1, 5, 10, 50, 100 ms) as repeated fixed factors. A random intercept for a subject- and electrode- specific variable was included, and to investigate the effect of habituation, the order in which the pulses were given was added to the model as a covariate. Due to unequal correlation between repeated measures the autoregressive (AR(1)) covariance structure was used. This model was selected based on the lowest Akaike information criterion. In case of a three-way interaction between the fixed factors, 2 x 2 LMM was used to analyze the effect of electrode and pulse shape for each of the five durations. The latter model accounted for the same random intercept, covariate and repeated measure as the three-way model. The strength-duration relationship for the rectangular pulse shape was analyzed by fitting the data to Weiss law for calculating the rheobase and chronaxie for both electrode types (Weiss 1901). The rheobase and chronaxie were compared between the paradigms by using a Friedman test in accordance with the data distribution.

Another analysis was carried out to investigate the perception threshold for the exponential pulse shape in more details. This model was a two-way LMM with the electrode type (pin and patch electrode) and pulse duration (1, 5, 10, 15, 20, 30, 40, 50, 60, 80, 100 ms) as fixed-effect factors. The same random intercept, covariate, and repeated measures were included as in previously described models. A two-way factorial interaction between the electrode and pulse duration was analyzed to investigate the difference in strength-duration relation between the electrodes. Furthermore, a factor-covariate interaction was analyzed between electrode and stimulation order to investigate the effect of habituation between the electrode types. The part of the strength-duration curve, which manifested a linear relationship between perception threshold (multiple of rheobase) and stimulation duration (T_S) was used to calculate the slope of accommodation. The duration was first transformed to the time constant of current increase (τ) according to: $\tau = T_S/2$, since the accommodation slope is defined as the relative threshold increase with respect to time constant of current increase (rheobase/τ) (Kugelberg 1944).

Experiment B

Stimulus-response curves: The stimulus-response curves were analyzed by fitting the data for each paradigm to sigmoidal curves, according to the following equation: $y(x) = \frac{c}{1+e^{a(b-x)}}$, where a was the slope of the curve, b was the stimulation factor for inducing 50% of maximum pain rating, and c was the maximum pain rating. The coefficients were considered statistically significant different between the paradigms in case of non-overlapping 95% confidence intervals.

3. Results

Experiment A

The strength-duration curves for both pulse shapes are shown for the patch- and the pin electrodes in Fig. 3. The LMM revealed a three-way interaction between the pulse shape, electrode, and duration ($F(4,213.11) = 10.49$, $p < 0.001$). Two-way interactions were observed for the two long durations (50 and 100 ms). For 50 ms and 100 ms, a larger perception threshold difference was observed between the patch- and the pin- electrode for the exponential pulse than for the rectangular pulse (Fig. 3). For the shorter durations, the threshold relations were not statistically different for the two pulse shapes and electrodes.

*Figure 3. Re-transformed perception threshold normalized to rheobase (mean ± standard error) vs. pulse duration is shown for a) the patch electrode and b) the pin electrode. Asterisk indicate interactions between pulse shape and electrode for the specific durations, * $p < 0.05$, ** $p < 0.01$. Exp = Exponential, Rec = rectangular.*

The raw strength-duration curves for the rectangular pulse used to estimate the rheobase and chronaxie for both electrodes are shown in Fig. 4. The rheobases and chronaxies are presented as median with interquartile range (IQR) for both electrodes in a table, which is included in Fig. 4. A larger rheobase was found for the patch electrode than for the pin electrode (Wilcoxon signed rank test, $p < 0.001$) and a larger chronaxie was found for the pin electrode than for the patch electrode (Wilcoxon signed rank test, $p < 0.001$).

Figure 4. Strength-duration curves (on the left) for the rectangular pulse were used to estimate the rheobase and chronaxie, which are presented as median and interquartile range in the table on the right. The perception threshold in mA on the y-axis of the strength-duration curve was used to represent the current strength needed for activation at different durations with the pin- and the patch electrodes.

A more detailed strength-duration curve for the exponential pulse is shown in Fig. 5. Analysis of these curves revealed an interaction between the pulse duration and electrode, $F(10,474.12) = 23.87$, $p < 0.001$ (Fig. 5). This interaction is explained by different strength-duration curves for the two electrode types. For the pin electrode, perception threshold decreased with increased duration, whereas a V-shaped strength-duration relation was observed for the patch electrode (accommodation curve, Fig. 5); The nadir of the curve was reached at 10 ms, followed by increase in perception threshold for longer durations. With respect to the 1 ms pulse duration, the interaction term was significant from 20-100 ms ($p < 0.001$), with an increased effect for the longer durations. The accommodation slope was calculated for the durations, which manifested a linear relationship from Fig. 5 (left) ($10 \text{ ms} < t < 60 \text{ ms}$) and transformed to be reported as rheobase/τ. The accommodation slope was 23.52 ± 2.84 rheobase/s.

Examination of the effect of habituation revealed a significant main effect of stimulation order, $F(1,435.55) = 193.21$, $p < 0.001$. Furthermore, an interaction between stimulation order and electrode was found, $F(1,435.55) = 72.26$, $p < 0.001$. This indicates that the perception threshold increased throughout the session and habituation affected the pin electrode to a greater extent than the patch electrode.

The variance due to the subject-and electrode specific intercept contributed profoundly to the model and accounted for 62.3% of the total variance. The standard deviation of the residuals from the LMM was higher for the pin electrode than for the patch electrode (log values: 0.14 and 0.06, respectively).

*Figure 5. Re-transformed perception thresholds normalized to rheobase (mean ± standard error) are shown for all durations of the exponential pulse for the patch electrode on the left and the pin electrode on the right. Asterisk indicate statistically significant interaction between electrode and duration for the pin electrode and t = 1 ms as reference. ** $p < 0.01$, *** $p < 0.001$.*

Experiment B

The pain ratings to stimulus of different intensities are shown in Fig. 6. In Tab. 1, coefficients of the fitting to sigmoidal-curve are shown. Similar maximum pain ratings were found for the pulse train stimulation of exponential– and rectangular pulse, which was higher than for single pulses. Lower intensity was needed to cause half of the maximum pain for rectangular pulse train compared to the other paradigms. This is related to the slope, which was also found to be steepest for the rectangular pulse train.

