University of Cape Town

Department of Health and Rehabilitation Sciences

Division of Physiotherapy

The effect of stimulus threat on experimentally induced secondary hyperalgesia

Gillian Jennifer Bedwell

BDWGIL001

Submitted to the University of Cape Town

In fulfilment of the requirements for the degree

MSc Physiotherapy (by dissertation only)

29 March 2020

Supervisor: Dr Victoria J Madden (PhD)

Co-supervisor: Associate Professor Romy Parker (PhD)

Plagiarism declaration

I, *Gillian Bedwell,* hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature: Signed by candidate

Date: 17 August 2020

Acknowledgements

I would like to take the opportunity to thank the following people. Without them this thesis would not have been possible:

Supervisors

I don't know how to even begin to express my gratitude to my primary supervisor, Dr Tory Madden. Tory invested so much time and effort into not only developing this project, but into developing me as a researcher. I am eternally grateful for her support, empathy and fantastic mentorship. My personal and professional growth during these two years are testament to your careful and meaningful mentorship – Thank you, Tory!

Thank you to my co-supervisor, Associate Professor Romy Parker, for her guidance and support during this project. Romy always availed herself to assist with providing additional feedback throughout the development of this project. Your experience and support were instrumental to the completion of this project.

Research assistant

I would like to thank Caron Louw for being an incredibly organised, reliable and proactive research assistant. I sincerely appreciated your support and hardwork during piloting procedures and data collection. Without you, this project would not have been possible.

Department of Anaesthesia and Perioperative Medicine

Thank you to the Department of Anaesthesia and Perioperative Medicine for providing me with a workspace and full access to all the equipment needed to run my experiment. I sincerely appreciated your generosity and support.

Participants

Thank you to all the participants who gave up their time to participate in this project. Without you, this project would not have been possible.

Librarian

Thank you to Mrs Gill Morgan, an experienced librarian at the Faculty of Health Science's library, University of Cape Town. I sincerely appreciated your time, effort and patience in helping me with developing the search strategy for the systematic review, navigating databases and referencing.

Family

I want to extend a massive thank you to my extremely supportive parents, Jimmy and Jenny Bedwell for always believing in me and encouraging me to pursue my dreams.

Thank you to my amazing partner, Stewart Steenkamp, for always supporting me and helping me through stressful times. Thank you for listening to me talk about this project endlessly and for pretending to be as enthusiastic about research as I am.

Funding

I would like to thank PainSA, the South African Society of Physiotherapy, the Division of Physiotherapy, and Postgraduate Funding Office at the University of Cape Town for providing me with financial support during my studies. I am grateful for these awards that have recognised and supported me to further my interest, knowledge, and skills as a researcher in the field of chronic pain. Without your support, this project would not have been possible – Thank you.

Abstract

Background

Neuropathic pain affects 7 – 10% of people and responds poorly to pharmacotherapy. Numbers needed to treat for first line drugs range from 4 – 8. Therefore, there is an obvious need for improved understanding of the mechanisms of neuropathic pain to inform improved treatments. Mechanistic research on neuropathic pain frequently uses a human surrogate model of secondary hyperalgesia that is a common feature of neuropathic pain. The value of experimentally inducing secondary hyperalgesia is that one can then test the influence of different pharmacological and non-pharmacological interventions. This may shed some light on the physiological mechanisms within the spinal cord, which possibly also translates to the effects of the interventions on other pathways that are involved in processing signals that may be related to pain.

Additionally, pain is known to be influenced by the threat value of the situation. Many South Africans live under constant threat: less than a third of South Africans feel safe walking alone at night. This constant threat may be perpetuating the pain problem in South Arica. However, the mechanism by which threat achieves this influence on pain is unclear. This project is focused on one possible mechanistic hypothesis: that threat influences pain by affecting central physiological changes within the dorsal horn of the spinal cord. These central changes often present clinically as secondary hyperalgesia. A thorough understanding of these mechanisms will inform improved treatment strategies.

Methods

Phase one: systematic review and meta-analysis

The aim of this systematic review was to identify, describe, and compare methods that have been used to manipulate experimentally induced secondary hyperalgesia in healthy humans. A systematic search strategy (conducted on 01 October 2019) was supplemented by reference list checks and direct contact with identified laboratories to maximise the identification of data reporting the experimental manipulation of experimentally induced secondary hyperalgesia in humans. Studies were only included if they were published and in-press or accepted records for which the title, abstract, and full-text versions were available in English. Duplicate screening, risk of bias assessment, and data extraction procedures were used. Risk of bias was appraised for the following domains: selection, performance, detection, attrition, measurement, reporting, and other sources of bias. Data were extracted using a standardised data extraction form. This form was piloted and refined beforehand. Authors were asked to provide data were necessary. Data were pooled by method of

manipulation and outcome (intensity of secondary hyperalgesia, area of secondary hyperalgesia, or both).

Phase two: experimental paradigm

An experimental study was developed and conducted to investigate the effect of a stimulus threat on secondary hyperalgesia. The aim of this study was to investigate the effect of a manipulation of the threat value of a stimulus on experimentally induced secondary hyperalgesia in healthy human volunteers. All participants underwent a sham skin examination (the threat stimulus) at both the experimental and control sites. Through this sham skin examination, participants were informed that their skin integrity was fragile at the experimental site and robust at the control site. Secondary hyperalgesia was induced with high-frequency electrical stimulation at both the experimental and control site. Sensory testing was conducted at the experimental and control site, providing a within-subject comparison of the intensity and area of secondary hyperalgesia at each site. It was hypothesised that greater threat will be associated with (hypothesis 1) greater intensity and (hypothesis 2) greater surface area of induced secondary hyperalgesia.

Results

Phase one: systematic review

Twenty-one studies with non-pharmacological manipulations were included. Nine (out of 21) studies assessed intensity of secondary hyperalgesia after manipulation. Nicotine deprivation and negative expectations about the induction increased the intensity of secondary hyperalgesia. Three studies using attentional modulation and cognitive loading reported conflicting results with two studies having no effect and the other reporting a decrease in the intensity of secondary hyperalgesia. Emotional disclosure decreased the intensity of secondary hyperalgesia at four days and at one month after the manipulation. Hot/cold application, and verbal suggestion had no effect on the intensity of secondary hyperalgesia.

Seventeen (out of 21) studies assessed area of secondary hyperalgesia after manipulation. Nicotine deprivation and sleep deprivation increased the area of secondary hyperalgesia. Hyperbaric oxygen therapy, cognitive behavioural therapy, emotional disclosure, spinal manipulation, transcranial direct current stimulation, and placebo analgesia decreased the area of secondary hyperalgesia. Interestingly, the effects of emotional disclosure and hyperbaric oxygen therapy were evident one month after manipulation. Acupuncture had no significant effect on the area of secondary hyperalgesia. Four studies assessed the effect of hot/cold application. Three studies reported no effect and one study reported an increase in the area of secondary hyperalgesia after cold application.

Phase two: experimental study

The threat manipulation (sham skin examination) was not effective in eliciting increased anxiety and threat of tissue damage at the experimental site, despite a thorough piloting procedure being conducted to ensure the effectiveness of the threat manipulation. Therefore, one would anticipate that intensity and area of secondary hyperalgesia would not be predicted by site allocation. The results in this current study confirmed that both intensity and area of secondary hyperalgesia were not predicted by site allocation (i.e. which arm received the high-frequency electrical stimulation under a condition of threat).

Although a similar sham skin examination has been used effectively as a threat manipulation before, it was not effective in eliciting threat of tissue damage in the participants in this present study. There are many theorised possibilities as to why this threat manipulation was not effective: 1) Safety requirements for the study, 2) Trust, 3) Safety cues, 4) Competing threats, 5) Sampling bias, and 6) South African context.

Conclusion

Manipulations primarily influencing peripheral mechanisms (hot/cold application) have little effect on the intensity and area of secondary hyperalgesia. Although there are conflicting data, these data indicate that non-pharmacological manipulation can manipulate secondary hyperalgesia – a typically considered highly physiologically driven outcome.

The results of the experimental study are inconclusive without an effective threat manipulation. Improvements need to be made to the threat manipulation. Further research is required to investigate the association between threat and chronic pain among South Africans.

Table of Contents

Table of tables

Table of figures

Abbreviations

CBT	Cognitive behavioural therapy
CD-RISC-10	10-item Connor Davidson Resilience Scale
CI	Confidence interval
CTQ-SF	Childhood Trauma Questionnaire
HBO_2	Hyperbaric oxygen therapy
HFS	High-frequency electrical stimulation
HIV	Human Immunodeficiency Virus
IQR	Interquartile range
NRS	Number ratings scale
PCS	Pain Catastrophising Scale
SD	Standard deviation
SEM	Standard error of the mean
SH	Secondary hyperalgesia
SPARS	Sensation and Pain Ratings Scale
Spinal LTP	Spinal long-term potentiation
VAS	Visual analogue scale
WMH-CIDI	World Health Organisation Composite International Diagnostic Interview

Chapter 1: Introduction

Theoretical pain models

Pain is a complex phenomenon which has baffled philosophers, doctors, and scientists for centuries. Over the years, many theories have been proposed to explain how and why humans experience pain (Moayedi and Davis, 2013). Descartes's 1664 Model of pain implies that the severity of pain is directly proportional to the magnitude of tissue damage (Moayedi and Davis, 2013). However, there are multiple anecdotes that dispute this proposed 1:1 relationship between pain and tissue damage, for example soldiers having extensive tissue damage but no pain (Beecher, 1946). Additionally, Descartes's Model does not account for persisting pain after the tissue has healed – phantom limb pain, for example, is when people experience pain in the limb that has been amputated (Flor, 2002) with the pain often lasting years after the initial tissue damage (i.e. the amputation) has healed (Ehde et al., 2000).

Ideas on physiological processes contributing to pain (i.e. the mechanisms of pain) progressed from Descartes's Model with the publication of the Melzack and Wall (1968) Gate Control Theory. This theory introduced the role of the dorsal horn of the spinal cord acting as a relay station, facilitating or inhibiting signals between the periphery and the brain. Transmission of these 'signals' is referred to as *nociception,* which is the "neural process of encoding noxious stimuli" (Merskey and Bogduk, 1994). The Gate Control Theory was one of the first pain models to explain that pain is not directly proportional to tissue damage and can be influenced by psychological variables (Melzack, 1996). However, this model still does not account for persistent pain without tissue damage.

More recently, the Pain Neuromatrix model was proposed (Melzack, 2001). This model describes pain as being a multidimensional experience produced by specific "neurosignatures": neural activity patterns in a widely distributed area of the brain (Melzack, 2001). This model does not rely on nociceptive input from the peripheries for pain to be perceived; it accounts for pain without tissue damage. In this understanding of pain, nociception from the peripheries is only one aspect which may contribute to pain. Other psychological and physical stressors can activate the pain neuromatrix and result in pain without any peripheral input. However, the Pain Neuromatrix model has been criticised as being unfalsifiable and therefore of limited scientific utility (Griffin and Tsao, 2012). Clearly, the mechanisms underlying pain and its persistence are complex. There is a need for an improved understanding of these mechanisms to alleviate the burden of persistent pain to the individual, their community and society. The burden of persistent pain will be discussed next.

Neuropathic is common, poorly understood and poorly managed

The prevalence of neuropathic pain - "pain caused by a lesion or disease of the somatosensory nervous system" (Merskey and Bogduk, 1994) - has been estimated to be between 6.9 – 10% in the general population (Van Hecke et al., 2014). However, wide variations in samples, settings, study methodology and the quality of studies investigating the prevalence of neuropathic pain make pooling of prevalence data difficult (Van Hecke et al., 2014). It has been proposed that reported prevalence data underestimates the true burden of neuropathic pain (Van Hecke et al., 2014).

