Capsaicin-induced changes in electrical pain perception threshold can be used to assess the magnitude of secondary hyperalgesia in humans

Running title: Electrocutaneous assessment of secondary hyperalgesia

Hughes, SW, Basra, M., Chan, C., Parr, C., Wong F., Strutton PH.

The Nick Davey Laboratory, Faculty of Medicine, Imperial College London, UK.

Corresponding author and Address: Dr Sam Hughes; The Nick Davey Laboratory, Human Performance Group, Division of Surgery, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, W6 8RF. Tel: +44 (0)20 331 38837 Email: sam.hughes@imperial.ac.uk

Funding: Imperial College London

Conflict of interest disclosure: The authors report no conflicts of interest.

Abstract

Objectives

Areas of secondary hyperalgesia can be assessed using quantitative sensory testing (QST). Delivering noxious electrocutaneous stimulation could provide added benefit by allowing multiple measurements of the magnitude of hyperalgesia. We aimed to characterise the use of electrical pain perception (EPP) thresholds alongside QST as a means by which to measure changes in pain thresholds within an area of secondary mechanical hyperalgesia.

Methods

EPP and heat pain thresholds (HPT) were measured at 5 distinct points at baseline and following 1% capsaicin cream application; 1 within a central zone and 4 within a secondary zone. Areas of mechanical secondary mechanical hyperalgesia were mapped using QST. In a further 14 participants, capsaicin-induced reduction in EPP thresholds was mapped using a radial lines approach across 24 points.

Results

There was a reduction in EPP threshold measured at the 4 points within the secondary zone which was within the mapped area of mechanical secondary hyperalgesia. The magnitude of secondary hyperalgesia could be split into a mild (~4% reduction) and severe (~21% reduction) area within an individual subject. There was no reduction in HPT within the secondary zone but there was a reduction in both HPT and EPP threshold within the primary zone. EPP mapping revealed differences in the magnitude and spread of hyperalgesia across all subjects.

Conclusions

Measuring capsaicin-induced reduction in EPP thresholds can be used to map hyperalgesic areas in humans. This semi-automated approach allows rapid assessment of the magnitude of hyperalgesia, both within an individual subject and across a study population.

Key words: Capsaicin; secondary hyperalgesia; electrocutaneous; sensitisation

Introduction

Chronic pain affects up to 28 million people in the UK and is associated with a low quality of life and poor pain control with conventional use of analgesics (1, 2). Understanding the mechanisms of chronic pain is imperative in the development of more effective personalised therapies (3). A common feature of chronic pain is the development of secondary hyperalgesia which is a spinally-mediated enhanced pain response in an area adjacent to the initial injury site (4, 5). Quantitative sensory testing (QST) techniques used in human surrogate pain models can be used to assess experimentally-induced secondary hyperalgesia have been key to furthering our understanding of the mechanisms of chronic pain (6). However, it is possible that adding further assessment methods to current QST-based approaches has the potential to provide new insight into the secondary hyperalgesia response.

The capsaicin model has been routinely used to investigate the mechanisms of secondary hyperalgesia (7-14). QST is used both clinically and experimentally to map the area of hyperalgesia using a radial lines approach, which manifests as selective changes in the function of mechanically sensitive Aδ-fibre afferents (7, 10, 14-16). As well as measuring the surface area on the skin affected by secondary hyperalgesia, it also imperative that the magnitude of static secondary hyperalgesia is also assessed at specific points within the affected area in order to achieve more detailed information. It is possible to do this by measuring changes in the mechanical pain threshold or stimulus-response functions (17); however this can be time consuming if more than one site is to be assessed within an area of secondary hyperalgesia.

The use of a computer controlled electrical stimulus has been shown to be a reliable method to assess cutaneous sensitivity (18) and has been used to measure changes in pain threshold in areas of capsaicin-induced secondary hyperalgesia (19, 20). By delivering incrementing

noxious stimuli with rapid onset and offset times it is possible to quickly measure electrical pain perception (EPP) thresholds using the methods of limits approach by increasing or decreasing the current intensity (21). Short square-wave electrical stimulation will directly excite the full spectrum of peripheral nerve fibres and therefore this approach cannot be readily used to assess specific nerve fibre function (22). However, it is possible that measuring EPP thresholds could be used alongside traditional QST methods to provide a detailed overview of the changes in magnitude of pain thresholds within a secondary hyperalgesia area associated with altered mechanical pain sensitivity.

The aim of this study was to first characterise the use of EPP as a means by which to capture capsaicin-induced changes in pain threshold across four different points within a mapped area of secondary mechanical hyperalgesia. It was anticipated that there would be within-subject differences in the degree of change in EPP threshold within the mapped surface area of mechanical hyperalgesia. In a follow-on study we aimed to map the changes in EPP threshold using a radial lines approach with a view to provide a more detailed overview of changes in pain thresholds measured across multiple points within an area of secondary hyperalgesia.

Methods

Subject screening and recruitment

All procedures were approved by the local research ethics committee. We recruited 26 healthy participants across 2 studies (study 1: n = 12; mean (SD) age 20.8 ± 3.1; 3 females; study 2: n = 14; mean age 24.7 ± 10.1; 5 females). All participants were informed of the experimental protocols and subsequently provided written consent in accordance with the principles of the declaration of Helsinki. All participants were initially screened to see if they met any of the exclusion criteria for pain testing (i.e. pregnancy, diabetes, blood disorders, neurological

conditions, immune-suppression, inflammatory disease, psychiatric conditions, taking steroid, antibiotic or pain medicines).

