ORIGINAL ARTICLE

Sympathetic efferent neurons are less sensitive than nociceptors to 4 Hz sinusoidal stimulation

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Abstract

Background: Sinusoidal current stimuli preferentially activate C-nociceptors. Sodium channel isoforms NaV1.7 and NaV1.8 have been implicated in this. Sympathetic efferent neurons lack NaV1.8 and were explored upon sinusoidal activation.

Methods: Quantitative Sudomotor Axon Reflex Test (QSART) was performed in hairy (n = 16) and glabrous (n = 12) skin. Responses of sympathetic efferents (n = 10) and nociceptive afferents (n = 21) to sinusoidal current stimulation (4 Hz, 0.05–0.15 mA) were recorded in humans by microneurography (n = 11). Activation of sympathetic units upon supra-threshold sinusoidal currents (>0.8 mA) was recorded in pigs (n = 8).

Results: Sinusoidal stimuli (4 Hz, 0.4 mA) evoked weak sweat output (30 ml/h/m2) in hairy skin compared to rectangular pulses (4 Hz, 5 mA, 53 ml/h/m2, p < .00001, ANOVA). No change in sweat output was recorded from glabrous skin to sine wave stimuli. Sinusoidal current at intensities ranging from 0.05 to 0.15 mA activated almost all (85%) nociceptors but only 40% of sympathetic units in human. Stimuli lead to a significantly lower activation in sympathetic versus nociceptive fibres as measured by activity-dependent slowing (ADS) of conduction (sympathetic efferents average ADS 100 ± 0.2% vs. C-nociceptors average ADS 113 ± 4%, p < .003, ANOVA).

Conclusions: Sympathetic efferent neurons are less apt to convert slow depolarizations into action potentials as compared to nociceptors. Distinctive sodium channel expression patterns between nociceptors and sympathetic efferent neurons may account for this difference. Sinusoidal stimulation therefore provokes weak sweat responses and provides no alternative for clinical assessment of autonomic function.

Significance: C-nociceptors in hairy skin are activated by 4 Hz sinusoidal current stimulation at lower intensities than myelinated fibres. Sympathetic efferent neurons—albeit also unmyelinated—are less responsive to sinusoidal activation than nociceptors within the same skin area. Cutaneous sympathetic efferent neurons apparently are less apt than nociceptors to convert slow depolarization into action potentials.

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1 | INTRODUCTION

Single nerve fibre recordings in vivo reveal that a sinusoidal current can produce a slow electrical depolarization that preferentially activates unmyelinated primary afferent C-nociceptors over A-delta fibres when applied transcutaneously and causes a pronounced axon reflex erythema (Jonas et al., 2018). Yet, it is unknown whether slow depolarization would stimulate also unmyelinated efferent sympathetic neurons in the skin. In analogy to the axon reflex erythema mediated upon C-nociceptor stimulation, the activation of sympathetic efferent fibres evokes a sweat response (Wada, Arai, Takagaki, & Nakagawa, 1952). For the assessment of peripheral autonomic nervous system function, a quantitative sudomotor axon reflex test (QSART) has been established (Low, Caskey, Tuck, Fealey, & Dyck, 1983) and an electrical stimulation protocol developed to activate sympathetic efferent neurons (Lang, Foerster, Pfannmuller, & Handwerker, 1993). Here, we used electrical QSART to functionally explore sympathetic efferent responses to sine wave stimulation. Based on the preferential activation of unmyelinated fibres by this stimulus protocol (Jonas et al., 2018), we hypothesized activation of sweat gland innervating nerve fibres without uncomfortable activation of A-delta nociceptors.