Importantly, prevalence of a disease does not provide insight into the impact of the disease on the individual, their community and society. Therefore, it is important for researchers, clinicians and health policy makers to not merely know the prevalence of neuropathic pain but to also understand the impact of neuropathic pain. Neuropathic pain substantially impacts people's quality of life, preventing participation in meaningful life roles, disturbing sleep (Gore et al., 2005) and contributing to mental health issues such as depression and anxiety (Jain et al., 2011). Furthermore, it burdens the health sector and substantially increases countries' health expenditure (Burke et al., 2017, Gierthmühlen and Baron, 2016).

Neuropathic pain is difficult to treat and often requires multiple therapeutic agents to achieve effective pain relief: only 30 – 40% of patients report satisfaction with monotherapy (Cruccu, 2007). Despite extensive research into therapeutic agents for neuropathic pain, a systematic review and meta-analysis reported that the numbers needed to treat to achieve a 50% reduction in pain severity were between 4 – 8 for first line pharmacological agents (Finnerup et al., 2015). Therefore, there is a clear need for improved understanding of the mechanisms of neuropathic pain to allow for the development of improved treatments directly targeting the pathology.

One mechanism underlying neuropathic pain may be structural and functional neuroplastic changes within the dorsal horn of the spinal cord, referred to as central sensitisation. These neuroplastic changes cause enhanced synaptic strength at the dorsal horn of the spinal cord. This enhanced synaptic strength is referred to as spinal long-term potentiation (LTP) (Ruscheweyh et al., 2011). Spinal LTP is the amplification and prolongation of an afferent signal at the dorsal horn in the spinal cord (Nickel et al., 2012). These neuroplastic changes result in increased sensitivity to painful stimuli, termed *hyperalgesia* and increased sensitivity to non-painful stimuli, termed *allodynia* (Merskey and Bogduk, 1994).

Spinal LTP, and therefore the presence of neuroplastic changes in the dorsal horn, can be assessed using three different outcomes: allodynia (increased sensitivity to a normally non-painful stimulus), primary hyperalgesia (increased pain to a normally painful stimulus within the area of tissue

damage) and secondary hyperalgesia (SH) (increased pain to a normally painful stimulus in the area surrounding the tissue damage) (Merskey and Bogduk, 1994). These three outcomes are common clinical findings in patients with chronic pain and are therefore frequently assessed in experimental pain research.

Experimental pain research

Experimental pain research is commonly used to improve researchers' understanding of pain mechanisms and to find improved treatment strategies to relieve pain suffering. In experimental pain research specifically investigating the mechanisms of neuropathic pain, neuroplastic changes are frequently experimentally induced. Specifically, a human surrogate model of SH – that is, a short-lived expression of SH that has been induced under controlled conditions in a laboratory – is commonly used in mechanistic experimental research on neuropathic pain (Klein et al., 2005).

There are many methods of experimentally inducing secondary hyperalgesia, including but not limited to burn injury (Salomons et al., 2014), high-frequency electrical stimulation (Henrich et al., 2015), low-frequency electrical stimulation (Klein et al., 2004), and intradermal and topical capsaicin (Serra et al., 2004). Furthermore, recent research has shown that SH can be manipulated (i.e. the magnitude of SH can be increased or decreased) with pharmacological modalities such as ketamine (Andersen et al., 1996), and non-pharmacological modalities such as cognitive behavioural therapy (Salomons et al., 2014). One of the aims of this thesis is to investigate the non-pharmacological methods used to manipulate experimentally induced SH (discussed in Chapter 2).

There are many benefits of conducting laboratory-based pain experiments. First, experimental pain research is 'clean' and controlled, for example sensation and pain ratings would be assessed at specific time points with specific equipment. Second, researchers can individually select and manipulate different variables such as the nature, localisation, intensity and frequency of the pain stimulus (Arendt-Nielsen et al., 2007). Third, a validated and reliable method of sensory testing called *Quantitative Sensory Testing* (Geber et al., 2011) has been developed, allowing for standardised assessment of specific outcomes, such as allodynia, and primary and secondary hyperalgesia. Standardised assessment of outcomes allows for accurate comparison across the literature base. Fourth, experimental pain research allows researchers to selectively add psychological manipulations, such as an emotional disclosure intervention (You et al., 2014) or cognitive behavioural therapy (Salomons et al., 2014), to investigate how these psychological manipulations may influence pain processing and/or pain itself. Lastly, experimental pain research with healthy human participants helps to bridge the gap between animal studies and clinical trials.

Commonly, research findings from animal studies are investigated in experimental studies on healthy humans before clinical trials are conducted (Arendt-Nielsen et al., 2007).

Although experimental pain research can be useful for researchers to gain insight into the mechanisms of pain, it has some limitations. Pain studies are subject to sampling bias: people who volunteer for pain studies may not be representative of the general population (Karos et al., 2018). A recent study compared the characteristics of volunteers for a pain-related study to those of volunteers for a non-pain related study (Karos et al., 2018). Volunteers for the pain-related study displayed higher levels of sensation seeking than volunteers for the non-pain related study. Additionally, low fear of pain and older age predicted the likelihood of someone volunteering for a pain-related study. Karos et al. (2018) proposed that individuals who display protective behaviours may be less likely to participate in pain-related studies. Another limitation of experimental pain research is that it often focuses solely on the sensory dimension of pain and naïvely disregards the affective dimension of pain. Pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Merskey and Bogduk, 1994). This definition explains that pain is not merely a biological sensory experience. Instead, pain also has an affective-motivational dimension (Price, 2000). The affective-motivational dimension encompasses the unpleasantness of pain and the motivational drive to do something about it (Talbot et al., 2019). It has been argued that assessing the severity of a pain stimulus with a numerical scale does not adequately assess the entire pain experience (Pryseley et al., 2009). A recent systematic review concluded that the sensory dimension cannot be selectively modulated by cognitive manipulations, but it may be possible to selectively modulate the affective dimension; however, evidence for this is weak (Talbot et al., 2019). Additionally, it was argued that it may be easier to selectively modulate the affective dimension in an experimental pain study than in a clinical setting. This may be due to pain being short-lived, unlikely to cause tissue damage and participants are aware that they can withdraw from the study at any time during an experimental pain study, thus decreasing the threat value of the situation (Price et al., 1987). It is important for researchers to be mindful of these limitations when designing and conducting experimental pain research.

The benefits and limitations of experimental pain research were taken into consideration when designing and conducting the experiment (discussed in Chapter 3) for this thesis. This author was interested in gaining further insights into the influence of threat on pain. Specifically, an aim for this thesis was to investigate the effects of a threat manipulation on experimentally induced secondary hyperalgesia.

Threat is associated with increased pain

Pain is known to be influenced by the threat value of the stimulus (Arntz and Claassens, 2004, Wiech et al., 2010a). In experimental research, participants report increased pain if they are informed the stimulus will be of high intensity (Moseley and Arntz, 2007). The extent to which a person feels threatened that a stimulus can cause tissue damage is subjective (Arntz and Claassens, 2004). Overall, threat is known to influence pain. What is less clear is the mechanism by which it achieves that influence. This author speculates that the mechanisms by which threat influences SH may be by descending facilitation, i.e. upregulation of nociception from the spinal cord to the brain. However, there is insufficient evidence to conclude this speculation accurate now. Threat may influence SH through a change in descending inhibition, or something entirely supratentorial.

There is well-established research investigating descending inhibition, that is cortical regions inhibiting nociception from the spinal cord, resulting in decreased pain severity (Suzuki et al., 2004). This forms a strong basis for many pharmacotherapies for pain, e.g. opioid medications. Cortical regions have also been reported to contribute to descending facilitation, i.e. upregulation of nociception from the spinal cord to the brain (Porreca et al., 2002, Urban and Gebhart, 1999, Ren and Dubner, 2002). Descending facilitation has been thought to contribute to the maintenance of SH (Urban and Gebhart, 1999) – a spinal cord driven phenomenon. Included in this thesis (in Chapter 3) is an experimental study investigating the effect of threat on experimentally induced secondary hyperalgesia. Specifically, this project aims to build on the work by Wiech et al. (2010a) by testing the effects of a threat manipulation (using a similar sham skin integrity test) on experimentally induced SH in healthy humans. A thorough understanding of how threat may influence spinal processing of nociception will allow for improved targeting of novel therapeutic interventions to decrease pain. Investigating the effect of threat has not been done within a South African context. Insight into the effects of threat on SH within a South African context is important because many South Africans live under threatening circumstances and many suffer from chronic pain. Research into the association between threat and chronic pain may help with the development of improved treatment modalities for chronic pain, specific to people living in threatening environments.

Contextual setting relevant to South Africa

The available data suggest that the prevalence of pain in the developing world is similar to that in the developed world. Data obtained from countries participating in the World Mental Health Surveys report the prevalence of chronic pain being 41.1% in developing countries and 37.3% in developed countries (Tsang et al., 2008). Importantly, the prevalence of chronic pain in South Africa was higher than the average for developing countries, with approximately half (51.8%) of the population reporting chronic pain within the 12 months prior to the survey being conducted. Furthermore,

musculoskeletal conditions resulting in pain are commonly reported at clinics in South Africa and can often be debilitating (Parker and Jelsma, 2010). Of those attending two clinics in under-resourced areas in Cape Town, South Africa, 36% were seeking care for peripheral and spinal joint pain (Parker and Jelsma, 2010). Despite the high prevalence of pain in South Africa, not enough resources are being pooled to address this major health concern (Louw et al., 2007). Instead, the focus of many researchers and healthcare professionals in South Africa is on infectious diseases due to the huge burden they place on the healthcare system (Parker and Jelsma, 2010). Importantly, prevalence data do no provide insight into the burden of the disease. For example, a disease could be highly prevalent but not be burdensome to the individual, their community and society. This is *not* the case with chronic pain. Chronic pain is burdensome in South Africa. It severely limits function and increases disability: back and neck pain is the second greatest cause of years lived with disability in Southern Sub-Saharan Africa, second only to HIV (Institute for Health Metrics and Evaluation, 2017). Therefore, it is vital that research on the topic of chronic pain is conducted to address this potential national health crisis. Research into the mechanisms of chronic pain is required to assist with the development of improved treatment modalities.

Furthermore, many South Africans live under constant threat: less than a third (31.8%) of South Africans feel safe walking alone at night (Statistics South Africa, 2019). This constant threat may be perpetuating the pain problem in South Arica. However, there is no research investigating the relationship between threat and pain in the South African setting. The magnitude of the pain problem in South Africa may be due to a limited understanding of pain mechanisms and the potential influence of threat such that optimising clinical treatment is difficult (Breivik et al., 2013, Briggs et al., 2015). A thorough understanding of these mechanisms will inform improved treatment strategies.

Terminology: neuropathic vs nociplastic

Neuroplastic changes have recently been termed 'nociplastic'. Nociplastic pain is defined as "pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain" (International Association for the Study of Pain, 2017). Prior to the development of this new terminology, the term 'neuropathic' pain was used to describe these neuroplastic changes. Neuropathic pain refers to *frank damage* to the nervous system (Merskey and Bogduk, 1994). This author argues that the 'nociplastic' rather than 'neuropathic', more accurately describes the neuroplastic changes induced in some experimental pain studies. This is because: 1) experimental pain studies that use modalities such as high-frequency electrical stimulation (a modality used in this study and outlined in Chapter 3 of this thesis) induces neuroplastic changes at the dorsal horn of the spinal cord *without* any tissue damage, and 2) often participants in experimental study are healthy and have no evidence for disease or lesion of the somatosensory system. However, 'nociplastic' has been criticised by the scientific pain community for being vague, imprecise and unhelpful for health professionals' clinical reasoning and for experimental pain research (Aydede and Shriver, 2018, Granan, 2017). A limitation with using 'nociplastic' that is specific to this thesis is that the definition outlines "no clear evidence of actual or *threatened* tissue damage". An aim of this thesis was to investigate effects of a threat manipulation on experimentally induced SH (discussed in Chapter 3). This threat manipulation was focused on inducing *threat of tissue damage*. Therefore, nociplastic is an inaccurate term for this study. Additionally, since nociplastic is a relatively new term, all published experimental studies inducing short-lived neuroplastic changes used the term 'neuropathic' when referring to pain elicited by these neuroplastic changes. Therefore, for the sake of clarity and succinctness, the term neuropathic pain, rather than nociplastic pain will be used throughout this thesis.