Capsaicin pain model

All Participants received topical application of capsaicin cream (1% w/w, Pharmacierge, London, UK). Using a 1 ml syringe, 50 µl was ejected onto a 9 mm diameter clear plastic disc which was then placed face-down on the skin, remaining in place for the remainder of the protocol (area of capsaicin skin contact: 64 mm^2) (16). The participants used a modified VAS used previously (16) where 0 = no sensation; 50 = pain threshold; 100 = worst pain imaginable. Following application of capsaicin cream, the participants were instructed to rate the sensation every 3 minutes for 120 minutes. The participants described the sensation initially as ''tingling'' which increased in intensity over approximately 45 minutes until a distinct ''stinging'' or ''burning'' pain was perceived (i.e. 50 VAS rating).

Mapping secondary mechanical hyperalgesia

The location of capsaicin cream application was standardised by measuring an area on the left L5 dermatome, one third the way along a line from the left lateral femoral epicondyle to the left lateral malleolus. Using the radial lines approach, 8 spokes were marked using a non-permanent marker that radiated outwards from the point of capsaicin cream application. Mapping of altered mechanical pain sensation was performed using a 128 mN pin prick stimulator starting at the point of capsaicin cream application and moving outwards at 1cm intervals at rate of 1 stimulus/s along the length of each of the 8 spokes and a point was marked on each spoke at the point when the sensation changed from a sharp pinprick to a blunt prodding sensation. During this procedure, the participant was instructed not to observe the testing site. The erythematous flare response (i.e. primary hyperalgesia zone) was defined as

the area of skin that was reddened around the capsaicin cream application. This was evaluated visually and the border between the detectable erythema and normal skin pigmentation was marked along each of the 8 spokes (23). The areas of primary and secondary hyperalgesia were subsequently traced using acetate and for each area, the points on the 8 spokes were connected to create separate 8 sided polygons. The area of each polygon was then measured using an image analysis program (ImageJ, US National Institute for Health) (24).

EPP threshold testing

Each transcutaneous electrical stimulus consisted of a standard, constant-current 1-ms duration square pulse using a constant current stimulator (DS7A, Digitimer, UK) (21). For testing carried out in study 1, four 4.5cm points from the capsaicin application were drawn in proximal, distal, medial and lateral directions. Modified cathodal electrodes (Ag/AgCl; self-adhesive, 1 cm diameter, CareFusion, UK) were used to measure EPP threshold at the point of capsaicin application and at each of point with an anode (Ag/AgCl ; self-adhesive, 25mm diameter, CareFusion, UK) placed over the patella. Pain thresholds (mA) were then determined using the methods of limits approach at each of the 5 points by increasing the current intensity in 0.5 mA steps at 1 Hz and was defined as the mean of 3 intensities logged as the point at which sensation transitioned from being a ''heavy tapping'' sensation (i.e. no pain) to a sharp ''pinprick'' pain (21). There was a 10 second-stimulus interval between each EPP test to avoid sensitisation. During study 2, eight radial 4.5 cm spokes were drawn from the point of capsaicin application and EPP thresholds were measured across 24 points radiating outwards from the capsaicin cream application.

Heat pain threshold testing

Heat pain thresholds were determined across the same 5 points used for EPP testing (i.e. 4 in a secondary zone and 1 in a primary zone) using a thermode (TSA-II, Medoc, Israel) placed over the skin on the leg. The baseline temperature was set to 32 °C and the temperature ramp increased at 1 °C/s and the participant pressed the stop button when the impression of warmth or heat changed towards an additional impression of burning, stinging, drilling or aching sensation. Heat pain thresholds were measured 3 times with a fixed inter-stimulus interval of 10 seconds.

Experimental protocols

For all experiments, participants were seated on a couch with knee extended to 180°. Before the experiment started, each participant was familiarised with all sensory testing procedures.

Study 1: Baseline EPP and heat pain threshold measurements were measured across 5 points; at the proximal, distal, medial and lateral points as well as the central capsaicin point with an inter-stimulus interval of 10 seconds (i.e. secondary and primary hyperalgesia zones, respectively; Figure 1A). Topical capsaicin cream (1% concentration; 50 µl) was then applied to a 9mm diameter clear plastic disc and placed faced down on the skin in the centre. VAS ratings were then recorded every 3 minutes to track the development of an ongoing pain state. Following the onset of capsaicin induced pain perception (i.e. >50 VAS rating), secondary mechanical hyperalgesia was mapped using a radial lines approach. EPP and heat pain thresholds were then re-measured at each of the proximal, distal, medial and lateral points in the secondary hyperalgesia zone. The capsaicin cream was then removed and EPP and heat pain thresholds were then re-measured within the primary hyperalgesia zone (Figure 1B).

Study 2: Baseline EPP thresholds were mapped across 24 points; 8 points within an inner circle (i.e. 1.5cm from the central point), 8 points within a middle circle (i.e. 3cm from the central point) and 8 points in an outer circle (i.e. 4.5cm from the central point; Figure 1C). Participants were asked to attend 2 visits (separated by ~1 week) where they received topical application of either capsaicin cream or sham cream (Aqueous Cream B.P, Boots pharmaceuticals UK), which was applied to a clear plastic disc and placed faced down on the skin at the centre of the 8 radial lines. Following the onset of capsaicin induced pain perception (i.e. when VAS ratings reached 50) EPP thresholds were measured in a clockwise fashion starting from the proximal position on the inner, middle and outer rings with an inter-stimulus interval of 10 seconds (Figure 1D).