Figure 6. Stimulus-response curves are shown for all paradigms. Pain ratings are shown as mean ± standard error. The fit for sigmoidal curve with 95% confidence interval is added to the plots.

Table 1. Sigmoidal model fit for the pain ratings (NRS). Model coefficients are shown (with 95% confidence interval)

4. Discussion

This study investigated the strength-duration relationship for an exponentially increasing current delivered to human skin for activating sensory afferent fibers. The current strength needed to activate large- and small fibers was indicated by the subjective perception threshold of a standard patch- and pin electrode, respectively. The results showed an increase in perception threshold of the patch- but not pin electrode when long duration

exponential current was applied. The increase in perception threshold was maximized for 100 ms duration. For the pin electrode, the strength-duration relation was similar for rectangular and exponential currents. The ability of the exponential pulse to elevate the threshold of the patch electrode exclusively, indicated that this stimulation shape at long durations caused accommodation of large fibers. Therefore, long duration exponential pulses enabled activation of small fibers with limited large fiber activation, especially when applied by the pin electrode. The study furthermore showed that the stimulus-response curves were similar for single long exponential and short rectangular pulses, but the slope of the curves were different for repetitive stimulation.

Neuronal Accommodation

Slowly rising depolarizing pulses have been used in earlier animal (Li and Bak 1976; Sassen and Zimmermann 1973) and human studies (Hennings et al. 2005a; Kugelberg 1944) to depolarize the nerve fiber membrane and inactivate mainly the large fibers to achieve preferential small fiber activation. The increase in perception threshold with the patch electrode in present study is an indication of accommodation of large sensory nerve fibers to the long duration exponential pulses ($T_S \geq 20$ ms, see Fig. 5). To compare the rate and degree of accommodation to earlier findings by Kugelberg et al. 1944 it was necessary to transform the pulse duration (T_s) to time constant of current increase (τ), according to $T_s = 2\ \tau$. The accommodation slope in the interval of 10 ms $< T_s <$ 60 ms was 23.53 ± 2.84 rheobase/s (see Fig. 5), which is comparable to motor neuron accommodation (21.2 ± 0.46 rheobase/s), calculated for $\tau <$ 100 ms in the study by Kugelberg and colleagues (Kugelberg 1944). Motor nerves have been shown to accommodate more rapidly than sensory fibers (Bretag and Stämpfli 1975) and maximum threshold increase is usually higher as accommodation breakdown of motor- and sensory fibers has been observed at 2.5-5 and 1.5-2 times rheobase strength, respectively (Kugelberg 1944). The degree of accommodation in the present study was ~ 1.9 times rheobase for the longest duration (T_s = 100 ms). A small degree of accommodation breakdown was noticed, but the perception threshold increased to some degree for the longest duration but not at the same increase rate as between 20 ms and 60 ms. It is expected that the perception threshold would not have increased further had durations > 100 ms been included, due to accommodation

breakdown (Hennings et al. 2005b; Kugelberg 1944). In another study on motor nerves, similar rate- and degree of accommodation was reported for linearly increasing current (Hennings et al. 2005a). However, the strength-duration curve showed a delayed accommodation initiating at duration of 50 ms (Hennings et al. 2005a) compared to 20 ms duration in the current study. This is further supported in a recent study, where accommodation was indicated for 50 ms exponential pulses (identical to the pulse shape in the present study), but not for linear pulses (Hugosdottir et al. 2017). The exponential pulse therefore produces relatively good accommodation in large sensory fibers, matching the degree and rate of increase when motor fibers are exposed to linear pulses (Hennings et al. 2005a). Taken together previous data and data from the present study, substantial accommodation of large sensory neurons can be obtained with exponential pulses of 20 ms-100 ms, with a maximum for 100 ms pulses. Sinusoidal currents of 5 Hz have been proposed to activate pain sensing fibers (Koga et al. 2005; Masson et al. 1989) and recently, evidence for C-fiber activation using a 4 Hz sinusoidal (i.e. a full wave-period of 250 ms) current were presented (Jonas et al. 2018). This supports the pulse shape and duration proposed in the present study and further matches early findings on time constant of current increase needed to accommodate sensory fibers (Kugelberg 1944).

Stimulation intensity

The intensity of the exponential pulse is critical for achieving the desired large fiber accommodation and concurrent small fiber activation. Accommodation is different between fiber types and depends on the fiber diameter (Bretag and Stämpfli 1975; Hennings et al. 2005a; Kugelberg 1944) and whether a slowly rising current activates or accommodates a nerve fiber depends on the rate of current increase (Hill 1936). Pain responses to different stimulus intensities were investigated in the current study and identical stimulus-response curves were observed for long exponential and short rectangular single pulse application. Stimulus-response curves with similar pain responses were found for intra epidermal stimulation in a previous study, but the pain ratings did not reach a plateau level as in present study (Lim et al. 2016). The shift in stimulus-response curve towards higher pain ratings for repeated pulses indicated that temporal summation, which most likely reflects

gradual depolarization of neurons in the dorsal horn (Sivilotti et al. 1993), played a role in producing maximum pain ratings. This is in accordance with previous studies using repeated electrical stimulation (Arendt-Nielsen et al. 2000). However, it seemed that relatively lower intensity was needed to cause maximum pain using the rectangular pulse compared to the exponential pulse as the curves raised with different rates, but towards identical maximum pain ratings (see Fig. 6). Potential explanations for this difference could be that repeated stimulation of slowly conducting small nociceptive fibers could have led to action potential broadening (Liu et al. 2017) and/or latency jitter at the dorsal horn causing less temporal summation. The absolute current was moreover lower for the exponential pulse compared to rectangular pulse, which may have caused more pronounced habituation (Arendt-Nielsen et al. 2000; Dimitrijević et al. 1972) and less pain facilitation for the lower intensities.