Voltage-gated ion channels play a key role for initiating and conducting action potentials in excitable cells. Nine voltage-gated sodium channel (NaV) isoforms have been identified in mammalian tissue (NaV1.1–1.9) of which NaV1.5, NaV1.8 and NaV1.9 show little sensitivity to tetrodotoxin (TTX) blockage (Goldin et al., 2000; Kwong & Carr, 2015; Narahashi, Moore, & Scott, 1964). TTX-sensitive currents of A-fibres, in particular NaV1.6 are subject to slow inactivation. Consequently, neurons dependent on these currents for action potential initiation will not fire action potentials in response to slow depolarizations. In contrast, the slow closed state inactivation property of NaV1.7 has been assumed to enable this channel to contribute to action potential initiation in the face of slowly depolarizing currents by the generation of ramp currents (Cummins, Howe, & Waxman, 1998; Dib-Hajj, Yang, Black, & Waxman, 2013; Herzog, Cummins, Ghassemi, Dib-Hajj, & Waxman, 2003). Reduced excitability of induced pluripotent stem cell (iPSC) nociceptors from patients with non-functional NaV1.7 (congenital insensitivity to pain) in particular to slow depolarizing stimuli suggests a crucial role of this channel (Han et al., 2015; McDermott et al., 2019).

As postganglionic sympathetic neurons are expressing NaV1.7 we expected them to respond also to slow depolarizing ramp stimuli. This prediction was tested in the present study by electrically induced QSART comparing standard rectangular and sine wave stimulation. Thereby, indirect evidence for functional activation of sweat gland innervating sympathetic efferent neurons should be provided. We additionally used single nerve fibre recordings (microneurography) in humans for direct verification of sympathetic efferent neuron activation versus C-nociceptor excitation during sine wave stimulation. Finally, we recorded from pig sympathetic efferent neurons in vivo in order to characterize thresholds to sinusoidal currents even at supra-threshold intensities (not applicable in human microneurography) activating these neurons.

2 | METHODS

Investigations in human subjects were performed according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) and informed written consent was obtained from all volunteers. Subjects attended either QSART and psycho-physics of nociception, or microneurography experiments. Animal experiments followed the guidelines of the Institutional Animal Care and Use Committee (IACUC) and the protocol was approved by the Committee on the Ethics of Animal Experiments, Baden-Württemberg (Protocol Number: 78–18).

2.1 | Stimulation parameter

C-nociceptor afferent and sympathetic efferent function was explored by square wave (rectangular) and sine wave (sinusoidal) stimuli. The stimulus shape and the applied parameters used for QSART/somato-sensory tests and microneurography are summarized in Table 1.

No particular skin cleaning preparation was required prior to the electrical stimulation. The delivered peak and average pulse current intensity was displayed on the front panel of the constant current stimulator (Digitimer DS5, Welwyn Garden City, United Kingdom) and error warnings were send if current was out of compliance.

2.2 | Quantitative sudomotor axon reflex test (QSART)

Postganglionic sympathetic neuron function was assessed by a quantitative sudomotor axon reflex test (QSART) as described before (Low et al., 1983). In principle, a two-compartment chamber was attached airtight to the medial aspect of the ventral forearm skin, 5–8 cm distal from the cubital fossa of 16 sex- and age-matched subjects (38 ± 3 years). The chamber was continuously aerated with filtered (FP10, Boge, Germany) and dried (MDK 6, KT 2016 M, Zander, Germany) air at a constant flow rate of 6 L/h adjusted by an air flow control unit (KDG 1,113 V 000, Kobold Messring GmbH, Germany) and a constant pressure of 1.5 bar controlled by a pressure gauge control unit (Heyer Medical AG, ...)
Assessment of QSART responses depolarization of sympathetic efferent fibres delivered at a current strength similar to 0.4 mA sinusoidal pulses. For this purpose, we requested the volunteer to estimate the pain sensation upon delivery of sine wave stimuli. In glabrous skin of the palm we recorded the sweat response evoked by sinusoidal stimuli at a frequency of 0.4 Hz and an intensity that induced similar pain intensity as 0.4 mA sinusoidal pulses. For this purpose, we requested the volunteer to estimate the pain sensation upon delivery of sine wave stimuli.

The actual values for absolute humidity and temperature were recorded for each measurement. The data were analyzed using statistical software (SPSS 22.0, IBM, Armonk, NY, USA).