Statistical analysis

All data were initially entered into Microsoft Excel before being analysed for normality and statistical significance in GraphPad Prism (GraphPad Software Inc., USA). The stability of the capsaicin-induced ongoing pain VAS ratings was analysed by one-way repeated measures (RM) ANOVA with Holm-Sidak multiple comparison post-hoc tests. Mild and severe areas of electrically-evoked secondary hyperalgesia were defined as the mean of the 2 smallest reductions in EPP threshold and the mean of the 2 greatest reductions in EPP threshold measured across the proximal, distal, medial and lateral points within each individual subject. Paired t-tests were used to analyse the difference in the capsaicin-induced reduction in EPP thresholds measured in the mild and severe areas in the secondary zone. The changes in HPT and EPP threshold measured before and after capsaicin were assessed using paired t-tests. During study 2, the effects of sham cream application were analysed by comparing pre- versus post- cream application EPP thresholds using paired t-tests. Participant-specific heat maps

were drawn using the percentage change in EPP threshold across the 24 measurement points in the inner, middle and outer rings following capsaicin or sham cream application using SigmaPlot 12.5 (Systat Software Inc, UK). Statistical significance was set at $p<0.05$ and all data in the text are presented as mean ± SD.

Results

Development of capsaicin-induced ongoing pain

Topical application of 1% capsaicin cream resulted in an initial very light tingling sensation after 3 minutes (VAS: 0.92 ± 2.8; Figure 2). There was an overall main effect of Time ($p<0.001$) and the tingling sensation increased further until it developed into a distinct burning pain at a mean time of 51 minutes post-capsaicin application (i.e. VAS: 53.75 ± 14.32). Post-hoc analysis revealed there was no significant difference in pain rating between 51 minutes and 90 minutes post-capsaicin application.

Capsaicin-induced changes in EPP threshold reveal within-subject differences in the magnitude of secondary hyperalgesia

The area of flare (i.e. primary hyperalgesia zone) and punctate secondary mechanical hyperalgesia are shown in Table 1. Within the area of mechanical secondary hyperalgesia, there were differences in the degree of change in EPP threshold measured across 4 points covering an area of 64 cm^2 within an individual subject (Figure 3A). These changes in EPP thresholds within the secondary zone could be categorised as the mean of the 2 smallest drop in threshold and mean of the 2 largest drop in threshold (i.e. mild: -4.28 ± 18.27% versus severe: -21.16 ± 18.25%; $p<0.001$; Figure 3B). There was no change in heat pain threshold within the points defined as mild (pre-capsaicin: 46.88 ± 2.64 °C versus post-capsaicin: 46.64 ±

2.40 °C; p>0.05; Figure 3C) and severe (pre-capsaicin: 47.23 ± 1.73 °C versus post-capsaicin: 46.60 ± 1.74 °C; Figure 3D) areas of secondary hyperalgesia.

Changes in heat pain threshold and EPP threshold in an area of capsaicin-induced primary hyperalgesia

Topical capsaicin application was associated with a robust drop in heat pain threshold in the area directly below the cream (pre-capsaicin: 46.59 ± 2.39 °C versus post-capsaicin: 36.04 ± 0.84 °C; p<0.001; Figure 4A). There was also a drop in EPP threshold measured over the same position (pre-capsaicin: 18.34 ± 5.38 mA versus post-capsaicin: 13.95 ± 4.98 mA; p<0.001; Figure 4B).

Capsaicin-induced changes in EPP threshold reveals between-subject differences in the magnitude of hyperalgesia

In a separate cohort of participants, changes in EPP threshold were then mapped at 24 points along 8 lines radiating outwards from either real or sham capsaicin cream application. There was a heterogeneous response to the real capsaicin cream which could be quantified using subject-specific heat map analysis which show the percentage change in EPP threshold at each point (Figure 5). There were distinct between-subject differences in the degree of change in EPP threshold as well as the direction to which sensitisation to electrical stimuli spread around the 8 spokes.

Sham cream application was associated with no change in mean EPP threshold measured across all 8 points within the inner (pre-sham: 7.7 ± 5.5 mA versus post-sham: 7.6 ± 5.3; p>0.05), middle (pre-sham: 7.7 ± 5.8 versus post-sham: 7.5 ± 5.5 mA; p>0.05) or outer (pre-

sham: 7.9 ± 5.5 versus post-sham: 7.8 ± 5.4 mA; $p>0.05$) and there was no tingling or burning pain sensation reported.

Discussion

In this study, we have shown that measuring capsaicin-induced changes in EPP thresholds can provide added benefit to existing QST-based methods of assessing secondary hyperalgesia responses. We have demonstrated the ability of EPP to rapidly capture differences in pain sensitivity which could be categorised into mild and severe within an individual subject. The changes in EPP threshold within a secondary zone were confirmed by defining the area of hyperalgesia to mechanical but not heat pain stimuli. There was also a change in EPP threshold within an area of primary heat hyperalgesia which suggests noxious electrocutaneous stimulation can be used to broadly assess sensitisation across sensory nerve fiber function. We show that using EPP is a new approach to measuring hyperalgesic responses and allows the rapid determination of pain thresholds for responses across multiple electrodes. By using this semi-automated approach, it is possible to programme stimulation of numerous sites making it more efficient than manual stimulation of the same number of sites using QST based approaches. These results indicate that by combining electrocutaneous stimulation with conventional methods used to assess the surface area affected by secondary hyperalgesia, it is be possible to rapidly investigate within and between subject differences in both the area and magnitude of the secondary hyperalgesia response.