Electrodes and habituation

The different strength-duration curves observed with the pin- and the patch electrodes support the view that the pin electrode mainly activates small fibers and patch electrodes mainly activate large fibers. The results revealed a 2.5-fold larger variance for the pin electrode than the patch electrode. The difference may be explained with different electrode properties. Both electrode-skin contact and size of stimulation area differed between the electrodes. The patch electrode has one relatively large stimulation area whereas the pin electrode is composed of 15 small contact areas. The two electrodes moreover elicit different sensation (Hugosdottir et al. 2017), and it may be speculated whether it is more difficult for the participants to sense the pricking sensation from the pin electrode than the artificial, blunt "shock" like sensation with the patch electrode when low intensities are applied. This factor might explain the different variances, but should however affect the different durations to a similar degree and therefore not affect the curve shape, which is the main outcome of the study. Recently, similar variance variation was observed for perception threshold found with identical electrode types (Hennings et al. 2017). Habituation could also have influenced the variance. The LMM used to analyze the perception threshold showed a linear increase in perception threshold with respect to in which order the pulses were

delivered, indicating habituation. Moreover, it was shown that the effect of stimulation order was more pronounced with the pin electrode than with the patch electrode, indicating that small fibers habituated to a larger degree than large fibers. Similar degree of habituation was shown in a study investigating nociceptive detection thresholds to intra-epidermal electro-cutaneous stimulation (van den Berg and Buitenweg 2018). The order of pulse duration and pulse shape was randomized to overcome this limitation.

Limitations

This study was based on subjective perception thresholds with different electrodes in experiment A and subjective pain ratings to different stimulation paradigms in experiment B. The subjective nature of the study is a limitation, but pain is a subjective experience and the method is well acknowledged to investigate pain-related mechanisms in humans (Hennings et al. 2017; Klein et al. 2004). Perception thresholds were used to measure the excitability of small and large fibers with pin- and patch electrodes, respectively. Using such an indirect measure is a limitation of the study, but the perception threshold gives good indication for fiber activation including unknown factors, which most likely are identical between stimulation paradigms. The perception threshold has been used in previous studies to assess membrane properties of nerve fibers (Hennings et al. 2017; Hoberg et al. 2019).

Conclusion

This study showed increased perception threshold (accommodation) when slowly rising exponential pulses were delivered with standard patch electrodes, mainly activating large fibers. No threshold increase was observed when identical stimulation pulses were delivered with a pin electrode, mainly activating small fibers. This implies that slowly rising currents can be used to activate small fibers more selectively than standard short pulses. A minimum duration of 20 ms was needed to elevate patch electrode perception thresholds, but longer durations are recommended as the effect increased with increased pulse duration and was most pronounced for 100 ms pulse. During repetitive stimulation, co-existing mechanisms might interfere with the small fiber

activation during slowly rising pulses and the present study can therefore only support and propose single pulses of slowly increasing exponential pulses for preferential small fiber activation.

Acknowledgements

Center for Neuroplasticity and Pain (CNAP) is supported by the Danish National Research Foundation (DNRF121)

5. References

Amaya F, **Decosterd I**, **Samad TA**, **Plumpton C**, **Tate S**, **Mannion RJ**, **Costigan M**, **Woolf CJ**. Diversity of expression of the sensory neuron-specific TTX-resistent voltage-gated sodium ion channels SNS and SNS2. *Mol Cell Neurosci* 342: 331–342, 2000.

Arendt-Nielsen L, **Sonnenborg FA**, **Andersen OK**. Facilitation of the withdrawal reflex by repeated transcutaneous electrical stimulation: an experimental study on central integration in humans. *Eur J Appl Physiol* 81: 165–173, 2000.

Baker M, **Bostock H**. Depolarization changes the mechanism of accommodation in rat and human motor neurons. *J Physiol* 411: 545–561, 1989.

van den Berg B, **Buitenweg JR**. Analysis of Nociceptive Evoked Potentials during Multi-Stimulus Experiments using Linear Mixed Models. In: *40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*. IEEE, 2018, p. 3048–3051.

Blair NT, **Bean BP**. Roles of tetrodotoxin (TTX)-sensitive Na+ current, TTX-resistant Na+ current, and Ca2+ current in the action potentials of nociceptive sensory neurons. *J Neurosci* 22: 10277–10290, 2002.

Bostock H, **Cikurel K**, **Burke D**, **Burke D**. Threshold tracking techniques in the study of human peripheral

nerve. [Online]. *Muscle Nerve* 21: 137–58, 1998http://www.ncbi.nlm.nih.gov/pubmed/9466589 [19 Sep. 2016].

Bretag AH, **Stämpfli R**. Differences in action potentials and accommodation of sensory and motor myelinated nerve fibres as computed on the basis of voltage clamp data. *Pflügers Arch Eur J Physiol* 354: 257–271, 1975.

Brindley G, **Craggs M**. A technique for anodally blocking large nerve fibres through chronically implanted electrodes. *J Neurol Neurosurg Psychiatry43* 43: 1083–1090, 1980.

Burke BYW, **Ginsborg BL**. The Electrical Properties of the Slow Muscle Fibre Membrane. *J Physiol* 132: 586–598, 1956.

Dimitrijević MR, **Faganel J**, **Gregorić M**, **Nathan PW**, **Trontelj JK**. Habituation: effects of regular and stochastic stimulation. *J Neurol Neurosurg Psychiatry* 35: 234–242, 1972.

Djouhri L, **Fang X**, **Okuse K**, **Wood JN**, **Berry CM**, **Lawson SN**. The TTX-resistant sodium channel Nav1. 8 (SNS/PN3): expression and correlation with membrane properties in rat nociceptive primary afferent neurons. *J Physiol* 550: 739–752, 2003.

Ebenezer GJ, **Mcarthur JC**, **Thomas D**, **Murinson B**, **Hauer P**, **Polydefkis M**, **Griffin JW**. Denervation of skin in neuropathies : the sequence of axonal and Schwann cell changes in skin biopsies. *Brain* 130: 2703–2714, 2007.

Fang X, **Djouhri L**, **Black JA**, **Dib-hajj SD**, **Waxman SG**, **Lawson SN**. The Presence and Role of the Tetrodotoxin-Resistant Sodium Channel Na v 1 . 9 (NaN) in Nociceptive Primary Afferent Neurons. 22: 7425–7433, 2002.