2.3 | Microneurography in human vivo

In order to verify the QSART responses, which are an indirect measure for sympathetic efferent activation, we recorded extracellular activity from single sympathetic nerve fibres in humans by microneurography and compared the excitation upon sine wave stimulation and comparison to the excitation of C-nociceptors. In principle, as described before (Schmidt et al., 1995; Torebjork & Hallin, 1974; Vallbo, Hagbarth, Torebjork, & Wallin, 1979), a tungsten electrode (FHC, Bowdoinham, USA) was placed into the superficial peroneal nerve fascicle at ankle level in 11 healthy human subjects (age 29 ± 5 years). A reference microelectrode was positioned intradermally nearby. Square wave pulses (width 0.5 ms, intensity ~ 7 mA) were delivered transcutaneously via a pointed electrode (2 mm diameter) to identify the receptive field of the unit. Once a time-locked action potential was recorded,
we inserted intradermally two tungsten electrodes at this site and determined the electrical excitation threshold (square wave pulse width 0.5 ms) of the unit. Recordings were obtained by 0.25 Hz electrical stimuli delivered with twofold threshold intensity. Sympathetic units were identified according to their unresponsiveness to mechanical stimuli, their activity-dependent latency shift (“ADS”) upon repetitive electrical stimulation (Serra, Campero, Ochoa, & Bostock, 1999), and their pronounced activation upon manoeuvres simulating the sympathetic nervous system, for instance mental stress (Schmelz, Schmidt, Bickel, Toebjork, & Handwerker, 1998; Serra et al., 1999; Weidner et al., 1999). Action potential signals were amplified, processed online and stored on a PC using a CED micro1401 interface (Cambridge Electronic Design Ltd., Cambridge, UK) and DAPSYS 8 software (www.dapsys.net). Sinusoidal 4 Hz stimuli were generated by a constant current stimulator (Digitimer DSS, Welwyn Garden City, UK) and analog pulse generator (NI USB-6221, National Instruments, Texas, US) controlled by DAPSYS 8 data acquisition processor system (www.dapsys.net) and delivered via the same stimulation needles through which the test pulses were delivered every 4 s. Sinusoidal pulses (4 Hz) were administered continuously for 12 s at intensities of 0.05, 0.1 and 0.15 mA. An activation of the sympathetic unit by the sinusoidal stimulation was identified by the unit's “marking” (i.e. the activity-dependent conductance latency shift, ADS), as described before in detail (Schmelz et al., 1995). The ADS response was calculated in percent (%) in relation to the conductance latency recorded upon 0.25 Hz electrical stimuli (set at 100%).

2.4 Single nerve fibre recordings in pig in vivo

We further characterized the thresholds to sinusoidal currents for activating sympathetic efferent neurons. Therefore, we recorded the excitation of sympathetic efferent units upon supra-threshold transdermal sinusoidal stimulation in pigs in vivo, hence at current strengths that would not have been tolerated in humans. To this purpose, the teased nerve fibre technique was used to record extracellular signals of eight postganglionic sympathetic efferent units in domestic male pigs (Sus scrofa, n = 8, average body weight 28.5 kg, age 12 weeks). Premedication of the animals comprised 2 mg/kg Stresnil® (Azaperon, Janssen Pharmaceutica, Beerse, Belgium) and 0.3 mg/kg Dormicum® (Midazolam, Roche, Basle, Switzerland). Following intubation and ventilation (75–50% N₂O and 25–50% O₂) the animals sedation was maintained by intravenous 9 mg kg⁻¹ h⁻¹ Narcoren® (Pentobarbital-Natrium, Rhone Merieux, Laupheim, Germany). The saphenous nerve was exposed and isolated nerve bundles teased into thin filaments until single action potentials could be recorded time-locked upon square wave pulses (width 0.5 ms, intensity 20 mA) delivered with a pair of non-insulated tungsten electrodes (FHC, Bowdoinham, ME, USA) inserted within the receptive field of the unit. Nerve fibre signals were amplified (Model 5,113, Ametek Inc., Berwyn, USA), filtered (Model 3,364, Krohn-Hite Corp., Brockton, USA) and recorded using DAPSYS 8 software (www.dapsys.net). Sympathetic nerve fibre units were identified according their unresponsiveness to mechanical stimuli, their activity-dependent slowing pattern during continuous 2 Hz stimulation, and their action potential inter-stimulus intervals recorded upon two supra-threshold electrical pulses separated by 50 ms (Campero, Serra, Bostock, & Ochoa, 2004; Obreja et al., 2010; Ringkamp et al., 2010). Sinusoidal pulses were generated by a linear stimulus isolator (Model A395, World Precision Instruments, Sarasota, USA) controlled by DAPSYS 8 (www.dapsys.net) and delivered transdermally using an L-shaped bipolar platinum/iridium electrode (diameter 0.4 mm, Cephalon, Maastricht, Netherlands,) placed on a length of 3 mm onto the skin surface and within the receptive field of the unit. Twelve sinusoidal pulses at 4 Hz were administered with increasing intensity (0.1–1 mA) in increments of 0.1 mA and responsiveness of the unit quantified by the number and instantaneous frequency of generated action potentials (current intensity dose response). An activation of the sympathetic nerve fibre was defined by the occurrence of at least two consecutive and time-locked action potentials recorded during ongoing sinusoidal stimulation. In addition, sinusoidal pulses (4 Hz) were delivered continuously for 12.5 s (50 pulses) at intensities of 0.05–0.1–0.2–0.4 mA and corresponding action potential generation assessed for sympathetic nerve fibre activation. Also, sinusoidal pulses were delivered for 60 s (4 Hz) at supra-threshold intensity of 1.2 mA and number of action potentials and their median instantaneous discharge frequency recorded.