Overview of thesis chapters

The remainder of this thesis is presented in three chapters:

Chapter 2: Systematic review. This systematic review critically appraises the literature base investigating non-pharmacological methods used to manipulate experimentally induced secondary hyperalgesia. Originally, the protocol for this systematic review included summarising all methods – non-pharmacological *and* pharmacological – used to manipulate experimentally induced secondary hyperalgesia. However, for the scope of this thesis, only data pertaining to non-pharmacological methods used to manipulate experimentally induced SH will be reported. The reasoning behind this decision was two-fold: Firstly, a non-pharmacological manipulation (a threat stimulus) was used to manipulate experimentally induced SH in the experimental study presented in Chapter 3 of this thesis. Therefore, it was appropriate to focus the systematic review on non-pharmacological manipulations. Secondly, a very large number (n = 137) of studies using pharmacological methods to manipulate SH was identified in the review search. Two systematic reviews – one using non-pharmacological manipulations only and one using pharmacological manipulations only – will be completed and published separately. However, this thesis focuses on studies using non-pharmacological manipulations.

Chapter 3: Experimental study. Chapter 3 presents a laboratory-based experimental study investigating the effects of a threat manipulation, as a non-pharmacological method, to manipulate experimentally induced SH in healthy human volunteers.

Chapter 4: Conclusion. This chapter summarises key findings from this project and proposes ideas for further research in the field of chronic pain.

Chapter 2: Systematic literature review

Introduction

Neuropathic pain is prevalent and hinders good quality of life. The prevalence of neuropathic pain varies among different population groups: it is estimated to be between 6.9 – 10% in the general population[1] (Van Hecke et al., 2014), 38% in patients with HIV (Ellis et al., 2010) and as high as 53% in patients with spinal cord injuries (Burke et al., 2017). As discussed in Chapter 1, neuropathic pain substantially impacts people's quality of life (Gore et al., 2005, Jain et al., 2011) and burdens the health sector and increases countries' health expenditure (Burke et al., 2017, Gierthmühlen and Baron, 2016).

Neuropathic pain is often misdiagnosed (Dieleman et al., 2008), under-treated (Torrance et al., 2013) or not managed with the latest evidence-based treatments (Dworkin et al., 2012). This poor management and high prevalence of neuropathic pain has driven a 66% increase in published trials between 2005 and 2010 investigating pharmacological treatments for neuropathic pain (Finnerup et al., 2010). However, despite this increase in research, neuropathic pain still often responds poorly to pharmacotherapy (Torrance et al., 2013).

There is a growing body of research investigating non-pharmacological treatments for neuropathic pain. Non-pharmacological treatments such as cognitive behavioural therapy (Evans et al., 2003), physical exercise (Dobson et al., 2014), invasive and non-invasive cortical stimulation (Moisset and Lefaucheur, 2019), and graded motor imagery (Bowering et al., 2013) have been reported to decrease symptoms of neuropathic pain. However, research into non-pharmacological treatments is limited, and often generates conflicting data. More research is required to provide a comprehensive evaluation of different non-pharmacological treatments for neuropathic pain.

There is a clear need for improved pharmacological and non-pharmacological treatment modalities to manage neuropathic pain. But first, an improved understanding of the mechanisms of neuropathic pain is needed to inform the development of improved treatment modalities.

Secondary hyperalgesia is a common feature of neuropathic pain and can be induced experimentally in a laboratory setting. Mechanistic experimental research on neuropathic pain frequently uses human surrogate models of SH - that is, a short-lived expression of SH that has been induced under controlled conditions in a laboratory. As discussed in Chapter 1, a variety of methods have been used to experimentally induce SH in humans, including but not limited to high-frequency electrical

[1] In this systematic review, "general population" included people diagnosed with postherpetic neuralgia, Herpes Zoster Virus, painful diabetic peripheral neuropathy, trigeminal neuralgia and/or glossopharyngeal neuralgia, as well people with no medical diagnoses.

stimulation (van den Broeke and Mouraux, 2014, Pfau et al., 2011), low-frequency electrical stimulation (Torta et al., 2019), topical capsaicin application (You et al., 2016), intradermal capsaicin injection (Baron et al., 1999) and burn injury (Wahl et al., 2019). The value of experimentally inducing SH is that the effect of different pharmacological and non-pharmacological interventions can then be explored. Experimentally induced SH can be manipulated (i.e. the magnitude of the SH can be increased or decreased) with both pharmacological and non-pharmacological modalities. Pharmacological modalities such as ketamine (Andersen et al., 1996) and non-pharmacological modalities such as cognitive behavioural therapy (Salomons et al., 2014) have been reported to decrease the intensity of secondary hyperalgesia. Non-pharmacological modalities such as negative suggestion have been reported to *increase* the intensity of SH (van den Broeke et al., 2014). A thorough examination of the effects of different pharmacological and non-pharmacological modalities used to manipulate experimentally induced SH is expected to shed light on the mechanisms of neuropathic pain and help with the development of improved treatment modalities. As far as this author is aware, there are no published reports of a systematic appraisal of methods used to manipulate experimentally induced secondary hyperalgesia. The aim for this study was to systematically identify, collate, and describe all the published studies that have applied non-pharmacological[2] manipulations intended to influence experimentally induced SH in healthy human participants.

Aims

The aim of this review was to systematically identify, collate, and describe all the published studies that have applied non-pharmacological manipulations intended to influence experimentally induced SH in healthy human participants. In doing so, this author hopes to provide a resource that will summarise the literature to date, provide pooled effect size estimates where possible, and identify gaps in knowledge and opportunities for further inquiry. Therefore, the primary aim of this systematic review was to identify, describe, and compare methods that have been used to manipulate experimentally induced SH in healthy humans.

[2] Systematic review of studies that used pharmacological manipulations was out of the scope of this thesis and will be prepared for publication separately to the studies that used non-pharmacological manipulations.

Objectives

1. To identify methods used to manipulate secondary hyperalgesia;
2. Describe each method in terms of procedure, essential equipment required, pain reported by participants during induction[3] and manipulation of SH and reported harm in studies using each method;
3. Determine the effect of each manipulation on:
    a. The magnitude of static mechanical SH (i.e. intensity of SH)
    b. The surface area affected by static mechanical SH (i.e. area of SH).

Methods

A systematic review and meta-analysis were planned and conducted, following the guidelines outlined by the Cochrane Collaboration (Higgins and Green, 2011). The protocol was peer-reviewed and published in the journal *Systematic Reviews* (Madden et al., 2019c) (Appendix 1).

Eligibility criteria

Types of studies

Prospective experimental studies were included, that is, studies that attempted to manipulate SH for the purpose of studying the effects of the manipulation on experimentally induced SH, and that did so in the context of an experiment, such that the SH was not a naturally occurring clinical phenomenon. In other words, participants must have begun the study without any SH present. Studies must have assessed SH within 120 minutes after induction (so as not to miss the anticipated peak of the effect). Published and in-press or accepted records for which title, abstract, and full-text versions were available in English were eligible for inclusion.

Types of study participants

Only data from healthy human participants were included. No restrictions were placed on the age of participants and data from adults were to be treated separately from data from children (< 18 years old). However, all included studies enrolled adult participants only.

[3]Pain during induction of SH was not reported in the protocol. Thus, there was a deviation from the protocol because this objective was added after the protocol was published to provide more information on the induction procedures.

Types of interventions

Data were included from experimental studies that aimed to manipulate SH (defined as "increased pain from a stimulus that normally provokes pain" (Merskey and Bogduk, 1994) in an area adjacent to the stimulated area). Studies that manipulated SH as one step in a larger study were considered eligible, provided that suitable baseline/control data were available to allow for estimation of the effect of the manipulation on secondary hyperalgesia.

Types of outcome measures

Pain was defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Merskey and Bogduk, 1994). Pain must have been measured by subjective self-report. Therefore, studies must have assessed the subjective (participant-reported) intensity of pain or sensation to somatosensory stimulation.

Primary outcome

The primary outcome was intensity of SH. Studies must have assessed mechanical SH (specifically, participants' self-report to punctate mechanical stimulation) applied to the area surrounding the induction site. Further, in order to qualify as "hyperalgesia", the post-manipulation assessment must have been compared to a within-subject control site (e.g. opposite limb on which induction but not manipulation was performed) or time point (e.g. after induction but before manipulation or, in the case of repeated inductions, to the same induction procedure performed without manipulation) or a between-subject control (e.g. group that underwent induction without the manipulation).

Secondary outcomes

- Surface area of SH, as measured using reproducible methods (such as a radial lines approach (You et al., 2016, Henrich et al., 2015, Andersen et al., 1996).
- Time course of SH.
- Pain (except to test stimulation) reported during and after manipulation procedure.
- Pain (except to test stimulation) reported during and after induction procedure (this was not initially a secondary outcome listed in the protocol. It was added to provide more information on the induction procedures.).
- Risks of the manipulation, defined as adverse events (e.g. skin damage, other adverse reaction).

Screening

Electronic searches

The following electronic databases were searched (on 24 June 2019 and rerun on 01 October 2019) with a strategy that spans the time from their inception to the date of the search:

- Biosis (via Web of Science)
- PubMed (includes MEDLINE)
- Scopus
- PsychArticles
- PsychInfo
- Cochrane library
- Web of Science Core (use to search and then use menu on left to filter for Core option and Biosis)

Initially, ScienceDirect was include in the list of databases outlined in the protocol. However, it was subsequently omitted after discussion with an experienced librarian at the Health Sciences Library, University of Cape Town. The reason for this deviation from the protocol was because all content of ScienceDirect is included in Scopus.

The search strategy was:
((“human*” OR “women” or “woman” OR “man” OR “men” OR “participant*” OR “volunteer* OR individual*”)
OR
“normal skin” OR “healthy skin”)
AND

(“secondary hyperalgesia” OR “punctate hyperalgesia” OR “pinprick pain” OR “pinprick hyperalgesia” OR “mechanical hyperalgesia” OR “mechanical pain” OR “heat hyperalgesia” OR “neurogenic hyperalgesia”))

with all terms searched for in the title, keywords, or abstract.

+ limit to humans when possible in each database

Other sources

Reference lists of eligible studies were screened to check for other eligible studies not identified by electronic searching. Experts in the field, and the corresponding authors of the most recent narrative reviews on experimental induction and manipulation of secondary hyperalgesia, were contacted to ask for their assistance in identifying any missed studies (including Walter Magerl, Rolf Baron, Jürgen Sandkühler, Mark Wallace, Peter Drummond, Emmanuel van den Broeke). The protocol specified that unpublished data would be requested from labs that have published extensively on these techniques, including data obtained during model develop or optimisation. However, this step was deemed to fall outside the scope of the current thesis and therefore is not reported here.

Data collection and analysis

Data management

Originally, the protocol specified the use of the online Systematic Review Facility (http://syrf.org.uk/) to manage the review process. However, this platform proved unnecessarily complicated to work with: there were difficulties with viewing how many studies were included and excluded at title/abstract screening and full-text screening, and the reasons for exclusion were not clearly displayed on this platform. Therefore, the authors opted to deviate from the protocol and use the Covidence (https://covidence.org/) online software to manage the review process.

Study selection

Two reviewers (GJB and PCC)[4] independently screened each identified record for eligibility in two sequential stages, screening (Stage 1) title and abstracts and (Stage 2) full texts. Initially, three reviewers had planned to undertake the screening process independently. However, time constraints led to a deviation from the protocol and only two reviewers undertook the screening process. A customised eligibility form (Table 1) was used to record decisions in Stage 2. Any disagreements about study inclusion were resolved by discussion or by adjudication from a third, independent reviewer (VJM)[5].