Grill WM, **Mortimer JT**. Stimulus Waveforms for Selective Neural Stimulation. *IEEE Eng Med Biol Mag* 14: 375–385, 1995.

Hennings K, **Arendt-Nielsen L**, **Andersen OK**. Orderly activation of human motor neurons using electrical

ramp prepulses. *Clin Neurophysiol* 116: 597–604, 2005a.

Hennings K, **Arendt-Nielsen L**, **Andersen OK**. Breakdown of accommodation in nerve: a possible role for persistent sodium current. *Theor Biol Med Model* 2: 16, 2005b.

Hennings K, **Arendt-Nielsen L**, **Christensen SS**, **Andersen OK**. Selective activation of smali-diameter motor fibres using exponentialiy rising waveforms: a theoretical study. *Med Biol Eng* 43: 493–500, 2005c.

Hennings K, **Frahm KS**, **Petrini L**, **Andersen OK**, **Arendt-Nielsen L**, **Mørch CD**. Membrane properties in small cutaneous nerve fibers. *Muscle Nerve* 55: 195–201, 2017.

Hill AV. Excitation and accommodation in nerve. *Proc R Soc Lond B* 119: 305–355, 1936.

Hilliges M, **Wang L**, **Johansson O**. Ultrastructural Evidence for Nerve Fibers Within All Vital Layers of the Human Epidermis. *J Invest Dermatol* 104: 134–137, 1995.

Hoberg TN, **Frahm S**, **Hennings K**, **Arendt-nielsen L**, **Mørch CD**. Assessing the modulation of cutaneous sensory fiber excitability using a fast perception threshold technique. *Muscle Nerve* 1–8, 2019.

Hugosdottir R, **Mørch CD**, **Andersen OK**, **Helgason T**, **Arendt-Nielsen L**. Evaluating the ability of non-rectangular electrical pulse forms to preferentially activate nociceptive fibers by comparing perception thresholds. *Scand J Pain* 16: 175, 2017.

Inui K, **Kakigi R**. Pain perception in humans: use of intraepidermal electrical stimulation. *J Neurol Neurosurg Psychiatry* 83: 551–556, 2012.

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

Jonas R, **Namer B**, **Stockinger L**, **Chisholm K**, **Schnakenberg M**, **Landmann G**, **Kucharczyk M**, **Konrad C**, **Schmidt R**, **Carr R**, **Mcmahon S**, **Schmelz M**, **Rukwied R**. Tuning in C-nociceptors to reveal mechanisms

406 in chronic neuropathic pain. *Ann Neurol* 1–13, 2018.

407 **Klein T**, **Magerl W**, **Hopf HC**, **Sandkuhler J**, **Treede RD**. Perceptual correlates of nociceptive long-term
408 potentiation and long-term depression in humans. *J Neurosci* 24: 964–971, 2004.

409 **Koga K**, **Furue H**, **Rashid H**, **Takaki A**, **Katafuchi T**, **Yoshimura M**. Selective activation of primary afferent
410 fibers evaluated by sine-wave electrical stimulation. *Mol Pain* 11: 1–11, 2005.

411 **Kugelberg E**. Accommodation in human nerves and its significance for the symptoms in circulatory
412 disturbances and tetany. *Acta Physiol Scand* 8, 1944.

413 **Lelic D**, **Mørch CD**, **Hennings K**, **Andersen OK**, **Drewes AM**, **Drewes AM**. Differences in perception and
414 brain activation following stimulation by large versus small area cutaneous surface electrodes. *Eur J Pain* 16:
415 827–37, 2012.

416 **Li C**, **Bak A**. Excitability Characteristics in a Peripheral of the A- and Nerve. *Exp Neur* 79: 67–79, 1976.

417 **Lim M**, **Roosink M**, **Kim JS**, **Kim HW**, **Lee EB**, **Son KM**, **Kim HA**, **Chung CK**. Augmented Pain Processing
418 in Primary and Secondary Somatosensory Cortex in Fibromyalgia: A Magnetoencephalography Study Using
419 Intra-Epidermal Electrical Stimulation. *PLoS One* 11, 2016.

420 **Liu PW**, **Blair NT**, **Bean BP**. Action Potential Broadening in Capsaicin-Sensitive DRG Neurons from
421 Frequency-Dependent Reduction of Kv3 Current. 37: 9705–9714, 2017.

422 **Lucas K**. On the rate of variation of the exciting current as a factor in electric excitation. *J Physiol* 36: 253–274,
423 1907.

424 **Masson EA**, **Veves A**, **Fernando D**, **Boulton AJM**. Current perception thresholds: a new, quick, and
425 reproducible method for the assessment of peripheral neuropathy in diabetes mellitus. *Diabetologia* 32: 724–
426 728, 1989.

Merrill DR, **Bikson M**, **Jefferys JGR**. Electrical stimulation of excitable tissue: Design of efficacious and safe protocols. *J Neurosci Methods* 141: 171–198, 2005.

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. *Med Biol Eng Comput* 49: 385–395, 2011.

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

Provitera V, **Nolano M**, **Pagano A**, **Caporaso G**, **Stancanelli A**, **Santoro L**. Myelinated nerve endings in human skin. *Muscle Nerve* 35: 767–775, 2007.

Sassen M, **Zimmermann M**. Differential Blocking of Myelinated Nerve Fibres by Transient Depolarization. *Pflügers Arch* 341: 179–195, 1973.

Sivilotti LG, **Thompson SWN**, **Woolf CJ**, **Kingdom U**. Potential Windup in Rat Spinal Neurons In Vitro. *J Neurophysiol* 69: 1621–1631, 1993.

Weiss G. Sur la possibilite de rendre comparables entre eux les appareils servant a l'excitation electrique. *Arch Ital Biol* 35: 413–445, 1901.

Xia W, **Mørch CD**, **Andersen OK**. Exploration of the conditioning electrical stimulation frequencies for induction of long-term potentiation-like pain amplification in humans. *Exp Brain Res* 234: 1–11, 2016a.

Xia W, **Mørch CD**, **Andersen OK**. Test-Retest Reliability of 10 Hz Conditioning Electrical Stimulation Inducing Long-Term Potentiation (LTP) -Like Pain Amplification in Humans. *PLoS One* 11: e0161117, 2016b.