2.5 Statistical analysis

Statistical analyses were performed with STATISTICA 7.1 (StatSoft Inc.) using analysis of variance (ANOVA) and Scheffe’s post hoc repeated measures to identify significant differences (p < .05) between the factorial groups “current” and “stimulation repetition” for sweat-output QSART measures, and an additional parameter “stimulation start-stop” for pain recordings. Correlation between “pain” and “QSART” was calculated by non-parametric Spearman rank test and Power analysis (Cohen’s effect size $d = 0.7$) performed to calculate the sample size ($G^*$Power 3.1, Kiel, Germany). QSART values are depicted as mean ± SD, values of pain (NRS) and nerve fibre recordings are given as mean ± SEM.

3 RESULTS

3.1 Quantitative Sudomotor Axon Reflex Test (QSART)

Sympathetic efferent neuron functionality was quantified by electrically induced QSART. Square pulses delivered
to hairy forearm skin with 4 Hz and 5 mA currents evoked a maximum sweat output from baseline 36 ± 5 ml/h/m² to 53 ± 7 ml/h/m² on average (p < .0001, ANOVA) and served as positive QSART response. Notably, delivery of square pulses evoked considerable pain, and correspondingly sweat output was significantly elevated at a non-stimulated distal skin site particularly during the first stimulation block (p < .02, ANOVA, Figure 1a). Sinusoidal stimuli delivered with 4 Hz and 0.4 mA currents evoked a weak but significant sweat output from 27 ± 0.5 ml/h/m² to about 30 ± 1 ml/h/m² on average (p < .002, ANOVA, Figure 1a). No significant sweat response was induced by sinusoidal stimuli delivered at 0.2 mA (27 ± 1 ml/h/m² on average, n.s., ANOVA). QSART upon square wave pulses was significantly higher as