[4] The two reviewers were Gillian J. Bedwell (this current author) and Prince C. Chikezie. GJB is associated with the Department of Health and Rehabilitation Sciences, University of Cape and the Pain Unit, Department of Anaesthesia and Perioperative Medicine, Neuroscience Institute, University of Cape Town, D23.30 Groote Schuur Hospital, Observatory, Cape Town 7925, South Africa Town, Cape Town, South Africa. PCC is associated with School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

[5] The third, independent reviewer was Victoria J. Madden. VJM supervised this project and is associated with the Pain Unit, Department of Anaesthesia and Perioperative Medicine, Neuroscience Institute, and the Department of Psychiatry and Mental Health, University of Cape Town, D23.30 Groote Schuur Hospital, Observatory, Cape Town 7925, South Africa Town, Cape Town, South Africa

Table 1 Inclusion/exclusion criteria and grouping table

	Inclusion	**Exclusion**	
Participants	Pain-free, healthy humans	Animals OR people with pain	
Study design	Used an experimental procedure with the aim of inducing secondary hyperalgesia AND manipulation secondary hyperalgesia (identifiable goal AND site AND induction procedure AND manipulation procedure)	Review (*set aside for cross-checking)* OR Not an experimental procedure OR No identifiable manipulation procedure	
Outcomes	Pain or sensitivity to provocation assessed subsequent to induction AND manipulation Acceptable: pain yes or no, self-report of intensity, quality, pain threshold	Subjective ratings not provided Unacceptable: facial expression, physical behaviour measurement, or psychophysiology in absence of self-report	
Include (yes/no)	Tick in *every* box above: include	Tick in *any* box above: exclude	Review (reference lists of reviews were screened for studies that may have been missed by the electronic search. Reviews were not eligible for inclusion in this systematic review.)

Risk of bias analysis

A risk of bias assessment was completed for each study by two reviewers[6] (GJB and FS), independently. A thorough risk of bias assessment is necessary to assess the quality of the methods and identify potential flaws in the data that might contribute to the results being unreliable for the purposes of answering the question of the current review (Lundh and Gøtzsche, 2008). Reviewers appraised the risk of bias for the domains of selection, performance, detection, attrition, measurement, reporting, and other sources of bias. The criteria used to rate the risk of bias were based on recommendations from the Cochrane collaboration (Higgins et al., 2011) known quality instruments (e.g. the CONSORT (Moher et al., 2010) and STROBE (Vandenbroucke et al., 2007) statements as relevant) and on known areas of bias relevant to the study design used (Sanderson et al., 2007), and were specified in the risk of bias assessment tool and guide (Appendices 2 and 3). Reviewers piloted the form on three studies and adapted it prior to formal application to all included studies. The focus of the risk of bias assessment was on the risk that the data to be extracted to answer the questions of this review could be biased. The appraisals of the two independent reviewers were compared and any disagreements resolved through discussion and consensus.

Data extraction

Two reviewers (GJB and FS) independently extracted data from each included study, using a standardised data extraction form (Appendix 4). The data extraction form was piloted and refined using three studies before formal data extraction commenced. Study authors were contacted a maximum of three times to obtain required data that were unavailable or unclear from the published texts. If no reply was received within six weeks, the data were considered unavailable. If the relevant data were not provided within six weeks of the first reply, the data were considered unavailable for this review. Any published data that seemed implausible was verified directly with the corresponding author where possible.

Data analysis

Data were analysed to 1) determine the potency of each manipulation method, 2) pool and compare data where possible and sensible, 3) facilitate relative ranking of interventions, and 4) detect publication bias. Data from studies that assessed intensity of SH were handled separately from those that assessed area of SH.

[6] The two reviewers conducting the risk of bias assessment and the data extraction were Gillian J. Bedwell (this current author) and Felicia Siboza. FS is associated with School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

Rescaling of ratings scales

A wide variety of ratings scales used to assess severity of pain are used in pain research studies. The most common ratings scales are the 0 – 10 and the 0 – 100 scales, where 0 = no pain and 10/100 = worst pain imaginable. To allow for descriptive comparison across ratings data, all pain ratings from 0 – 100 ratings scales were rescaled to 0 – 10, by dividing by 10.

Pooling of data

Data were pooled where possible and sensible, by grouping by outcome (i.e. intensity and area of SH), and subgrouping by class of manipulation. The outcome measure used was the standardised mean difference. A random effects model was used to allow for anticipated heterogeneity between studies. The RevMan software (Review Manager, 2014), version 5.3, was used to pool data and generate forest plots.

Relative ranking of manipulations

The protocol outlined that if the quantity and quality of data allowed, the pooled effect sizes (where available) would be compared to rank the different manipulations in the order of potency and risk.

Publication bias

The protocol outlined that funnel plots would be examined for publication bias.

Measures of manipulation effects

The potency of each manipulation was estimated by comparing the post-manipulation outcomes with the pre-manipulation outcomes and/or a control site/condition/group to generate a mean difference between post-manipulation (or with-manipulation) outcomes and pre-manipulation (or without-manipulation) outcomes, where possible. The standardised mean difference was used to measure manipulation effects because the standardised mean difference is recommended for continuous data where difference scales have been used. If all ratings scales were exactly the same among the included studies, the mean difference would have been more appropriate to use (Higgins and Green, 2011).

The protocol outlined subgrouping the studies into manipulations with localised effects, systemic effects and time-limited effects for determining the potency of manipulation methods. However, after careful consideration this author opted to deviate from the protocol and instead subgroup by the hypothesised direction (i.e. increase or decrease) of manipulation effect on 1) intensity and 2) area of SH. An aim for the outcome of this review is to provide readers with an improved understanding of the mechanisms of SH. Subgrouping by hypothesised direction of manipulation effect provides a more comprehensive overview of the effects of the manipulation on intensity and area of SH than the previously planned subgroups.

Assessment of the quality of body of evidence

The quality of the body of evidence for each manipulation was assessed using the GRADE criteria (Guyatt et al., 2008) and the GRADEpro GDT software (www.gradepro.org). The quality of the body of evidence was estimated for each outcome, where more than one study was available for a certain manipulation. The assessment was determined based on the following four factors:

1) Risk of bias, i.e. is there a low risk of bias associated with the study design?
2) Indirectness, i.e. does the data directly answer the research question?
3) Inconsistency, i.e. are the results consistent across all studies?
4) Imprecision, i.e. are all results calculated and reported correctly?

For each factor, studies are categorised as having *'no', 'serious' or 'very serious' limitations*. Factors graded as having *'serious'* limitations results in a downgrade of 1 level for the body of evidence. Factors graded as having *'very serious'* limitations result in a downgrade of 2 levels for the body of evidence.

Results

Results of search

An initial literature search (conducted on 24 June 2019) yielded a total of 4809 records, of which 2251 remained after duplicates were removed. Thirty-one additional studies were identified – 30 through rerunning the search (01 October 2019) and one through direct communication with experts in the field – resulting in a total of 2283 records included in title/abstract screening. The two reviewers independently identified 229 studies eligible for full text screening, with Cohen's Kappa statistic (view working out in Appendix 5) revealing moderate agreement (0.47) between the two reviewers. After screening the full texts, a total of 153 studies were identified as eligible for inclusion in this review, with Cohen's kappa coefficient (view working out in Appendix 5) revealing moderate agreement (0.59) between the two reviewers. A PRIMSA flow diagram (Fig 1) outlines the study selection process.

Due to the large number of included studies, it was decided to sub-group the studies by manipulation: 1) pharmacological manipulations of SH and 2) non-pharmacological manipulations of SH. This subgrouping had not been planned in the protocol. Acknowledging that both pharmacological and non-pharmacological manipulations influence normal physiological functioning, we used the mode of administration to define the subgroups. For a study to be classified as having used a pharmacological manipulation, participants had to have received a chemical substance via ingestion, injection or topical administration. For example, nicotine deprivation in smokers would be considered a non-pharmacological manipulation because, although nicotine deprivation would influence normal physiological functioning, it does not involve ingestion, injection or topical administration of a chemical substance. Conversely, ingestion of a liquid containing a high concentration of lipids would be classified as a pharmacological manipulation.

A large number (n = 137) of pharmacological studies were identified and included in this review. As outlined in Chapter 1, this thesis will focus on the studies that used non-pharmacological manipulations of SH only.

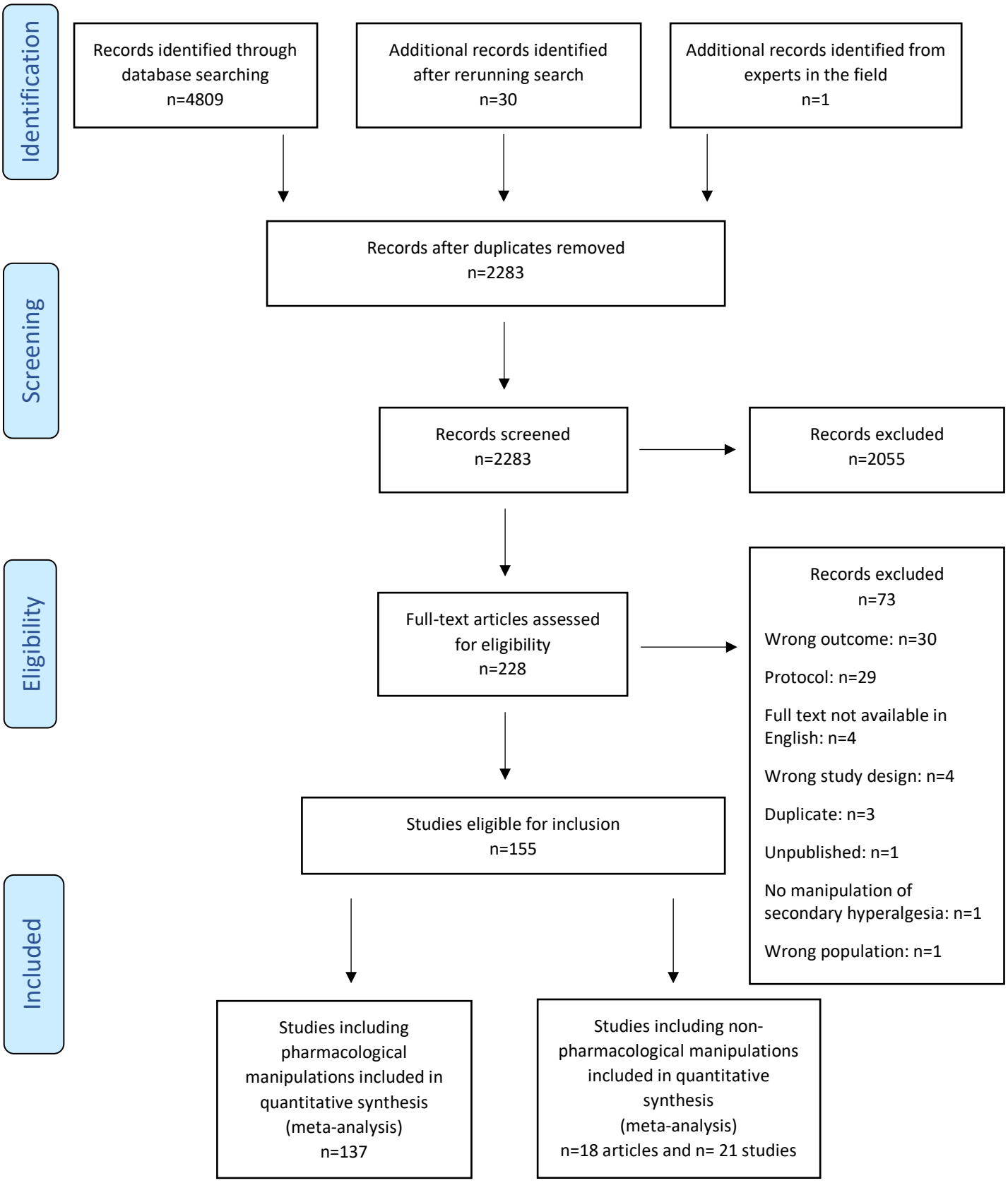


Figure 1 PRISMA flow diagram

Included studies

Types of studies

Eighteen articles reporting on non-pharmacological manipulations were eligible for this systematic review. Two articles reported on more than one study which were eligible for inclusion for this review: Torta et al. (2019) reported on three studies, of which two were eligible for inclusion in this review and Yucel et al. (2001) reported on three studies, of which all were eligible for inclusion in this review. Therefore, the total number of studies included in this review were 21. Table 3 summarises the characteristics of the studies. The study designs included between-group (n=11) and within-subject comparisons (n=9). One study (van den Broeke et al., 2014) used both between-group and within-subject comparisons. Six studies using between-group comparisons were crossover studies and one study using within-subject comparisons was a crossover study.