448

449 *Figure 1. The electrodes used in the experiment. Left) Pin electrode is composed of 15 small cathodal pins and a*
450 *concentric anode. Right) The patch electrode cathode*

451 *Figure 2. Examples of the shape of the exponential pulse are shown for durations of 1, 5, 50, and 100 ms. The*
452 *illustrations show the proportion of the maximal stimulation current intensity.*

453 *Figure 3. Re-transformed perception threshold normalized to rheobase (mean ± standard error) vs. pulse*
454 *duration is shown for a) the patch electrode and b) the pin electrode. Asterisk indicate interactions between*
455 *pulse shape and electrode for the specific durations, * $p < 0.05$, ** $p < 0.01$. Exp = Exponential, Rec =*
456 *rectangular.*

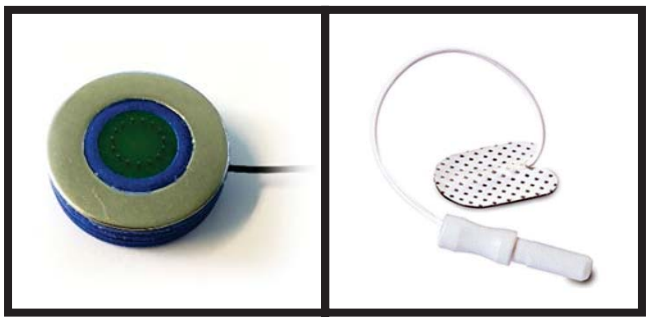
457 *Figure 4. Strength-duration curves (on the left) for the rectangular pulse were used to estimate the rheobase and*
458 *chronaxie, which are presented as median and interquartile range in the table on the right. The perception*
459 *threshold in mA on the y-axis of the strength-duration curve was used to represent the current strength needed*
460 *for activation at different durations with the pin- and the patch electrodes.*

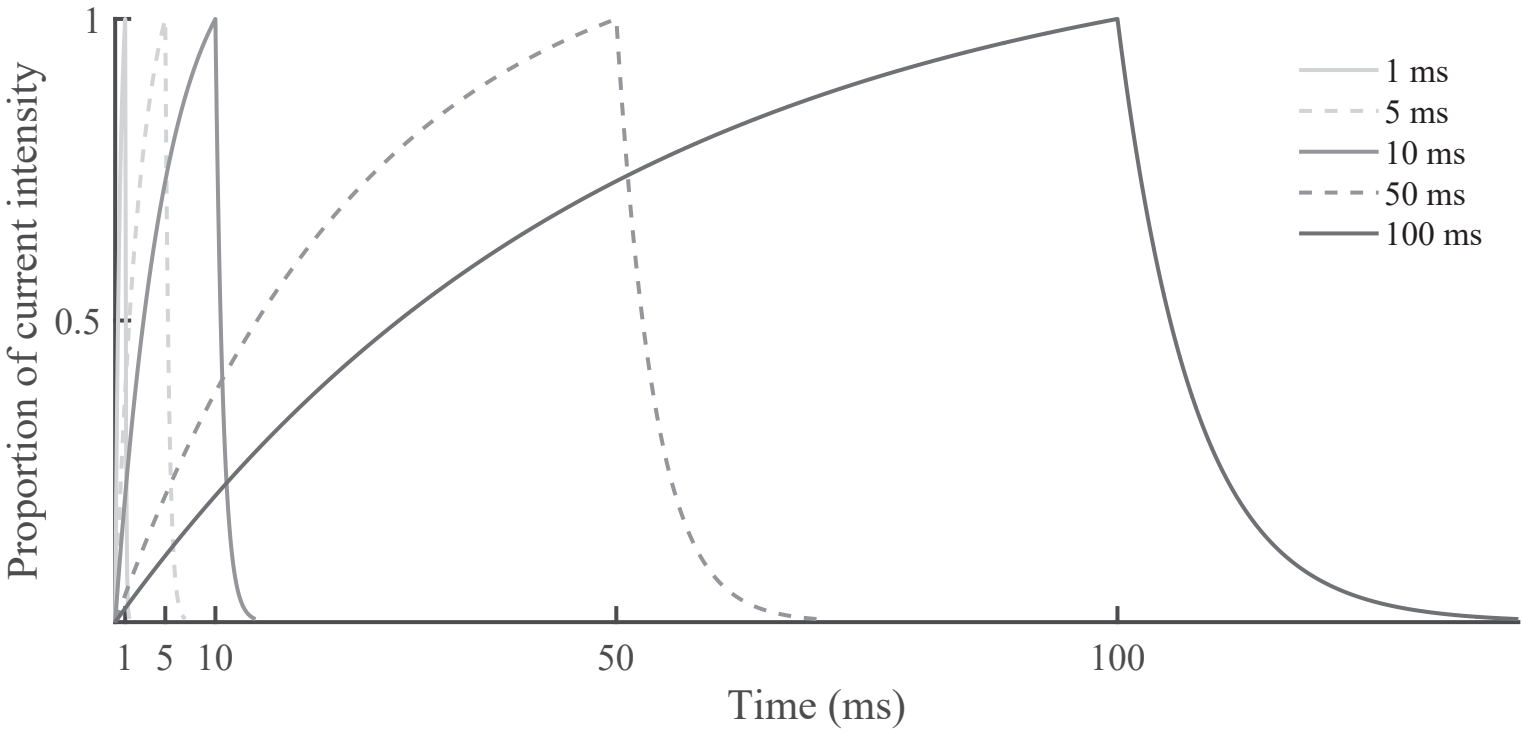
461 *Figure 5: Re-transformed perception thresholds normalized to rheobase (mean ± standard error) are shown for*
462 *all durations of the exponential pulse for the patch electrode on the left and the pin electrode on the right.*
463 *Asterisk indicate statistically significant interaction between electrode and duration for the pin electrode and t =*
464 *1 ms as reference. ** $p < 0.01$, *** $p < 0.001$.*