It is recognised that there is a heterogeneous response to the induction of secondary hyperalgesia in healthy subjects which is typically reflected in differences in the size of the secondary hyperalgesia area (13). Using a burn injury model, others have previously grouped subjects into high- and low-sensitisation phenotypes based on the size of the mapped area of

secondary hyperalgesia (25). In the current study we found a mean area of secondary mechanical hyperalgesia to be ~112 cm^2 which is in line with a previous report using this approach (16). By doing this characterisation, we could confirm that EPP thresholds, which were measured within a pre-defined area of 64 cm^2, were assessed within the mean secondary hyperalgesia zone seen using this model. However, it is important to use confirmatory QST assessments to determine the borders between primary and secondary hyperalgesia zones. It is possible that in some participants the area of secondary mechanical hyperalgesia may not spread evenly around the radial lines and therefore areas which are associated with no change in EPP threshold from baseline could in fact be measuring pain thresholds outside of the secondary hyperalgesia zone. Nevertheless, measuring capsaicin-induced changes in EPP thresholds provides the opportunity to map areas of altered pain thresholds which can be confirmed as either primary or secondary hyperalgesic response through QST assessments.

We have shown that by measuring changes in EPP threshold across multiple sites within an area of secondary hyperalgesia it is also possible to identify areas of high and low sensitivity within an individual subject. This indicates different levels of heterosynaptic sensitisation of afferent inputs coming from 4 distinct points from the secondary hyperalgesia zone. Central sensitisation corresponds to an overall increase in the activity of central nociceptive pathways caused by increases in membrane excitability, synaptic efficacy and reduced local and descending inhibitory control (26). Nociceptive input from a specific receptive field is highly malleable and open to centrally-mediated plasticity which manifest as changes in pain threshold and many other features of central sensitisation (27, 28). It is therefore possible that dorsal horn neurons which are subject to central sensitisation may exhibit localised differences in the levels of sensitivity which manifest as large or small reductions in pain threshold within the area of secondary hyperalgesia.

It is currently unclear why the response to secondary hyperalgesia varies despite each participant undergoing the same induction procedure. Results from human neuroimaging studies have shown that secondary hyperalgesia is associated with changes in the activation of brainstem regions involved in top-down inhibitory control (29-31) which has shown to be different based on the presence of high or low sensitisation phenotypes (25). It is therefore possible that changes in these spinally-projecting pathways may dictate the overall levels of sensitisation in the dorsal horn (12). Similar top-down inhibitory mechanisms have been attributed to the enhanced areas of capsaicin-induced secondary hyperalgesia seen in women with a history of life stressors (32). The efficiency of psychophysical measures of endogenous pain modulation has also been shown to vary considerably within populations of chronic pain patients (33-35) which may contribute to the differences seen in the severity of secondary hyperalgesia within the sample population of the present study. By mapping detailed changes in EPP threshold around an area of secondary hyperalgesia we demonstrated a spectrum of different severities across the participants ranging from high to low sensitivity. Future experiments which aim to investigate the relationship between the severity of secondary hyperalgesia and endogenous pain modulation may help to understand why participants fall into high and low sensitivity profiles. Indeed, measuring EPP thresholds alongside psychophysical or neuroimaging experiments may also help to improve our mechanistic understanding of the heterogeneity often seen in populations of chronic patients.

Nerve block experiments have indicated a role for Aδ-fibre nociceptors in mediating the secondary mechanical hyperalgesia response which is thought to occur via a heterosynaptic facilitation in the dorsal horn (10, 14). Interestingly, we also saw changes in EPP threshold but no changes in heat pain threshold within the area of secondary mechanical hyperalgesia which suggests the electrocutaneous stimulation montage used might activate a population of

sensitised Aδ-fibres. It is possible that more selectivity could be achieved by delivering a slowly incrementing exponential pulse through a cutaneous pin electrode, which has been shown to preferentially activate small diameter nerve fibres (36, 37). However, the electrocutaneous stimulation used in the current study was delivered using a standard patch electrode which is thought to activate both small and large diameter nerve fibres in an unnatural and synchronised manner with a bypassing of the sensory nerve endings and as such, losing the vital modality-specific information gained from sensory transduction (22). It is therefore feasible that the changes in EPP threshold seen in the primary zone could be due to activation of sensitised C-fibres as we all also saw concurrent changes in heat pain threshold, which is known to be predominantly caused as a result of C-fibre sensitisation (7, 38). Together, this provides evidence that the EPP test can be used to broadly assess sensitisation across the full spectrum of sensory nerve fibres and may provide an efficient and cost-effective alternative to measuring changes in pain sensitivity in experimental and clinical pain studies assessing mechanisms and efficacy of analgesics.

In conclusion, this study reveals that measuring capsaicin-induced changes in EPP thresholds can be used to assess changes in both primary and secondary hyperalgesia. By combining with QST methods used to map areas of altered mechanical sensitivity, it is possible to rapidly add further information on the magnitude of the secondary hyperalgesia response within an affected dermatome, by measuring differences in the degree of change in EPP threshold across numerous points using a semi-automated approach. Electrocutaneous stimulation may help to better understand individual differences in the magnitude of secondary hyperalgesia in human surrogate models and in chronic pain patients.

Acknowledgements

We would like to thank Imperial College London for funding this study and all participants for taking part.

Conflict of interest disclosure

The authors report no conflicts of interest.