Participants

A total of 698 participants (396 males, 302 females) were included in the 21 eligible studies. All participants were adults (> 18 years old). Age data could not be pooled because studies used a variety of statistical summaries for reporting age. Age has been reported individually per study in Table 3. Only one study included an obviously biased sample consisting of participants with a history of trauma (You et al., 2014). However, this biased sample was purposeful of their study design.

Types of interventions

Six different methods were used to induce SH: topical capsaicin (n=7), burn injury (n=6), intradermal capsaicin injection (n=4), low-frequency electrical stimulation (n=2), high-frequency electrical stimulation (n=1) and topical high-concentration menthol (n=1). A variety of manipulations were used to influence the intensity and/or area of the experimentally induced SH: application of heat or cold (n=6); verbal suggestion, negative suggestion, and placebo analgesia (n=3); hyperbaric oxygen therapy (n=2); modulation of attention or cognitive load (n=3); nicotine deprivation (n=1); transcranial direct current stimulation (n=1); spinal manipulation (n=1); acupuncture (n=1); cognitive behavioural therapy (n=1); sleep disruption (n=1) and emotional disclosure (n=1). Table 4 provides a summary of each study's induction and manipulation methodology.

Outcome measures

Most studies (12 of 21) assessed only the surface area of SH (Matre et al., 2006, Mohammadian et al., 2004, Meeker et al., 2019, Pud et al., 2006, Rasmussen et al., 2015, Salomons et al., 2014, Wahl et al., 2019, Yucel et al., 2001, Smith et al., 2018, Rebhorn et al., 2012)[7]. Five studies assessed both the intensity and surface area of SH (Baron et al., 1999, Ditre et al., 2018, Helfert et al., 2018, Werner et al., 2002, You et al., 2014). Four studies assessed the intensity of SH only (Kóbor et al., 2009, Torta et al., 2019, van den Broeke et al., 2014)[8].

Adverse events

No adverse effects were reported in any of the studies. Meeker et al. (2019) assessed the side effects of the manipulation (transcranial direct current stimulation) using a standardised questionnaire (Fregni et al., 2008). However, the results from this questionnaire were not reported, adding to the high risk of reporting bias outlined in the risk of bias assessment section below. Rasmussen et al. (2015) and Wahl et al. (2019) reported no adverse events to both induction (burn injury) and manipulation (hyperbaric oxygen therapy). As a precaution, Smith et al. (2018) excluded participants with a history of adverse reactions to capsaicin (induction method). However, three participants withdrew from the study because the pain from topical capsaicin induction was "too intense". The rest of the included studies (17 of 21) did not assess for adverse events after induction and manipulation.

Rescaling of scales

To assess change in sensation or pain, most (n = 12 of 21) studies reported using 0 – 10 rating scales (Baron et al., 1999, Ditre et al., 2018, Kóbor et al., 2009, Mohammadian et al., 2004, Rasmussen et al., 2015, Salomons et al., 2014, Wahl et al., 2019, You et al., 2014, Yucel et al., 2001, van den Broeke et al., 2014). Six studies reported using 0 – 100 rating scales with 0 = "no pain" and 100 = a version of the phrase *worst pain imaginable* (Matre et al., 2006, Pud et al., 2006, Rebhorn et al., 2012, Torta et al., 2019, Werner et al., 2002, Meeker et al., 2019). The remaining two studies did not report which ratings scale was used in their experiment (Helfert et al., 2018) or did not assess pain severity at any point in the experiment, therefore, no ratings scale was used (Smith et al., 2018). Table 2 summarises the ratings scales used for each study, except Helfert et al. (2018) and Smith et al. (2018). To allow for comparability across ratings data, all ratings from 0 – 100 ratings scales were rescaled to 0 – 10, by dividing by a factor of 10.

Table 2 Summary of pain and sensation ratings scales used in each study

Study	Scale used	Anchors

[7] Reminder that Yucel et al., 2001 reported on three studies, all of which were included in this current review.
[8] Reminder that Torta et al., 2019 reported on three studies, of which two were included in this current review.

Baron et al. (1999)	0 - 10	Anchors not reported
Ditre et al. (2018)	0 - 10	0 = “no pain” 10 = “pain as bad as you can imagine”
Kóbor et al. (2009)	0 – 10	0 = “no pain” 10 = “highest tolerable pain”
Mohammadian et al. (2004)	0 - 10	0 = “no pain” 10 = “intolerable pain”
Rasmussen et al. (2015)	0 - 10	0 = “no pain” 10 = “worst imaginable pain”
Salomons et al. (2014)	0 - 10	0 = “no pain” 10 = “most intense pain imaginable”
Wahl et al. (2019)	0 – 10	0 = “no pain” 10 = “worst imaginable pain”
You et al. (2014)	0 – 10	0 = “no pain sensation” 10 = “most intense pain sensation imaginable”
Yucel et al. (2001) Experiment 1	0 – 10	0 = “no pain” 10 = “most intense pain imaginable”
Yucel et al. (2001) Experiment 2	0 – 10	0 = “no pain” 10 = “most intense pain imaginable”
Yucel et al. (2001) Experiment 3	0 – 10	0 = “no pain” 10 = “most intense pain imaginable”
van den Broeke et al. (2014)	0 – 10	0 = “no pain” 10 = “unbearable pain”
Matre et al. (2006)	0 - 100	0 = “no sensation” 40 = “pain threshold” 100 = “worst pain imaginable”.
Pud et al. (2006)	0 - 100	0 = “no pain at all” 100 = “the worst pain one can imagine”
Rebhorn et al. (2012)	0 - 100	Anchors not reported
Torta et al. (2019) Experiment 1	0 - 100	0 = “no sensation” 50 = “separating non-painful to painful” 100 = “the most intense pain imaginable”
Torta et al. (2019) Experiment 2	0 - 100	0 = “no sensation” 50 = “separating non-painful to painful” 100 = “the most intense pain imaginable”
Werner et al. (2002)	0 - 100	0 = “no pain” 100 = “the worst imaginable pain”
Meeker et al. (2019)	0 - 100	Anchors not reported

Table 3 Summary of included studies, grouped by outcome measurement (intensity of secondary hyperalgesia and area of secondary hyperalgesia) and sub-grouped by manipulation procedure.

Outcome measurement: intensity of secondary hyperalgesia (n=9)						
Reference	Study design	Total sample size (male; female)	Age of participants	Secondary hyperalgesia induction method	Secondary hyperalgesia manipulation method	Outcome measurement methods
Manipulation: heat or cold stimulation (n=2)						
Baron et al. (1999)	Within-subject comparison	10 (10;0)	Not reported	Intradermal capsaicin	Whole-body cooling and heating with a thermal suit.	250 mN stiff von Frey filament.
Werner et al. (2002)	Within-subject comparison	24 (24;0)	Not reported	Burn injury	Local cooling	535 mN stiff von Frey filament
Manipulation: nicotine deprivation (n=1)						
Ditre et al. (2018)	Between-group comparison	165 (94;71)	Mean ± SD 41.12 ± 12.66	Topical capsaicin	Nicotine deprivation	300 g von Frey filament
Manipulation: emotional disclosure (n=1)						
You et al. (2014)	Between-group comparison	78 (0;78)	Mean ± SD With trauma history: 18.7 (0.6) Without trauma history: 18.8 (0.8)	Topical capsaicin	Disclosure intervention	2.9 N Stiff von Frey filament
Manipulation: verbal suggestions (n=1)						
Helfert et al. (2018)	Crossover and between-group comparison	16 (16;0)	Mean (range) 24 (20 – 29)	Topical high-concentration menthol	Verbal suggestion	128 mN pinprick stimulator
Manipulation: modulation of attention or cognitive loading (n=3)						
Torta et al. (2019) Experiment 1	Within-subject comparison	19 (4:15)	Median (range) 22 (18 – 40)	Low-frequency electrical stimulation	Cognitive load using Eriksen Flanker test	128 mN pinprick stimulator
Torta et al. (2019) Experiment 2	Within-subject comparison	21 (11:10)	Median (range) 26 (19 – 36)	Low-frequency electrical stimulation	Cognitive load using modified version of an N-back task	128 mN pinprick stimulator
Kóbor et al. (2009)	Crossover and within-subject comparison	16 (11;5)	Mean (range) 22.9 (19 - 25)	Heat-capsaicin model (topical)	Attentional modulation	180 g and 300 g von Frey filament.
Manipulation: negative expectation (n=1)						
van den Broeke et al. (2014)	Within-subject and between-group comparisons	30 (11;19)	Mean (range) 23.5 (18 – 59)	High-frequency electrical stimulation	Negative expectations	256 mN pinprick stimulator

Outcome measurement: area of secondary hyperalgesia (n=17)						
Manipulation: heat or cold stimulation (n=6)						
Baron et al. (1999)	Within-subject comparison	10 (10;0)	Not reported	Intradermal capsaicin	Whole-body cooling and heating with a thermal suit.	"Stimulations were done along four linear paths arranged radially around the capsaicin-sensitised skin in steps of 5 mm at intervals of 1 sec." Sensitivity was assessed with 250 mN stiff von Frey filament.
Werner et al. (2002)	Within-subject comparison	24 (24;0)	Not reported	Burn injury	Localised application of cold	8 radial lines at 45° angles. Sensitivity was assessed with 535 mN stiff von Frey filament.
Yucel et al. (2001) Experiment 1	Within-subject	10 (7;3)	Mean ± SD (range) 25 ± 5.7 (21 – 30)	Topical capsaicin	Heat conditioning	"Starting in the periphery and along the vector towards the center of the injury". Sensitivity was assessed using 75.9 g von Frey filament
Yucel et al. (2001) Experiment 2	Within-subject	10 (8;2)	Mean ± SD (range) 25 ± 4.2 (21 – 34)	intradermal capsaicin injection	Heat conditioning	"Starting in the periphery and along the vector towards the center of the injury". Sensitivity was assessed using 75.9 g von Frey filament
Yucel et al. (2001) Experiment 3	Within-subject	10 (7;3)	Mean ± SD (range) 25 ± 3.7 (21 – 34)	Burn injury	Heat conditioning	"Starting in the periphery and along the vector towards the center of the injury". Sensitivity was assessed using 75.9 g von Frey filament
Pud et al. (2006)	Within-subject comparison	14 (9;5)	Mean (range) 34.5 (20 – 35)	Intradermal capsaicin	Localised application of cold	6 radial lines at 60° angles. Sensitivity was assessed using 60.0 g von Frey filament.
Manipulation: nicotine deprivation (n=1)						
Ditre et al. (2018)	Between-group comparison	165 (94;71)	Mean ± SD 41.12 ± 12.66	Topical capsaicin	Nicotine deprivation	8 radial lines "radiating from the centre of the application site, forming eight concentric von Frey rings". Sensitivity was assessed with 6.65 von Frey filament