**FIGURE 1** QSART and Pain upon transcutaneous sinusoidal stimulation (hairy skin). (a) Quantitative sudomotor axon reflex test (QSART) assessed in 16 subjects upon 152 transcutaneous electrical pulses delivered with 4 Hz and repetitively within three stimulation blocks (black bar), each separated by 2 min intervals. Square wave pulses of 5 mA served as positive sweat output control (solid squares). Sinusoidal stimuli of 4 Hz were delivered at intensities of 0.4 mA (solid circles) and 0.2 mA (open circles), respectively and in randomized order. QSART was calculated in (ml/h/m²) and recorded from both the stimulated (left panel) and non-stimulated (ipsi-lateral) skin site (right panel). Sinusoidal pulses at 0.4 mA induced a significant QSART increase as compared to baseline condition (p < .002, ANOVA, marked by asterisks). Sinusoidal sweat response was significantly lower as compared to square pulse QSART (4 Hz, 5 mA, left panel). Notably, square wave pulses induced also at the non-stimulated (ipsi-lateral) skin site a significant sweat output compared to baseline (p < .02, ANOVA, marked by hashtag). Sinusoidal sweet response was significantly lower as compared to square pulse QSART (4 Hz, 5 mA, left panel). Notably, square wave pulses induced also at the non-stimulated (ipsi-lateral) skin site a significant sweat output compared to baseline (p < .02, ANOVA, marked by hashtag). (b) Pain responses (numeric rating scale, NRS 0–10) recorded during QSART measures in response to 5 mA square pulses (solid squares), 0.4 mA sinusoidal stimuli (solid circles) and 0.2 mA sine waves (open circles) at the beginning (“start”) and the end (“stop”) of each repetitively in 2 min intervals administered stimulation blocks (left-middle-right panel). Note that pain declined significantly at the end of the stimulation period (interaction stimulus condition × time p < .02, ANOVA, marked by asterisks) and was perceived significantly stronger upon square pulses as compared to the sinusoidal stimulation (p < .05, ANOVA, marked by hashtag).
compared to sinusoidal QSART \((p < .00001, \text{ANOVA})\). No significant differences were observed between subjects’ sex (n.s., ANOVA).

Maximum pain recorded in response to the 152 electrical pulses used for QSART was significantly different for the electrical stimulation condition (square wave vs. sine wave, \(p < .00001, \text{ANOVA}\)). No significant difference was identified between the three repetitions of stimulation (n.s., ANOVA). Between the stimulation onset (“start”) and the end of stimulation (“stop”) a significant interaction (stimulus condition x time) was calculated \((p < .02, \text{ANOVA, Figure 1b})\). Square pulses (4 Hz, 5 mA) were perceived most painful and rated with NRS \(6 \pm 1\) at the beginning and NRS \(5 \pm 1\) at stimulation termination. Significantly lower pain ratings were recorded upon 4 Hz sinusoidal stimuli at 0.4 mA \((p < .0001, \text{Scheffe’s post hoc test})\) and estimated with NRS \(3.8 \pm 1.7\) (“start”) and NRS \(2 \pm 1.5\) (“stop”). Sinusoidal stimuli delivered with 0.2 mA were rated NRS \(2 \pm 0.7\) (“start”) and NRS \(1.4 \pm 0.9\) (“stop”) on average. When comparing “start” to “stop” NRS-values as indicator for pain adaptation, we noticed a significantly less pronounced pain accommodation during square pulse stimulation (NRS declined by \(-1 \pm 0.3\), n.s.) as compared to 0.4 mA sine waves (NRS reduction by \(-1.6 \pm 0.2\), \(p < 0.05, \text{Scheffe’s post hoc test})\). Correlation between “average NRS” and “average QSART” was \(r = 0.33\) for square pulse stimulation and \(r = 0.068\) for 0.4 mA sinusoidal stimulation (n.s., not shown).

We additionally administered to hairy skin of 12 age- and sex-matched subjects square wave pulse intensities evoking similar pain ratings as 0.4 mA sinusoidal stimuli and compared the effect on QSART and nociceptor/sympathetic efferent activation (Figure 2a). Square wave pulses of 4 Hz delivered at 2.5 \(\pm 0.3\) mA on average caused pain of NRS \(5 \pm 0.2\) at start of stimulation and NRS \(4 \pm 0.3\) at the end of the stimulation (NRS sine wave \(5 \pm 0.9\) at start and NRS \(3 \pm 0.4\) at stimulation stop). Sweat output upon square wave stimulation increased on average (three repetitive cycles) from \(21 \pm 5\) (baseline) to \(29 \pm 10 \text{ ml/h/m}^2\) (stimulation). Sine wave pulses (4 Hz, 0.4 mA) failed to induce a significant sweat output, which was recorded on average \(18 \pm 2 \text{ ml/h/m}^2\) at baseline and \(19 \pm 3 \text{ ml/h/m}^2\) during stimulation (interaction stimulus condition x time \(p < .005, \text{ANOVA, Figure 2a})\).