Figure legends

Figure 1. Experimental protocols. A) Measurement points used during study 1 included 4 in the secondary zone (proximal, medial, lateral and distal) and 1 in the primary zone. B) Baseline EPP and heat pain thresholds were measured in the primary and secondary zone before application of 1% capsaicin cream. Post-capsaicin EPP and heat pain thresholds were first measured across the 4 points in the secondary zone before the cream was removed and post-capsaicin measurements were made in the primary zone. C) Measurement points used during study 2 included 8 within an inner ring, 8 within a middle ring and 8 within an outer ring. D) Baseline EPP thresholds were measured across the inner, middle and outer rings before and after application of either 1% capsaicin or sham cream. For A) and C) Light grey = secondary zone, dark grey = primary zone, black circle = position of capsaicin application.

Figure 2. Development of capsaicin induced ongoing pain. Time course of changes in pain perception following topical application of 50µl 1% capsaicin cream. The intensity of sensation increased until pain threshold was reached at ~51 minutes (i.e. dashed line; 50 VAS). Data expressed as mean and shaded area around the curve depict the standard deviation. n = 12.

Figure 3. Capsaicin-induced changes in EPP threshold within an area of mechanical secondary hyperalgesia. A) Representative trace showing the mapped area of mechanical secondary

hyperalgesia with the percentage change in EPP thresholds measured across 4 points within the secondary hyperalgesia zone. B) Mild and severe areas of electrically-evoked secondary hyperalgesia. There was no change in heat pain thresholds measured at C) mild and D) severe areas of secondary hyperalgesia. Data expressed as mean with individual data points. *** - $p<0.001$; ns – not significant. n = 12.

Figure 4. Capsaicin-induced primary hyperalgesia. There was a drop in A) heat pain threshold and B) EPP threshold in the area directly below the capsaicin cream application, i.e. the primary hyperalgesia zone. Data expressed as mean with individual data points. *** - $p<0.001$. n = 12.

Figure 5. Mapping between-subject differences in the severity of hyperalgesia using EPP thresholds. Individual heat maps showing the percentage change in EPP threshold measured across 24 points along 8 spokes radiating outwards from capsaicin cream application. Red = greatest reduction in EPP threshold; yellow = smallest reduction in EPP threshold; green = no change from baseline; blue/purple = increase in EPP threshold. n = 14.

Table 1. Mapped areas of primary and secondary hyperalgesia. The primary zone was defined as the border between the detectable erythema and normal skin pigmentation. The secondary zone was mapped by measuring changes in mechanical pain sensitivity. Data expressed as mean ± SD. n = 12.

References

1. van Hecke O, Austin SK, Khan RA, Smith BH, Torrance N. Neuropathic pain in the general population: a systematic review of epidemiological studies. Pain. 2014;155(4):654-62.

2. Finnerup NB, Attal N, Haroutounian S, McNicol E, Baron R, Dworkin RH, et al. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. Lancet Neurol. 2015;14(2):162-73.

3. Finnerup NB, Sindrup SH, Jensen TS. The evidence for pharmacological treatment of neuropathic pain. Pain. 2010;150(3):573-81.

4. Arendt-Nielsen L, Morlion B, Perrot S, Dahan A, Dickenson A, Kress HG, et al. Assessment and manifestation of central sensitisation across different chronic pain conditions. Eur J Pain. 2018;22(2):216-41.

5. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain. 2011;152(3 Suppl):S2-15.

6. Vollert J, Magerl W, Baron R, Binder A, Enax-Krumova EK, Geisslinger G, et al. Pathophysiological mechanisms of neuropathic pain: comparison of sensory phenotypes in patients and human surrogate pain models. Pain. 2018;159(6):1090-102.

7. Ali Z, Meyer RA, Campbell JN. Secondary hyperalgesia to mechanical but not heat stimuli following a capsaicin injection in hairy skin. Pain. 1996;68(2-3):401-11.

8. Andersen OK, Felsby S, Nicolaisen L, Bjerring P, Jensen TS, Arendt-Nielsen L. The effect of Ketamine on stimulation of primary and secondary hyperalgesic areas induced by capsaicin--a double-blind, placebo-controlled, human experimental study. Pain. 1996;66(1):51-62.

9. Kilo S, Schmelz M, Koltzenburg M, Handwerker HO. Different patterns of hyperalgesia induced by experimental inflammation in human skin. Brain. 1994;117 (Pt 2):385-96.

10. Magerl W, Fuchs PN, Meyer RA, Treede RD. Roles of capsaicin-insensitive nociceptors in cutaneous pain and secondary hyperalgesia. Brain. 2001;124(Pt 9):1754-64.

11. Magerl W, Wilk SH, Treede RD. Secondary hyperalgesia and perceptual wind-up following intradermal injection of capsaicin in humans. Pain. 1998;74(2-3):257-68.

12. Rempe T, Wolff S, Riedel C, Baron R, Stroman PW, Jansen O, et al. Spinal fMRI reveals decreased descending inhibition during secondary mechanical hyperalgesia. PLoS One. 2014;9(11):e112325.

13. Werner MU, Petersen KL, Rowbotham MC, Dahl JB. Healthy volunteers can be phenotyped using cutaneous sensitization pain models. PLoS One. 2013;8(5):e62733.

14. Ziegler EA, Magerl W, Meyer RA, Treede RD. Secondary hyperalgesia to punctate mechanical stimuli. Central sensitization to A-fibre nociceptor input. Brain. 1999;122 (Pt 12):2245-57.