Manipulation: emotional disclosure (n=1)						
You et al. (2014)	Between-group comparison	78 (0;78)	Mean ± SD With trauma history: 18.7 (0.6) Without trauma history: 18.8 (0.8)	Topical capsaicin	Emotional disclosure	8 radial lines at 45° angles. Sensitivity was assessed with 2.9 N Stiff von Frey filament.
Manipulation: verbal suggestion (n=1)						
Helfert et al. (2018)	Crossover and between-group comparison	16 (16;0)	Mean (range) 24 (20 – 29)	Topical high-concentration menthol	Verbal suggestion	8 radial lines "arranged horizontally and vertically" at the site of induction. Sensitivity was assessed with 128 mN pinprick stimulator
Manipulation: hyperbaric oxygen therapy (n=2)						
Rasmussen et al. (2015)	Crossover and between-group comparison	17 (17;0)	Mean (95% CI) 27.6 (25.1 – 30.2)	Burn injury	Hyperbaric oxygen therapy	8 radial lines at 45° angles. Sensitivity was assessed using 895 mN polyamide nonfilament
Wahl et al. (2019)	Crossover and between-group comparison	19 (19;0)	Median (95% CI) 26.1 (24.7 – 28.8)	Burn injury	Hyperbaric oxygen therapy	8 radial lines at 45° angles. Sensitivity was assessed using 512 mN pinprick stimulator.
Manipulation: placebo analgesia (n=1)						
Matre et al. (2006)	Between-group comparison	29 (17;12)	Range 20 - 45	Burn injury	Placebo analgesia	8 radial lines. Sensitivity was assessed using 84.4 g/mm^2 (pressure) von Frey filament.
Manipulation: cognitive behavioural therapy (n=1)						
Salomons et al. (2014)	Between-group comparison	34 (18;16)	Range 21 - 38	Burn injury	Cognitive behavioural therapy	8 radial lines at 45° angles. Sensitivity was assessed using 256 mN von Frey filament.
Manipulation: transcranial direct current stimulation (n=1)						
Meeker et al. (2019)	Crossover and between-group comparison	27 (16;11)	Mean (range) 25 (20 – 35)	Topical capsaicin	Transcranial direct current stimulation	8 radial lines. Sensitivity was assessed using 128 mN pinprick stimulator.
Manipulation: spinal manipulation (n=1)						
Mohammadian et al. (2004)	Crossover and between-group comparison	20 (14;6)	Mean (range) 27 (21 – 37)	Topical capsaicin	Spinal manipulation	6 radial lines at 60° angles. Sensitivity was assessed using 20.9 g von Frey filament.
Manipulation: acupuncture (n=1)						

Rebhorn et al. (2012)	Between-group comparison	50 (50; 0)	Mean (range) 24.8 (20 – 30)	Intradermal capsaicin	Acupuncture	Sensitivity was assessed using 256 mN von Frey filament. Specific methods of assessing area were not reported.
Manipulation: sleep disruption (n=1)						
Smith et al. (2018)	Crossover and between-group comparison	79 (33;46)	Mean ± SD 27.18 ± 6.98	Heat-capsaicin model (topical)	Sleep disruption	8 radial lines. Sensitivity was assessed using 5.18 (15.0 g) von Frey filament.

Table 4 Summary of induction and manipulation methods. Studies have been pooled by manipulation methods.

Reference	Induction of secondary hyperalgesia				Manipulation of secondary hyperalgesia					
	Method/modality	**Site**	**Duration**	**Dosage**	**Method/modality Experimental group**	**Duration Experimental group**	**Dosage Experimental group**	**Method/modality Control group**	**Duration Control group**	**Dosage Control group**
Manipulation: heat or cold stimulation (n=6)										
Baron et al. (1999)	Intradermal capsaicin	Volar forearm		20 µL of a solution containing 0.5% capsaicin (100µg)	Whole-body cooling and whole-body heating	Not reported	Cooling: 12°C, Heating: 50°C			
Werner et al. (2002)	Burn injury	Bilateral calf	7 minutes	47 °C	Localised application of cold	30 minutes	8 °C			
Yucel et al. (2001) Experiment 1	Topical capsaicin (and pre-heating)	Forearm	5 minutes pre-heating and 30 minutes topical capsaicin	1.5 g of 1% capsaicin cream	Heat conditioning	2 minutes x 2	1st conditioning: 39.2 ± 1.3 °C 2nd conditioning: 39.9 ± 2.8 °C			
Yucel et al. (2001) Experiment 2	Intradermal capsaicin (and pre-heating)	Forearm		50 µg in a volume of 0.2 ml	Heat conditioning	2 minutes x 2	1st conditioning: 40.9 ± 2.3 °C 2nd conditioning: 41.8 ± 2.9 °C			
Yucel et al. (2001) Experiment3	Burn injury (and pre-heating)	Forearm	7 minutes	47 °C	Heat conditioning	2 minutes x 2	1st conditioning: 40.1 ± 2.8 °C 2nd conditioning: 41.9 ± 1.8 °C.			
Pud et al. (2006)	Intradermal capsaicin	Right volar forearm		50 µg/50µl capsaicin	Localised application of cold	Before and 8 minutes after induction	Starting at 30 °C decreasing temperature by 2 °C/second			
Manipulation: nicotine deprivation (n=1)										
Ditre et al. (2018)	Topical capsaicin	Non-dominant volar forearm	15 – 20 minutes	10% capsaicin solution	Nicotine deprivation	12 – 24 hours prior to experiment		Continued smoking		
Manipulation: emotional disclosure (n=1)										
You et al. (2014)	Topical capsaicin	Dominate volar forearm	30 minutes	6% capsaicin solution (3g in 50ml of 50% ethanol)	Emotional disclosure	20 minutes	"Were asked to write about the most traumatic	Sham	20 minutes	"Were asked to write about how they manage their time"

							experience of their lives"			
Manipulation: verbal suggestions (n=1)										
Helfert et al. (2018)	Topical high-concentration menthol	Volar forearm	20 minutes	1 mL aliquot of a solution containing 400 mg of L-menthol (40%) dissolved in 90% ethanol	Verbal suggestion		Correct and incorrect information about the contents of the substance used for induction			
Manipulation: attentional modulation and cognitive loading (n=2)										
Torta et al. (2019) Experiment 1	Low-frequency electrical stimulation	Volar forearm	2 minutes	2 Hz, pulse width 2 ms, intensity 15x detection threshold	Eriksen Flanker Task	The task started 90 seconds before LFS and continued for approximately 90 seconds after LFS				
Torta et al. (2019) Experiment 2	Low-frequency electrical stimulation	Volar forearm	2 minutes	2 Hz, pulse width 2 ms, intensity 15x detection threshold	Modified version of an N-back task	The task started 90 seconds before LFS and continued for approximately 90 seconds after LFS				
Kóbor et al. (2009)	Topical capsaicin (and pre-heating)	Medial side right lower leg	45 minutes	45° C. 0.075% capsaicin cream	High attentional load			Low attentional load		
Manipulation: negative expectation (n=1)										
van den Broeke et al. (2014)	High-frequency electrical stimulation	Volar forearm	5 x 1 second trains with 10 seconds intervals	100Hz, pulse width 2 ms, intensity 20x detection threshold	Negative expectation		"After the HFS stimulation, your skin will become more sensitive to the pinprick stimulation'. The words 'more sensitive' were marked bold."			

Manipulation: hyperbaric oxygen therapy (n=2)										
Rasmussen et al. (2015)	Burn injury	Non dominant calf	7 minutes	47° C	Hyperbaric oxygen procedure	90 minutes with ±5 minutes for compression and decompression	2.4 ATM, breathing 100% oxygen	Control conditions		ambient pressure, FIO2 = 0.21
Wahl et al. (2019)	Burn injury	Calf	7 minutes	47° C	Hyperbaric oxygen procedure	90 minutes with ±5 minutes for compression and decompression	2.4 ATM, breathing 100% oxygen	Control conditions		1ATA, FIO2 = 0.21
Manipulation: placebo analgesia (n=1)										
Matre et al. (2006)	Burn injury	Medial volar arms	5 minutes	46° C	Placebo analgesia		Participants were informed that a magnet had analgesic properties.			Participants were not informed about the magnet. They were told that the aim of the study was to investigate the hypersensitivity of the skin to painful stimuli.
Manipulation: cognitive behavioural therapy (n=1)										
Salomons et al. (2014)	Burn injury	Left volar forearm	28 minutes	Individualised intensity: temperature started at heat pain threshold +1°C and slowly increased by 0.5°C until "a tolerable temperature <50°C and rated ≤ 6/10 NRS on 6 consecutive trails was found".	Cognitive behavioural therapy	5 minutes prior to intervention	"They were taught about the relationship between sensory, cognitive and emotional responses to pain and were trained to reduce their stress response to the painful stimuli by identifying negative cognitions that arose and reappraising their situation to	Sham		"Trained in interpersonal effectiveness after the pain stimuli. This training focused on managing the demands of others by effectively balancing goals and expectations and communicating assertively but respectfully with others."

							focus on potential benefits of the training (e.g. ability to cope with future pain stimuli, financial compensation). They were encouraged to use their training to cope with the painful experimental stimuli."			
Manipulation: transcranial direct current stimulation (n=1)										
Meeker et al. (2019)	Topical capsaicin (and pre-heating)	Lower left foreleg	35 minutes capsaicin cream	35°C. 1 g of 10% capsaicin cream	Transcranial direct current stimulation	20 minutes	1 mA	Sham transcranial direct current stimulation	20 minutes	"" Ramped current up for 30 s and then down for 30 sec and repeated for 20 min duration"
Manipulation: spinal manipulation (n=1)										
Mohammadian et al. (2004)	Topical capsaicin	Forearm	20 minutes	1% capsaicin, 1.5g applied to skin	Spinal manipulation treatment	15 minutes	"All chiropractic treatments were performed by the same chiropractor. SMT consisted of a short-lever, prestressed, high-velocity, low-amplitude sustained thrust and was applied at areas of vertebral subluxation in the thoracic spine."	Non-spinal manipulation treatment	15 minutes	"The non-spinal treatment involved the same interaction between the chiropractor and the subjects as in the SMT treatment. It reproduced the manual contact and setting procedure used in the treatment but without the actual adjustment"

Manipulation: acupuncture (n=1)										
Rebhorn et al. (2012)	Intradermal capsaicin	Dominate volar forearm	Injection administrated 30 minutes after cold-pressor test	25 lg dissolved in 50 lL ethanol 80%	Traditional Chinese Medicine acupuncture	20 minutes + 80 minutes during procedure	Sterile 0.30 x 30 mm needles. 8 positions in legs, arms and neck.	Sham acupuncture	20 minutes + 80 minutes during procedure	“Fitted with a blunt tip, Streitberger placebo needles did not penetrate skin but induced a pricking sensation. By moving inside the handle, the needles shortened and thus simulated penetration when being pressed against the skin"
Manipulation: sleep disruption (n=1)										
Smith et al. (2018)	Topical capsaicin (and pre-heating)	Upper or lower ventral forearm	5 minutes heat. 30 minutes capsaicin	45°C. 0.35 - 0.40g capsaicin cream (0.1% capsaicin)	Forced awakenings	Two consecutive nights maximum total sleep possible 280 minutes		Uninterrupted sleep	Maximum total sleep possible 480 minutes	

Risk of bias in included studies

Table 5 summarises the risk of bias results. Most of the studies (11 of 21) were judged to have a high risk of bias overall; only one study (You et al., 2014) was judged to have a low risk of bias overall. The remaining nine studies were judged to have unclear risk of bias overall.

Table 5 Summary of risk of bias assessment.