In order to assess sine wave induced responses from another skin site, we recorded QSART and pain from glabrous skin of the palm (Figure 2b). Square wave pulses of 5 mA induced on average (three repetitive cycles) a significant sweat output from \(64 \pm 1\) (baseline) to \(69 \pm 3 \text{ ml/h/m}^2\) (stimulation, \(p < .005, \text{ANOVA}\)). No significant changes were measured upon 2.5 mA square wave pulses (baseline \(66 \pm 3\) and stimulation \(68 \pm 2 \text{ ml/h/m}^2\)) or 0.4 mA sinusoidal pulses (baseline \(56 \pm 1\) and stimulation \(57 \pm 1 \text{ ml/h/m}^2\), Figure 2 B, left panel). Pain monitored from glabrous skin was significantly lower during sinusoidal stimulation (average NRS \(1 \pm 2.6\)) as compared to pain estimates recorded upon 2.5 mA (average NRS \(3 \pm 1.3\)) and 5 mA square wave pulses (average NRS \(3.5 \pm 1.9\), \(p < .002, \text{ANOVA, Figure 2b, right panel})\).

### 3.2 Microneurography in human

We recorded from 11 subjects 21 “polymodal” (mechanically sensitive) C-nociceptors (average conduction velocity 0.8 m/s) and 10 sympathetic efferent neurons (average conduction velocity 0.5 m/s). Intradermal electrical excitation thresholds for rectangular pulses (0.5 ms width, 0.25 Hz) were \(0.2 \pm 0.04\) mA (C-nociceptors) and \(0.5 \pm 0.2\) mA (sympathetic efferents, n.s., Table 1). Figure 3 A depicts a specimen recording of a polymodal C-nociceptor (left, response latency \(-110\) ms) and a sympathetic efferent unit (right, response latency \(-170\) ms) recorded for 39 traces (supra-threshold rectangular pulses delivered in 4 s intervals) and upon sinusoidal stimuli of 0.05, 0.1 and 0.15 mA, respectively, delivered for 12 s (three traces, indicated by solid circles). An increase in response latency (shift to the right, “marking”) indicates the activation of the recorded unit. Excitation thresholds to intradermal sinusoidal stimuli (4 Hz) were \(0.06 \pm 0.04\) mA for all polymodal C-nociceptors \((n = 21)\) and \(0.1 \pm 0.04\) mA for sine wave responding sympathetic units \((n = 4, \text{n.s., Table 1})\).

Sinusoidal pulses (4 Hz) delivered intradermally at 0.05 mA for 12 s evoked a positive response in 18 C-nociceptors \((\sim 85\%)\) and an activity-dependent latency increase of on average \(17 \pm 5\) ms (or 8.5% of baseline response latency). Only three of eight sympathetic units were activated with an ADS of on average \(0.4 \pm 0.2\) ms. When including all eight sympathetic units, mean change in response latency during 0.05 mA sinusoidal stimulation was \(-0.4\%\) (Figure 3b). Increasing the current intensity to 0.1 mA evoked an ADS of about \(28 \pm 9\) ms in C-nociceptors (average conductance latency increase by 14% of baseline latency, \(n = 14\)). Four sympathetic efferent units \((40\%)\) were activated with a mean ADS of \(0.9 \pm 0.4\) ms. At intensities of 0.15 mA we recorded from C-nociceptors an activation of about ADS \(31 \pm 12\) ms (average latency increase by 19%, \(n = 6\)) and in 1 sympathetic unit a latency shift of 0.5 ms (average ADS 100% in \(n = 4\) fibres, Figure 3b).