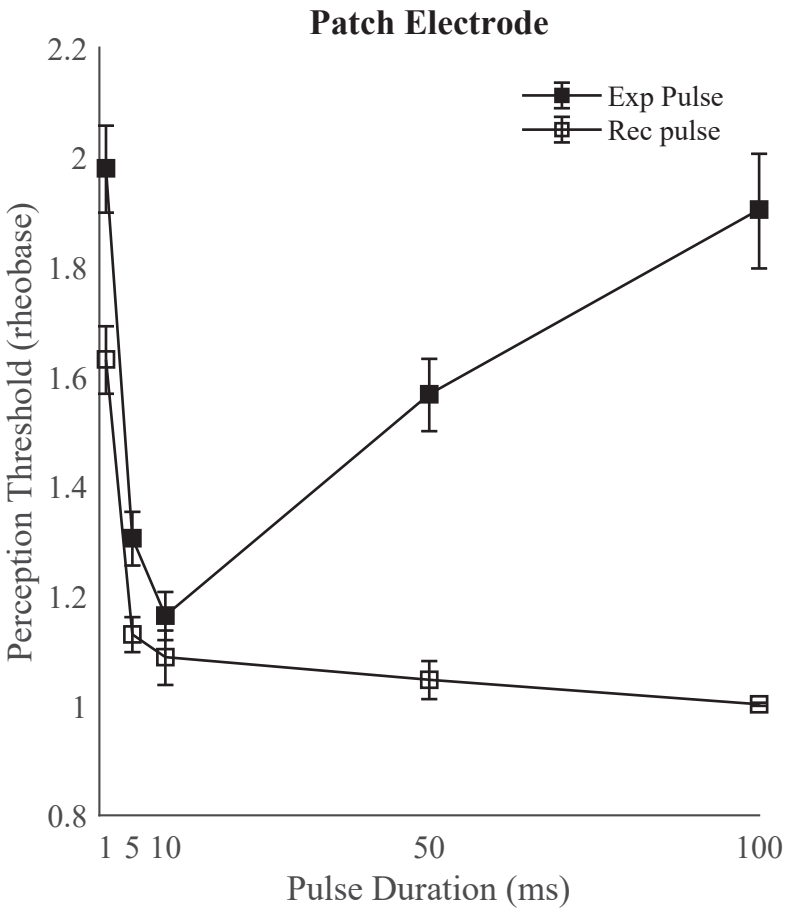
465 *Figure 6. Stimulus-response curves are shown for all paradigms. Pain ratings are shown as mean ± standard*
466 *error. The fit for sigmoidal curve with 95% confidence interval is added to the plots.*