	Selection bias	Performance bias	Detection bias	Risk of manipulation veracity problem	Attrition bias	Measurement bias SH	Measurement bias SA	Reporting bias	Overall risk of bias
Baron et al. (1999)									
Ditre et al. (2018)									
Helfert et al. (2018)									
Kóbor et al. (2009)							N/A		
Matre et al. (2006)						N/A			
Meeker et al. (2019)						N/A			
Mohammadian et al. (2004)						N/A			
Pud et al. (2006)						N/A			
Rasmussen et al. (2015)						N/A			
Rebhorn et al. (2012)						N/A			
Salomons et al. (2014)						N/A			
Smith et al. (2018)						N/A			
Torta et al. (2019) Experiment 1							N/A		
Torta et al. (2019) Experiment 2							N/A		
van den Broeke et al. (2014)							N/A		
Wahl et al. (2019)						N/A			
Werner et al. (2002)									
You et al. (2014)									
Yucel et al. (2001) Experiment 1						N/A			
Yucel et al. (2001) Experiment 2						N/A			
Yucel et al. (2001) Experiment 3						N/A			

Green = low risk of bias, red = high risk of bias, and orange = unclear risk of bias. SH = secondary hyperalgesia. SA = area of secondary hyperalgesia

Selection bias

Six studies (van den Broeke et al., 2014, Torta et al., 2019, Smith et al., 2018, Helfert et al., 2018, Rebhorn et al., 2012) were judged to be at low risk of selection bias. Most studies failed to screen participants for chronic and current pain and/or did not clearly explain their randomisation procedure. Importantly, Matre et al. (2006) and Meeker et al. (2019) reported excluding participants who did not develop SH after induction. Meeker et al. (2019) also excluded participants with large variances - "more than 25% increase or decrease in pinprick hyperalgesic area" - in hyperalgesia expression between testing sessions. For these reasons, both studies were judged to have a high risk of selection bias.

Performance bias

No studies were judged to be at low risk of performance bias. Most of the studies (17 of 21) failed to assess the effectiveness of their blinding procedure. Therefore, it was unclear whether there was performance bias or not. The rest of the studies (n=4) were judged to be at high risk of performance bias for reporting that participants were not blinded to group allocation and the research question. It was reported that blinding of participants to group allocation was not possible with studies using hyperbaric oxygen therapy for their manipulation (Rasmussen et al., 2015, Wahl et al., 2019), therefore high risk of performance bias was inevitable in these studies.

Detection bias

No studies were judged to be at low risk of detection bias. Most studies (17 of 21) did not clearly assess whether outcome assessors were blinded to the research question and whether the analysing researchers were blinded to group/site allocation of participants. Four studies (Werner et al., 2002, Rasmussen et al., 2015, Meeker et al., 2019, Matre et al., 2006) were judged to be at high risk of detection bias.

Risk of manipulation veracity problem

Thirteen of the 21 included studies were judged to be at low risk of having problems with the veracity of the manipulation. Five studies were judged to have unclear risk of having problems with the veracity of the manipulation due to ambiguous information given about the manipulation procedure. The remaining studies (n=3) were judged to have high risk of having problems with the veracity of the manipulation due to missing information about the manipulation procedure. van den Broeke et al. (2014) called their manipulation "negative expectation". However, they did not specifically assess participants' expectations and report whether their manipulations indeed did induce negative expectations. Therefore, in this current study, this manipulation was renamed "negative suggestion".

Attrition bias

All 21 studies were judged to be at low risk of attrition bias for either having no withdrawals, or clearly accounting for withdrawals in their statistical analysis.

Measurement bias

Risk of measurement bias was assessed separately for intensity and area of SH. Twenty of the 21 studies used valid and reliable outcome measurements to assess intensity and area of SH. Smith et al. (2018) used a 5.18 (15.0g) von Frey filament to assess intensity of hyperalgesia but reported that the filament was perceived as a "non-painful…light touch". This does not correspond to the definition for hyperalgesia used in this review: "increased pain from a stimulus that normally provokes pain" (Merskey and Bogduk, 1994). Therefore, the study by Smith et al. (2018) was judged to have an unclear risk of measurement bias.

Most studies (18 of 21) did not clarify whether the same assessor conducted all the assessments between group/site/time points. Only three studies (Matre et al., 2006, Meeker et al., 2019, Rasmussen et al., 2015) reported that the same assessor conducted all assessments, and were therefore judged to be at low risk of measurement bias. All studies used valid and reliable outcome measures.

Reporting bias

Ten studies (of 21) were judged to be at high risk of reporting bias, for either failing to report on all outcome measurements (n=8) or failing to disclose any funding sources or conflicts of interest (n=2) (Torta et al., 2019).

Primary outcome

The effect of manipulation on the intensity of secondary hyperalgesia (n=9)

Nine (of 21) studies assessed the intensity of SH after manipulation. Studies have been grouped by the hypothesised direction of the effect of the manipulation on the intensity of SH (i.e. either increase or decrease in the intensity of SH). Thereafter, studies were subgrouped by the class of manipulation procedure, where possible. Those that could not be subgrouped have been reported on individually. Table 6 summarises the hypothesised and actual directions of the effect of each study's manipulation on the intensity of SH.

Table 6 A summary of the hypothesised and actual directions of the effect of each study's manipulation on intensity of secondary hyperalgesia.

Study	Manipulation	Hypothesised direction of the effect of manipulation on the intensity of secondary hyperalgesia	Actual direction of the effect of manipulation on the intensity of secondary hyperalgesia
Baron et al. (1999)	Whole-body cooling and whole-body heating	Decrease*	No effect for both whole-body heat and whole-body cooling
Werner et al. (2002)	Localised cold application	Decrease*	No effect
Kóbor et al. (2009)	High attentional loading	Decrease*	No effect
Torta et al. (2019) Experiment 1	High cognitive loading (Eriksen Flanker task)	Decrease	No effect
Torta et al. (2019) Experiment 2	High cognitive loading (modified N-back task)	Decrease	Decrease
You et al. (2014)	Emotional disclosure	Decrease*	Decrease
Ditre et al. (2018)	Nicotine deprivation	Increase	Increase
Helfert et al. (2018)	Verbal suggestion	Increase*	No effect
van den Broeke et al. (2014)	Negative suggestion	Increase	Increase

*Not all studies reported a clear hypothesised direction of effect. In these cases, this author hypothesised the direction of effect based on published literature. Studies that failed to report a clear hypothesised direction of the effect and where this author hypothesised the direction of the effect have been denoted with an asterisk.

Baseline data

None of the studies that assessed the intensity of SH reported baseline data from the time prior to manipulation.

Manipulations hypothesised to decrease the intensity of secondary hyperalgesia (n=6)

Six studies (of 9) used manipulations hypothesised to decrease the intensity of SH. Interestingly, only one of the six studies successfully decreased the intensity of SH, with high cognitive loading using a modified version of the N-back Task (Torta et al., 2019) (Experiment 2). Whole-body cooling and heating (Baron et al., 1999), localised cold application (Werner et al., 2002), high attentional loading (Kóbor et al., 2009), high cognitive loading using the Eriksen Flanker task (Torta et al., 2019) (Experiment 1), and emotional disclosure (You et al., 2014) failed to decrease the intensity of SH.

Effect of heat or cold stimulation on the intensity of secondary hyperalgesia (n=2)

Two studies used a version of heat or cold stimulation to manipulate the intensity of SH (Baron et al., 1999, Werner et al., 2002). Baron et al. (1999) used an intradermal capsaicin injection induction

while exposing participants to a thermal suit to induce whole-body cooling and whole-body heating and reported the intensity of SH 11 minutes after the induction. The temperature of the skin at the induction and measurement site was kept at a constant 35°C. Whole-body cooling was intended to stimulate sympathetic vasoconstrictor activity (high sympathetic activity) and whole-body heating was intended to stimulate sympathetic vasodilator activity (low sympathetic activity). The extent of vasoconstriction and vasodilation was determined by measuring skin blood flow using laser Doppler and temperature using infrared thermometry at the index finger. It is unclear whether there was a statistically significant difference in perfusion and temperature measurements between whole-body cooling and whole-body heating. Mean (SEM) intensity ratings (on 0 – 10 ratings scale) stimulated by 250 mN stiff von Frey filament were 3.2 (0.5) during whole-body cooling (high sympathetic activity) and 3.4 (0.7) during whole-body heating (low sympathetic activity). These data suggest that high and low sympathetic activity did not influence the intensity of SH induced by an intradermal capsaicin injection.

Werner et al. (2002) used a burn injury induction using a hot contact thermode, and then applied contact cold stimulation of 8 °C (experimental) or non-active sham (control) for 30 minutes from eight minutes after induction. The median (IQR) (data extracted from plot) intensity ratings (reported on 0 – 100 ratings scale, rescaled to 0 – 10) were provided at 10, 40, 80, 120 and 160 minutes after the manipulation for each group (view Appendix 6, Section 6.1 for intensity ratings at each time point for each group). For the current review, mean intensity ratings were calculated to facilitate comparison with intensity ratings from Baron et al. (1999). Overall, the mean intensity rating were lower than intensity ratings from Baron et al. (1999): 1.2 at the experimental site and 1.16 at the control site. This may indicate a difference in the potency of these two induction procedures (intradermal capsaicin vs a burn injury). Localised application of cold did not have a statistically significant effect on reducing SH ($p > 0.4$. The specific p-value was no reported.) in comparison to the control site. These data suggest that application of cold may not influence the intensity of SH induced by burn injury.

Effect of modulation of attention or cognitive load on the intensity of secondary hyperalgesia (n=3)

Kóbor et al. (2009) used a heat and topical capsaicin induction and then exposed participants to either high attentional load tasks (experimental manipulation) or low attentional load tasks (control manipulation). Forty-five minutes after the induction, static mechanical stimulation was provided using 180 g and 300 g von Frey filaments and intensity ratings (on 0 – 10 ratings scale) were recorded during experimental and control manipulations. Intensity ratings were reported as mean (SEM) (data extracted from a bar graph), but after being normalised to a range of 0 – 1. Original pain ratings from the 0 – 10 scale were not reported. These data were requested from the corresponding author, but the author did not respond. Therefore, these data were considered unavailable. Intensity ratings were reported on a bar plot only with the y-axis of the plot ranging from 0 – 0.5, which contradicts the explanation of intensity ratings being normalised to a range of 0 – 1. This contributed to high risk of reporting bias, discussed above. The author of this current review requested clarification on the discrepancy in the plot's range and the normalised intensity ratings range, but the corresponding author did not respond. Kóbor et al. (2009) reported that attentional loading did not influence intensity of secondary hyperalgesia, but this could not be verified without the raw data.

Torta et al. (2019) (Experiment 1 and 2) assessed the effect of cognitive loading on the intensity of SH. In Experiment 1 participants engaged in the well-validated Eriksen Flanker task (Eriksen and Eriksen, 1974), which is known to require a lot of focus. In Experiment 2 a different cohort engaged in a modified version of the N-back task, which is known to require a lot of attention and working memory (Kane et al., 2007). Both tasks were performed while low-frequency electrical stimulation was simultaneously used to induce SH on one of the participants' arms (experimental site). SH was not induced on the other arm (control site), providing a within-subject comparison. Participants reported the extent of engagement required for each task on a 0 – 10 scale, where 0 = "not difficult/engaging at all" and 10 = "as difficult/engaging as possible". The mean (SD) extent of engagement was 4.28 ± 2.24 for the Eriksen Flanker Task and 6.76 ± 1.37 for the N-back task. This indicates that the Modified version of an N-back was a harder task than the Eriksen Flanker task, i.e. induced higher cognitive loading.

In Experiment 1, the mean intensity (reported on 0 – 100 ratings scale, rescaled to 0 - 10) was reported to be 2.5 at the experimental site and 2.0 at the control site 20 minutes after induction. At 45 minutes after the induction, the intensity ratings were 2.2 at the experimental site and 1.8 at the control site (data extracted from a plot). There was a statically significant increase ($p < 0.001$. The exact p-value was not reported) in intensity ratings over time at the experimental site. Conversely, there was not a statically significant ($p = 0.326$) increase in ratings over time at the control site. These data indicate that the manipulation (Eriksen Flanker task) was ineffective in preventing the development of SH.