### 3.3 Single nerve fibre recordings in pig

Neuronal responses were recorded from eight sympathetic nerve fibres in pig in vivo. According to previous experiments (Obreja et al., 2010), we should not expect an effect of general anaesthesia on the responses of sympathetic efferent units to electrical stimulation. Activation thresholds of the units to intracutaneous rectangular pulses were on average \(2.4 \pm 1.2\) mA. Four of the eight sympathetic fibres could be stimulated by 4 Hz transcutaneous sinusoidal pulses (specimen Figure 4a). Current thresholds of sinusoidal pulses
required for their activation were on average 0.65 ± 0.4 mA (n = 4, n.s. compared to rectangular pulses). A current intensity response was recorded for action potential number (AP) and instantaneous discharge frequency (Hz) from three units. Sympathetic fibres responded to supra-threshold sinusoidal stimuli of 0.8 mA (12 pulses delivered) with a discharge frequency of about 14 Hz and 18 action potentials on average (Figure 4 B). One unit responded to sinusoidal stimuli delivered continuously for 12.5 s (50 pulses) with an instantaneous frequency of 8 Hz and 98 AP’s at 0.2 mA (not shown). Also, two sympathetic units were recorded during continuous 60 s sinusoidal stimulation at supra-threshold intensity of 1.2 mA. The units responded with an instantaneous frequency of about 8 ± 5 Hz without accommodation of action potential generation (not shown).

4 | DISCUSSION AND CONCLUSIONS

Transdermally administered sinusoidal stimulation beyond 0.1 mA evokes substantial non-pulsating burning pain in...
hairy skin and about 85% polymodal C-nociceptors are activated by intracutaneously delivered 4 Hz sine wave stimuli at intensities of 0.05 mA. As shown by single nerve fibre recordings in pig and human, fewer sympathetic efferent neurons can be activated by sinusoidal pulses and their response to this stimulus is markedly weaker. Thus, 4 Hz sinusoidal stimuli even at 0.4 mA peak currents induce only a very weak sweat response (QSART) contrasting the development of an axon reflex erythema and pain already at 0.05 mA (Jonas et al., 2018). This mismatch might result from different levels of neuronal activity required to evoke nociceptor-driven pain and axon reflex erythema versus sympathetically driven sweat response or from different axonal excitability related to the sine wave stimulation.
4.1 Quantitative Sudomotor Axon Reflex Test (QSART)

To validate the excitability of sympathetic efferent neurons to slowly depolarizing sinusoidal stimulation we employed electrical QSART in human forearm skin and glabrous skin of the palm. Previously, sympathetic efferents had been activated by transcutaneous rectangular pulses of high current intensity (Lang et al., 1993; Low et al., 1983; Namer, Bickel, Kramer, Birklein, & Schmelz, 2004), but the QSART accompanying pain sensation may hamper the clinical evaluation of patients suffering not only an autonomic dysfunction but also neuropathic pain. It therefore would be clinically beneficial to have a different type of functional autonomous nervous system test that is less painful. Sinusoidal pulses (4 Hz, 0.4 mA), however, induced only weak sweat responses in forearm and none in glabrous skin. The lower efficacy for induction of sweating might be related to differences in optimum stimulation frequency: while maximum electrically induced sweating required around 50 Hz (Sommer et al., 2011), nociceptor-driven axon reflex flare is maximum already at 5 Hz (Dusch, Schley, Rukwied, & Schmelz, 2007). However, as we could elicit strong sweat responses at 5 Hz using rectangular pulses, mode of stimulation rather than frequency appears to be crucial. It also may be suggested that the sinusoidal current directly interacts with sweat glands or influences the transport of sweat along ducts, but this explanation can be dismissed by our microneurography data and the fact that we were measuring the sweat output in a chamber of 25 mm inner diameter but the locally applied current (pair of 3 mm electrodes) effects only a small proportion of the skin, thus mainly widespread axon reflex sweating was assessed.

Neuronal innervation pattern varies considerably between skin regions. Sympathetic efferent neurons are located deeper in the skin as they are associated with the sweat glands as compared to nociceptive nerve fibres abundantly distributed in superficial (epidermal) layers of human hairy skin (Kennedy et al., 2005). Also, very few unmyelinated fibres but abundant myelinated fibres are found around mechanoreceptors in...
glabrous skin (e.g., Meissner corpuscles), whereas unmyelinated fibres dominate in hairy skin (Provitera et al., 2007). For our present results from glabrous versus hairy skin upon transdermal sinusoidal stimulation it is important to consider the very thick non-innervated stratum corneum in glabrous skin that might reach 1 mm compared to 20–40 μm in hairy skin (Sandby-Moller, Poulsen, & Wulf, 2003). Thereby, the distance between the epicutaneous electrodes and the nerve fibres increases but the stimulation efficacy is reduced. Even with intracutaneous stimulation we required high current intensities to stimulate sympathetic efferent units, whereas “silent” nociceptors, innervating deep dermal arterioles to mediate the axon reflex erythema (Schmelz et al., 2000), were sufficiently activated by low-intensity sinusoidal pulses (Jonas et al., 2018). Hence, the deeper location of sympathetic efferent neurons in the skin cannot account alone for the differential responses recorded between nociceptors and sympathetic efferent neurons during sinusoidal stimulation.