15. Treede RD, Magerl W. Multiple mechanisms of secondary hyperalgesia. Prog Brain Res. 2000;129:331-41.

16. Harding LM, Murphy A, Kinnman E, Baranowski AP. Characterization of secondary hyperalgesia produced by topical capsaicin jelly--a new experimental tool for pain research. Eur J Pain. 2001;5(4):363-71.

17. Rolke R, Baron R, Maier C, Tolle TR, Treede RD, Beyer A, et al. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. Pain. 2006;123(3):231-43.

18. Ellaway PH, Catley M. Reliability of the electrical perceptual threshold and Semmes-Weinstein monofilament tests of cutaneous sensibility. Spinal Cord. 2013;51(2):120-5.

19. Doll RJ, van Amerongen G, Hay JL, Groeneveld GJ, Veltink PH, Buitenweg JR. Responsiveness of electrical nociceptive detection thresholds to capsaicin (8 %)-induced

changes in nociceptive processing. Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale. 2016;234(9):2505-14.

20. Hughes SW, Zhao H, Strutton PH. Attenuation of capsaicin-induced ongoing pain and secondary hyperalgesia during exposure to an immersive virtual reality environment. Pain Reports. 2019;In Press.

21. Hughes SW, Ali M, Sharma P, Insan N, Strutton PH. Frequency-dependent top-down modulation of temporal summation by anodal transcranial direct-current stimulation of the primary motor cortex in healthy adults. Eur J Pain. 2018.

22. Handwerker HO, Kobal G. Psychophysiology of experimentally induced pain. Physiol Rev. 1993;73(3):639-71.

23. Bishop T, Ballard A, Holmes H, Young AR, McMahon SB. Ultraviolet-B induced inflammation of human skin: characterisation and comparison with traditional models of hyperalgesia. Eur J Pain. 2009;13(5):524-32.

24. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012;9(7):671-5.

25. Asghar MS, Pereira MP, Werner MU, Martensson J, Larsson HB, Dahl JB. Secondary hyperalgesia phenotypes exhibit differences in brain activation during noxious stimulation. PLoS One. 2015;10(1):e0114840.

26. Latremoliere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. J Pain. 2009;10(9):895-926.

27. Woolf CJ, King AE. Subthreshold components of the cutaneous mechanoreceptive fields of dorsal horn neurons in the rat lumbar spinal cord. J Neurophysiol. 1989;62(4):907-16.

28. Sivilotti L, Woolf CJ. The contribution of GABAA and glycine receptors to central sensitization: disinhibition and touch-evoked allodynia in the spinal cord. J Neurophysiol. 1994;72(1):169-79.

29. Lee MC, Zambreanu L, Menon DK, Tracey I. Identifying brain activity specifically related to the maintenance and perceptual consequence of central sensitization in humans. J Neurosci. 2008;28(45):11642-9.

30. Martucci KT, Mackey SC. Neuroimaging of Pain: Human Evidence and Clinical Relevance of Central Nervous System Processes and Modulation. Anesthesiology. 2018;128(6):1241-54.

31. Zambreanu L, Wise RG, Brooks JC, Iannetti GD, Tracey I. A role for the brainstem in central sensitisation in humans. Evidence from functional magnetic resonance imaging. Pain. 2005;114(3):397-407.

32. You DS, Creech SK, Meagher MW. Enhanced Area of Secondary Hyperalgesia in Women with Multiple Stressful Life Events: A Pilot Study. Pain Med. 2016;17(10):1859-64.

33. Pas R, Rheel E, Van Oosterwijck S, Leysen L, Vijver E, Nijs J, et al. Endogenous pain modulation in children with functional abdominal pain disorders. Pain. 2019.

34. Albu S, Gomez-Soriano J, Avila-Martin G, Taylor J. Deficient conditioned pain modulation after spinal cord injury correlates with clinical spontaneous pain measures. Pain. 2015;156(2):260-72.

35. Mlekusch S, Neziri AY, Limacher A, Juni P, Arendt-Nielsen L, Curatolo M. Conditioned Pain Modulation in Patients With Acute and Chronic Low Back Pain. Clin J Pain. 2016;32(2):116-21.

36. Hugosdottir R, Morch CD, Andersen OK, Arendt-Nielsen L. Investigating stimulation parameters for preferential small fiber activation using exponentially rising electrical currents. J Neurophysiol. 2019.

37. Henrich F, Magerl W, Klein T, Greffrath W, Treede RD. Capsaicin-sensitive C- and A-fibre nociceptors control long-term potentiation-like pain amplification in humans. Brain. 2015;138(Pt 9):2505-20.

38. Taguchi T, Ota H, Matsuda T, Murase S, Mizumura K. Cutaneous C-fiber nociceptor responses and nociceptive behaviors in aged Sprague-Dawley rats. Pain. 2010;151(3):771-82.

Figure 1.