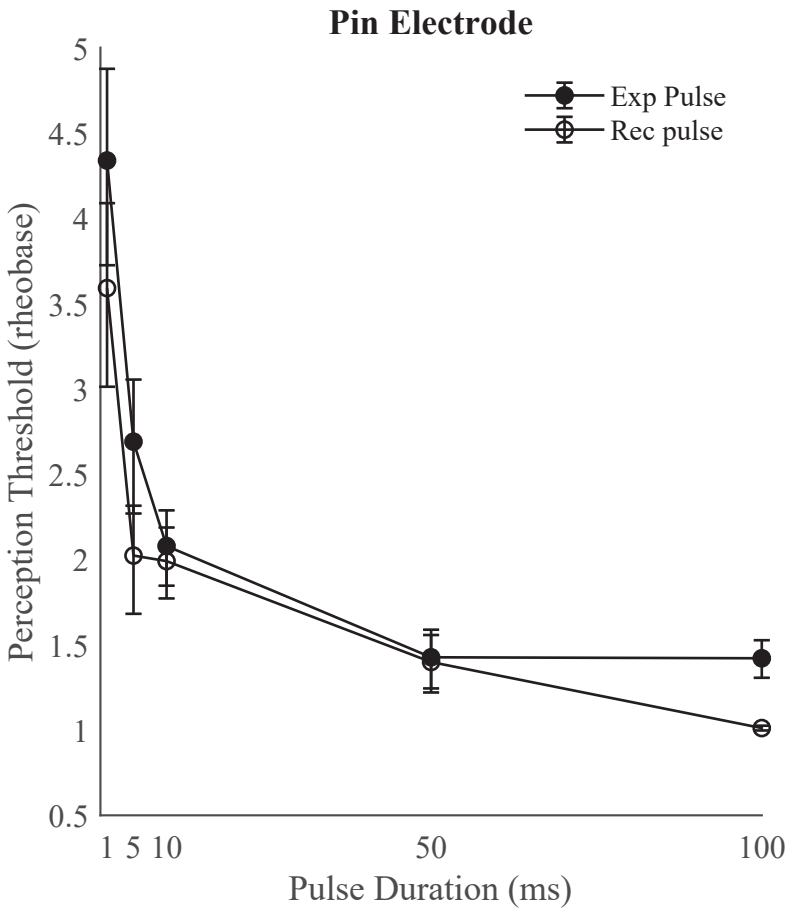
467

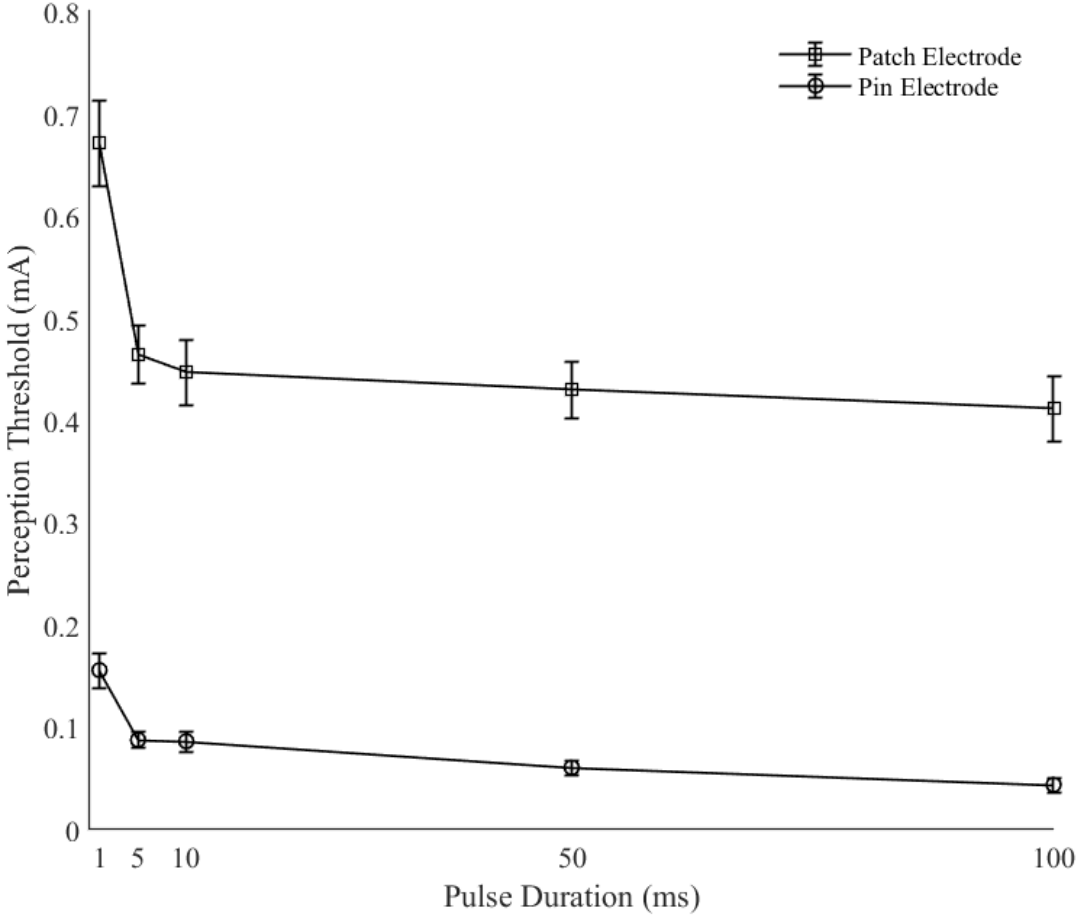
468



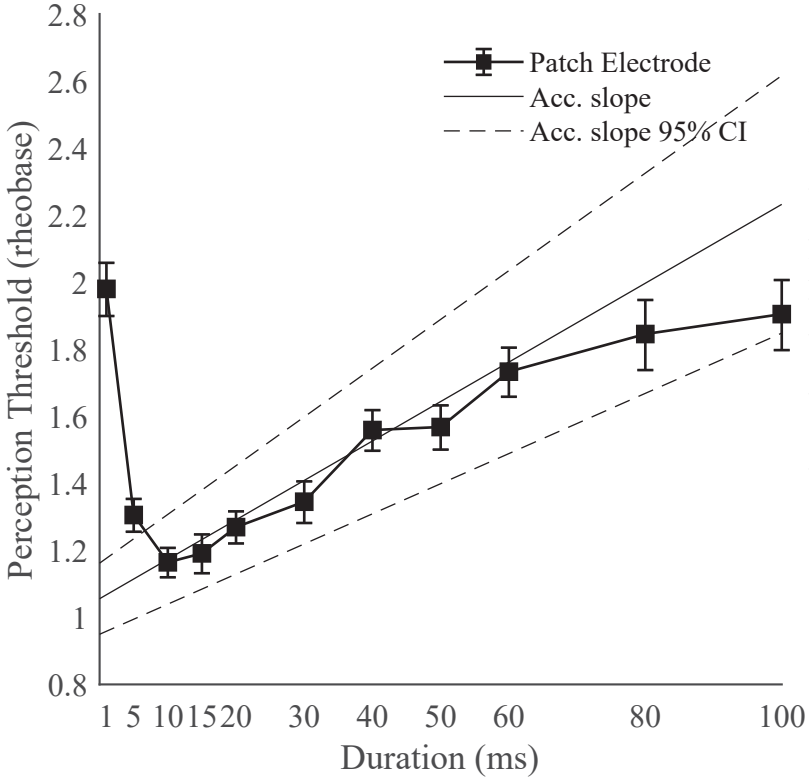


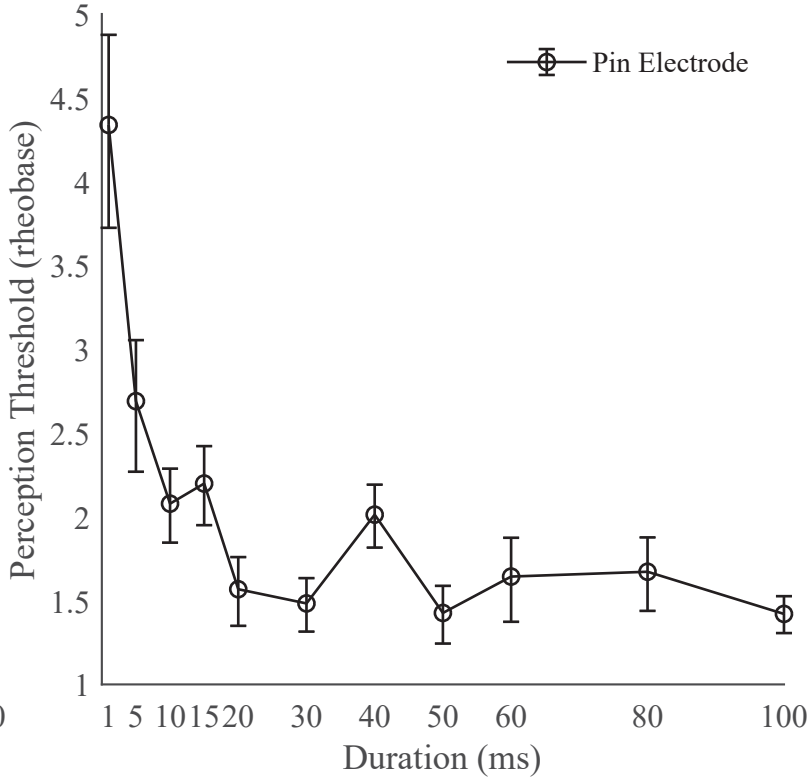


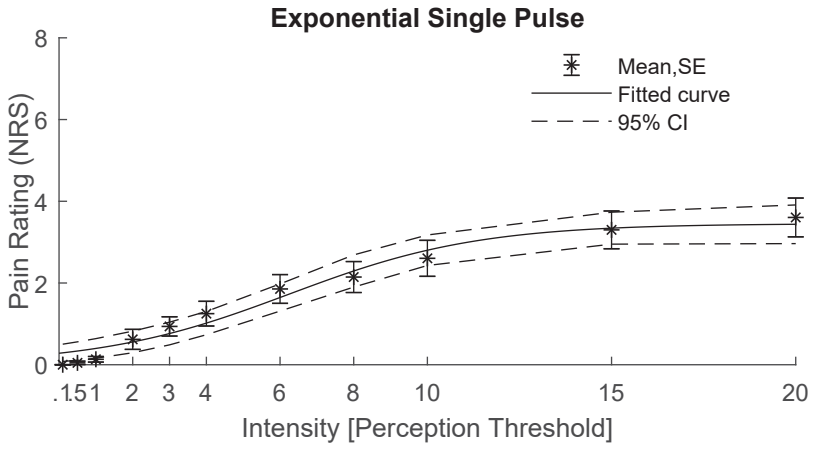


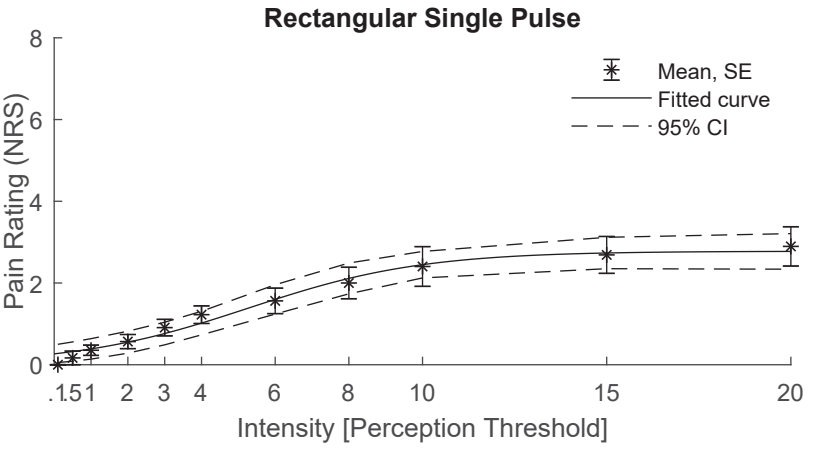


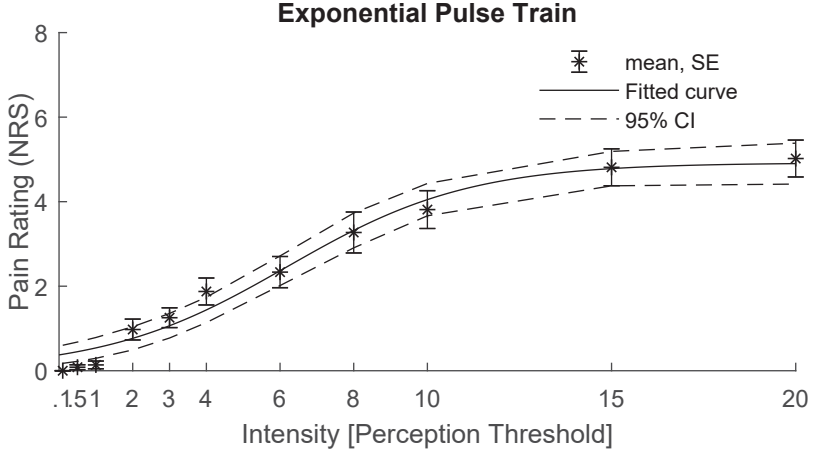
	Rheobase	
	Median (mA)	**IQR (mA)**
Pin Electrode	0.06	0.05
Patch Electrode	0.43	0.17
	Chronaxie	
	Median (ms)	**IQR (ms)**
Pin Electrode	1.33	2.04
Patch Electrode	0.594	0.19











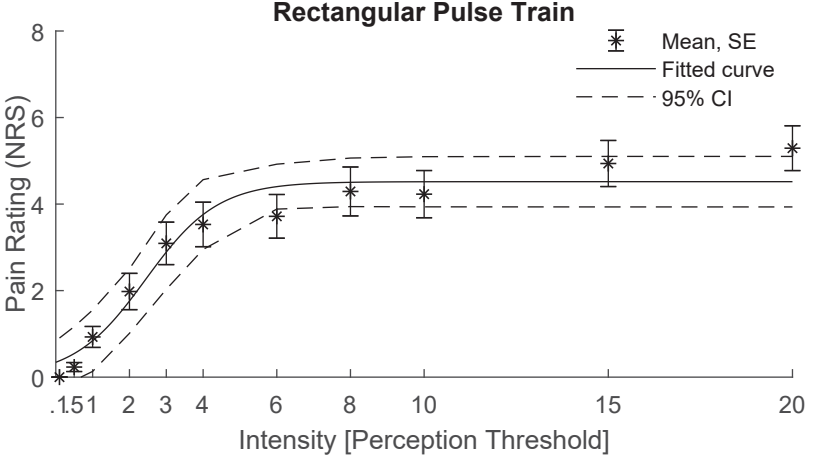


Table 1. Sigmoidal model fit for the pain ratings (NRS). Model coefficients are shown (with 95% confidence interval)

Paradigm	***Adj. R^2***	***A - slope***	***B – NRS max/2***	***C – NRS max***
Exponential single pulse	0.38	0.39 (0.24, 0.54)	6.24 (4.82, 7.67)	3.45 (2.96, 3.95)
Rectangular single pulse	0.29	0.42 (0.23, 0.62)	5.29 (3.76, 6.81)	2.78 (2.33, 3.23)
Exponential pulse train	0.54	0.40 (0.29, 0.52)	6.19 (5.20, 7.19)	4.92 (4.41, 5.42)
Rectangular pulse train	0.39	1.02 (0.56, 1.48)	2.44 (1.91, 2.97)	4.52 (4.11, 4.93)