In summary, sinusoidal stimulation apparently is no viable alternative to conventional rectangular pulses for functional sympathetic efferent tests, particularly in patients associated with reduced sympathetic function and neuropathic pain (for instance erythromelalgia (Han et al., 2012)).

4.2 Activation of nociceptors versus sympathetic efferent neurons upon sinusoidal currents

Our single nerve fibre recordings in the pig indicate that sinusoidal stimuli at intensities greater than even 1 mA activate less than 50% of sympathetic efferent neurons. In humans, intradermal stimuli delivered at up to 0.15 mA activated about 40% sympathetic efferents and about 85% nociceptors. Moreover, the number of action potentials induced by sinusoidal stimulation is much lower in sympathetic fibres as based on the difference in activity-dependent slowing (see Figure 3). Thus, our data clearly demonstrate that sympathetic efferent fibres are less sensitive to sinusoidal stimulation as compared to nociceptors. This difference may be unexpected based on the role of NaV1.7 for the induction of ramp currents (Cummins et al., 1998; Dib-Hajj et al., 2013; Herzog et al., 2003; McDermott et al., 2019) and its expression in sympathetic efferent neurons. A major difference between nociceptors and sympathetic efferents is the expression of NaV1.8 for which about 85% of nociceptors are positive (Abrahamsen et al., 2008), but which is not present in sympathetic efferent neurons (Akopian, Sivilotti, & Wood, 1996). Interestingly, gain of function mutations of NaV1.7 that render nociceptors hyperexcitable leave postganglionic sympathetic neurons hypoexcitable (Rush et al., 2006) unless they are transfected with NaV1.8 (Rush et al., 2006). Thus, one might conclude NaV1.8 has a crucial role to convert NaV1.7 ramp currents into action potentials, hence facilitating activation in nociceptors, which would be less dominant in sympathetic neurons lacking NaV1.8. Alternatively, inactivation of NaV1.6 that is also present in sympathetic neurons [at least in adult superior cervical ganglia of the rat (Rush et al., 2006)] or absent ramp currents mediated by NaV1.8 [in humans (Han et al., 2015)] might contribute to the lower sensitivity of sympathetic neurons to sinusoidal stimulation and further argues against an exclusive role of NaV1.7 in sympathetic excitation.

Finally, it might be expected that sensory endings are particularly optimized to process slow depolarizations into action potentials. Lipid rafts with clustered channels possibly creating some kind of spike initiation zone. There would be no need for sympathetic efferent fibres for such spike initiation zones and thus, stronger stimuli might be required to provoke antidromic action potentials. However, it is well-known that iontophoresis of acetylcholine readily and robustly activates human sympathetic fibres (Schmidt, Weidner, & Schmelz, 2011), indicating that their endings are able to mimic the function of a sensory afferent.

In conclusion, we demonstrate that sympathetic efferent neurons are less sensitive but nevertheless responsive to sinusoidal stimuli. Differential expression of NaV isoforms amongst nociceptors and sympathetic efferent neurons, in particular the lack of NaV1.8 in the autonomic fibres, may contribute to lower sensitivity to sinusoidal stimulation in these fibres. Future mechanistic studies are required to understand the contributions of different axonal ion channels to the slow depolarizing electrical stimulation, but regardless of the mechanism, this difference impedes sinusoidal stimulation as new tool for electrically induced QSART tests.

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Author Contributions

Study design, data recording and analysis (R.J., B.N., M.S., S.S., J.P. and R.R.), all authors discussed the results and commented on the manuscript. The authors declare no further conflicts of interest.
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