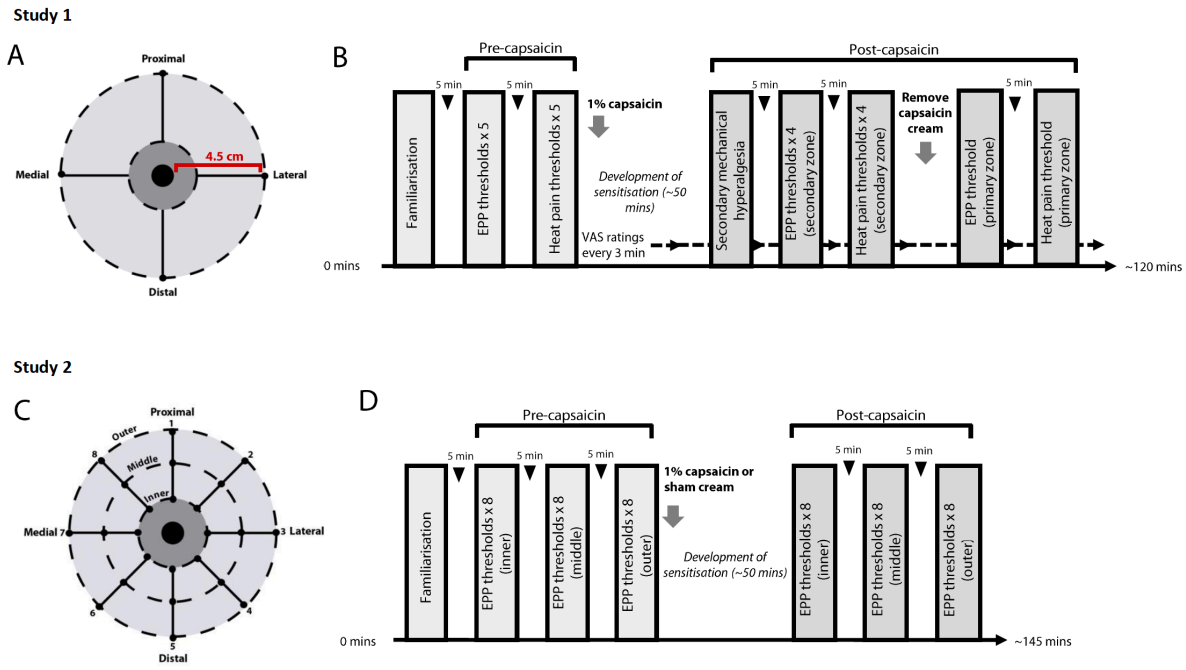
Study 1
A
Proximal
Medial
Lateral
Distal
4.5 cm
B
Pre-capsaicin
Post-capsaicin
5 min
Familiarisation
EPP thresholds x 5
Heat pain thresholds x 5
1% capsaicin
Development of sensitisation (~50 mins)
VAS ratings every 3 min
Secondary mechanical hyperalgesia
EPP thresholds x 4 (secondary zone)
Heat pain thresholds x 4 (secondary zone)
Remove capsaicin cream
EPP threshold (primary zone)
Heat pain threshold (primary zone)
0 mins
~120 mins
Study 2
C
Proximal
Outer
Middle
Inner
Medial
Lateral
Distal
D
Pre-capsaicin
Post-capsaicin
Familiarisation
EPP thresholds x 8 (inner)
EPP thresholds x 8 (middle)
EPP thresholds x 8 (outer)
1% capsaicin or sham cream
Development of sensitisation (~50 mins)
0 mins
~145 mins

Table 1.

	Area (cm^2) ± SD		EPP threshold (mA) pre-cap (range)	EPP threshold (mA) post-cap (range)	p-value
Primary zone	6.4 ± 3.4		18.3 (7.9 – 26.7)	13.9 (5.6 – 19.8)	0.0003
Secondary zone	112.6 ± 35.7	Mild Severe	17.2 (6.7 – 31.4) 18.8 (7.2 – 29.4)	16.5 (6.5 – 28.8) 14.9 (5.2 – 24.4)	0.4 0.003

Figure 2.

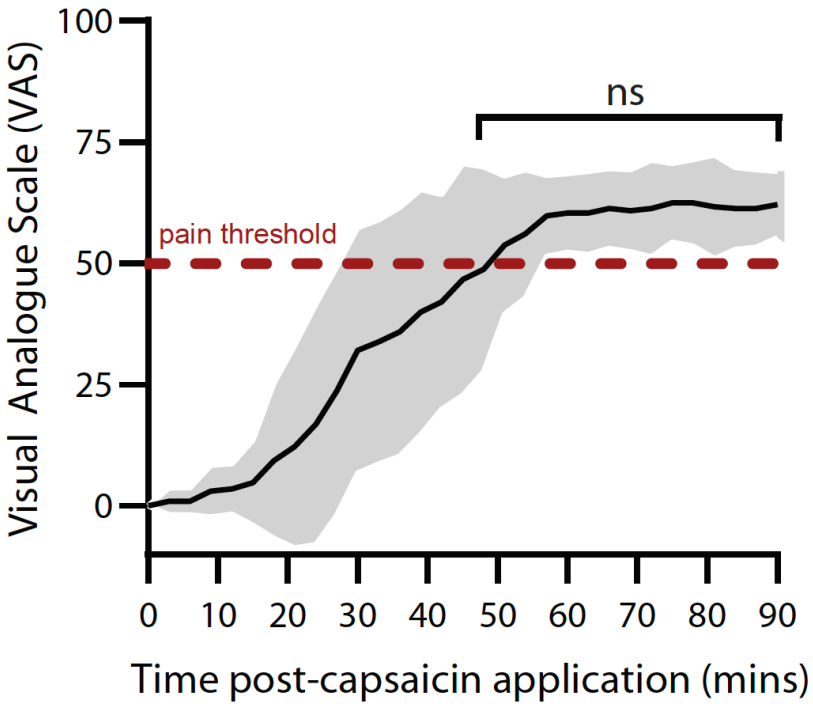
100
75
50
25
0
Visual Analogue Scale (VAS)
ns
pain threshold
0
10
20
30
40
50
60
70
80
90
Time post-capsaicin application (mins)

Figure 3.

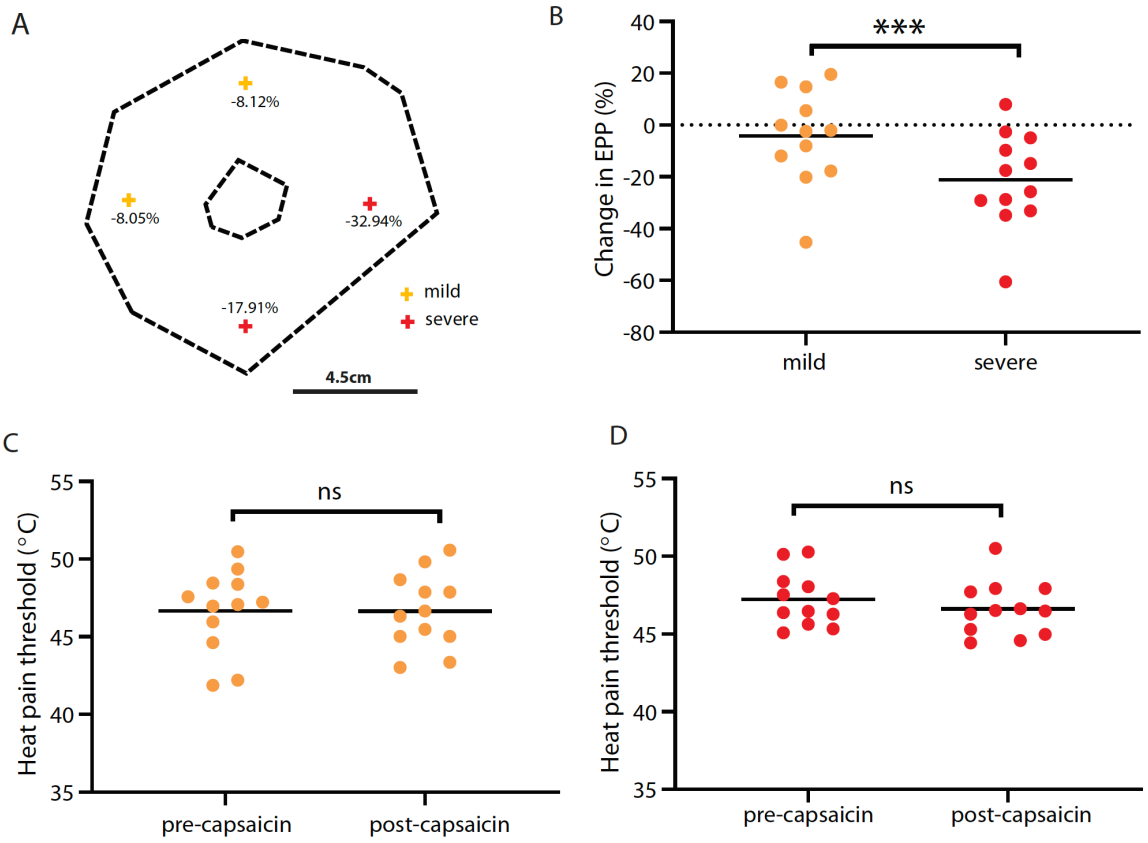
A
-8.12%
-8.05%
-32.94%
-17.91%
mild
severe
4.5cm
B
***
Change in EPP (%)
40
20
0
-20
-40
-60
-80
mild
severe
C
ns
Heat pain threshold (°C)
55
50
45
40
35
pre-capsaicin
post-capsaicin
D
ns
Heat pain threshold (°C)
55
50
45
40
35
pre-capsaicin
post-capsaicin

Figure 4.

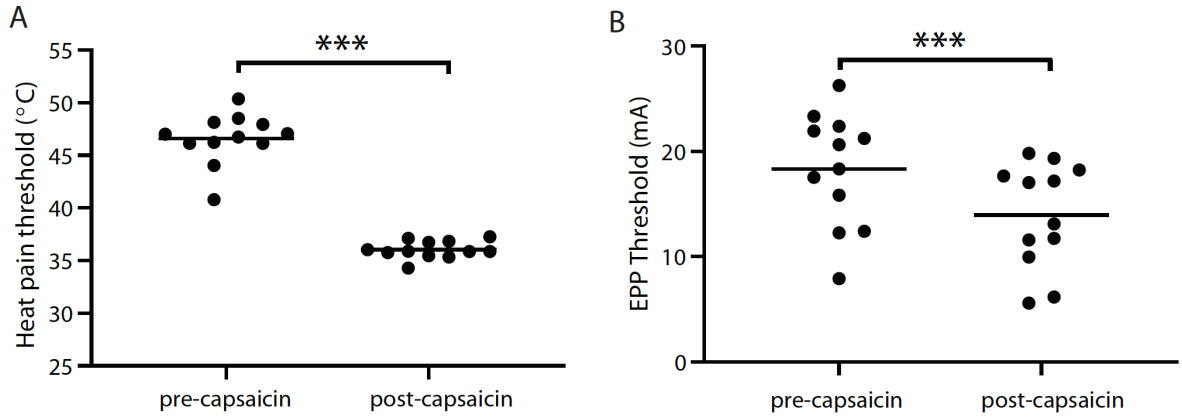
A
***
55
50
45
40
35
30
25
Heat pain threshold (°C)
pre-capsaicin
post-capsaicin
B
***
30
20
10
0
EPP Threshold (mA)
pre-capsaicin
post-capsaicin

Figure 5.