In Experiment 2, the mean intensity (reported on 0 – 100 ratings scale, rescaled to 0 – 10) was reported to be 3.0 at the experimental site and 2.0 at the control site 20 minutes after induction. At 45 minutes after the induction, the intensity ratings were 2.5 at the experimental site and 1.9 at the control site (data extracted from a plot). There was not a statically significant ($p = 0.108$) increase in ratings over time at the experimental site. Therefore, this higher cognitive loading task (modified version of an N-back) was effective (at a group level) in preventing the development of hyperalgesia induced by low-frequency electrical stimulation.

The effect of emotional disclosure on the intensity of secondary hyperalgesia (n=1)

You et al. (2014) used a topical capsaicin induction four days and one month after exposing participants to once-off emotional disclosure (experimental manipulation group) or sham intervention (control manipulation group). Experimental and control groups both consisted of a cohort of women with and without a history of trauma. Four days after the manipulations, the mean (SEM) intensity ratings (reported on 0 – 10 ratings scale. Data extracted from a bar graph) were 2.7 (2.3 – 2.9) for participants in the experimental group with a history of trauma and 1.3 (0.8 – 2.8) for participants in the experimental group without a history of trauma. The mean (SEM) intensity ratings were 2.1 (1.5 – 2.5) for participants in the control group with a history of trauma and 2.2 (1.9 – 2.4) for participants in the control group without a history of trauma. The difference in intensity ratings between the experimental and control group was statistically significant ($p <0.050$. The exact p-value was not reported), suggesting that the emotional disclosure was effective in reducing the intensity of SH at four days. In the experimental group, there was also a statistically significant difference ($p < 0.025$. The exact p-value was not reported) in the intensity of SH between those with a history of trauma and those without a history of trauma, indicating that emotional disclosure was more effective in reducing the intensity of SH in participants with a history of trauma at four days.

Intensity ratings were repeated at one month after the manipulation. Intensity ratings were 1 (0.7 – 1.3) for participants in the experimental group with a history of trauma and 2 (1.6 – 2.3) for participants in the experimental group without a history of trauma. Intensity ratings were 1.7 (1.2 – 2.1) for participants in the control group with a history of trauma and 1.2 (0.8 – 1.4) for participants in the control group without a history of trauma. There difference between the experimental and control group was statistically significant ($p < 0.025$. The exact p-value was not reported), suggesting that the emotional disclosure was effective in reducing the intensity of SH at one month. In the experimental group, there was also a statistically significant difference ($p < 0.050$. The exact p-value was not reported) in the intensity of SH between those with a history of trauma and those without a history of trauma, indicating that emotional disclosure was more effective in reducing the intensity of SH in participants with a history of trauma at one month.

Manipulations hypothesised to increase the intensity of secondary hyperalgesia (n=3)

Three studies used manipulations hypothesised to increase the intensity of SH. Two studies successfully increased the intensity of SH with nicotine deprivation (Ditre et al., 2018) and negative suggestion about the induction procedure (van den Broeke et al., 2014). Verbal suggestion (Helfert et al., 2018) failed to increase the intensity of SH.

The effect of nicotine deprivation on the intensity of secondary hyperalgesia (n=1)

Ditre et al. (2018) used a topical capsaicin induction in a cohort of smokers after exposing them to 12 – 24 hours of nicotine deprivation (experimental group) and compared it participants allowed to continue smoking (control group). They used an unusual approach to assess the intensity of secondary hyperalgesia: Pinprick stimuli, using a 6.65 con Frey filament, were “applied at points one centimetre apart alone eight linear paths radiating from the centre of the application site, forming eight concentric rings”. At each ring, the pinprick stimulus was applied eight times. The sum of the eight pinprick stimuli (rated on a 0 – 10 ratings scale) was calculated for the intensity of SH for each participant. Therefore, the intensity ratings ranged from 0 – 80 at each ring. The mean (SD) were reported for the summed intensity ratings at eight concentric rings one centimetre apart, starting at the centre of the induction site (view Appendix, Section 6.2 for summed intensity ratings at each ring for each group). The induction site was 1.5 centimetres by 1.5 centimetres. Therefore, the ratings at the innermost ring represents primary hyperalgesia and ring 2 – 8, at a diameter of two to eight centimetres around the centre of the application site represents secondary hyperalgesia. This distinction between primary and SH was not reported by the researchers; it is this author’s interpretation of the data. The overall mean (SD) intensity was 17, 05 (11.0) for the nicotine-deprived group and 4.04 (4.12) for the continued smoking group. Ditre et al. (2018) reported a statistically significant ($p < 0.05$. The exact p value was not reported.) difference in SH intensity

ratings at each concentric ring between nicotine-deprived group and continued smoking group. These data suggest that nicotine deprivation is effective in manipulating the intensity of SH induced by topical capsaicin application.

The effect of verbal suggestion and negative suggestion on the intensity of secondary hyperalgesia (n=2)

Helfert et al. (2018) used a high-concentration topical menthol induction while exposing participants to verbal suggestion about the induction procedure. Participants underwent four testing sessions where they received high-topical menthol application twice and topical ethanol application twice, in a randomised order. Participants were blinded to group allocation and therefore were unaware of specifically which substance was being applied. The verbal suggestion manipulation consisted of participants being told correct information for two of the four sessions and incorrect information for the other two sessions about which substance was being applied. Specifically, there were four testing conditions: 1) participants were told that menthol was being applied and menthol was actually applied (told correct information), 2) participants were told that menthol was being applied when in fact ethanol was being applied (told incorrect information), 3) participants were told that ethanol was being applied and ethanol was actually applied (told correct information), and 4) participants were told that ethanol was being applied when in fact menthol was being applied (told incorrect information). No individual nor group results were reported, despite the aim of the study being to investigate the effect of verbal suggestion on experimentally induced SH. These data were requested from the corresponding author; however, the author did not respond. These missing data added to the high risk of reporting bias outlined in the risk of bias assessment section above. Although it was reported that there was no significant change in the intensity of SH after manipulation, this can not be verified without access to the missing data.

van den Broeke et al. (2014) used a high-frequency electrical stimulation induction after exposing participants to a negative suggestion about the induction procedure (experimental) or control conditions. Participants were randomly assigned to either the negative suggestion or control group. The negative suggestion was achieved by informing participants that: “After the HFS stimulation, your skin will become **more sensitive** to the pinprick stimulation*”.* Mean intensity ratings (pain ratings were measured on a 0 cm – 10 cm scale but were then reported on a 0 mm – 100 mm scale. Therefore, in this current review, ratings were rescaled to 0 – 10 ratings scale, to allow for comparability) were recorded at 10 minutes and 20 minutes after induction for both the negative suggestion and control groups at both the conditioned site (receiving the induction). At 10 minutes after the induction, the mean intensity ratings were 3.81 for the experimental group and 2.62 for the control group. At 20 minutes after the induction, the mean intensity ratings were 3.82 for the

experimental group and 2.31 for the control group. These data indicate that negative suggestion about the HFS induction procedure was effective in increasing the intensity of SH.

Pooling of data

Two subgroups of classes of manipulation were identified and explored for pooling: 1) attentional and cognitive loading, and 2) verbal suggestion and negative suggestion. Three studies were included in Subgroup 1 (Kóbor et al., 2009, Torta et al., 2019) (Experiment 1 and 2) and two studies were included in Subgroups 2 (Helfert et al., 2018, van den Broeke et al., 2014). No other studies could be pooled by class of manipulation. However, pooling of data was not possible for both subgroups due to the unavailable intensity ratings data in Kóbor et al. (2009) (Subgroup 1) and Helfert et al. (2018) (Subgroup 2).

Relative ranking of manipulations

It was not possible to pool many studies that investigated the effect of manipulations on intensity of secondary hyperalgesia, owing to the high clinical heterogeneity among the studies and unavailable data. Therefore, the quantity and quality of data did not allow for determining the pooled effect sizes relative ranking of the different manipulations in the order of potency and risk.

Publication bias

It was outlined in the protocol that funnel plots will be examined for publication bias. However, there was vast clinical heterogeneity among the studies. Therefore, it was not possible to pool more than 10 studies and assess for publication bias (Higgins and Green, 2011). Alternative methods for assessing publication bias were explored. One such method is the Fail-safe N method, which "identifies the number of additional negative studies that would be needed to increase the P-value in a meta-analysis to above 0.5" (Dalton et al., 2016). However, this method is not recommended by The Cochrane Group because it focuses on an "arbitrary threshold" (the p-value) rather than the estimated size of the interventions' effect (Higgins and Green, 2011). Therefore, the Fail-safe N method was deemed inappropriate to assess for publication bias among included studies in this review. No other appropriate methods of measuring publication bias were identified.

Measures of manipulation effect

Studies were subgrouped by the hypothesised direction (i.e. decrease or increase) of manipulation effect on intensity of SH. There was high clinical heterogeneity among the studies in each group; therefore, it was not possible to measure the manipulation effect and estimate the potencies of the manipulation methods. Appendix 6, Section 6.3 describes the differences in the reporting of the intensity ratings across the studies in such subgroup.

Assessment of the quality of body of evidence

Only one class of manipulation was used by more than one study: attentional and cognitive loading (Torta et al., 2019, Kóbor et al., 2009). Tables 7 summarises the assessment of the quality of body of evidence for the effect of attentional and cognitive loading on intensity of SH. There were serious limitations in the study designs and reporting results in both studies having an overall unclear risk of bias. Therefore, the quality of evidence was downgraded by 1 level for risk of bias. This small body (n = 3 studies) of evidence was assessed as having *moderate* quality.

Table 7 Assessment of the quality of body of evidence for the effect of attentional and cognitive loading on intensity of secondary hyperalgesia

Population: healthy human adults (>18 years old) **Setting:** experimental laboratory **Intervention (manipulation):** attentional and cognitive loading **Comparison (control):** N/A (within-subject comparison)						
Outcome measurement: intensity of secondary hyperalgesia						Test accuracy of certainty of evidence
Number of studies (number of participants)	Study design	Factors that may decrease certainty of evidence				
		Risk of bias	Indirectness	Inconsistency	Imprecision	
2 (56 participants) (Torta et al., 2019, Kóbor et al., 2009)	Crossover, within-subject comparison, experimental	Serious limitations	No	No	No	Moderate

Secondary outcomes

The effect of manipulation on area of secondary hyperalgesia (n=17)

Seventeen (of 21) studies assessed area of SH after manipulation. Studies have been grouped by the hypothesised direction of the effect of the manipulation on the area of SH (i.e. either increase or decrease area of SH). Thereafter, Studies were pooled by manipulation procedures, where possible. The remaining manipulation procedures have been reported individually. Table 8 the hypothesised and actual directions of the effect of each study's manipulation on the area of SH.

Table 8 A summary of the hypothesised and actual directions of the effect of each study's manipulation on the area of secondary hyperalgesia

Study reference	**Manipulation**	**Hypothesised direction of the effect of manipulation on surface area of secondary hyperalgesia**	**Actual direction of the effect of manipulation on surface area of secondary hyperalgesia**
Baron et al. (1999)	Whole-body cooling and whole-body heating	Decrease*	No effect for both whole-body heat and whole-body cooling
Werner et al. (2002)	Localised cold application	Decrease*	No effect
Pud et al. (2006)	Localised cold application	Decrease*	Increase
Yucel et al. (2001) Experiment 1	Localised cold application	Decrease*	Data unavailable
Yucel et al. (2001) Experiment 2	Localised cold application	Decrease*	Data unavailable
Yucel et al. (2001) Experiment 3	Localised cold application	Decrease*	Data unavailable
Rasmussen et al. (2015)	Hyperbaric oxygen therapy	Decrease	Decrease
Wahl et al. (2019)	Hyperbaric oxygen therapy	Decrease	Decrease
You et al. (2014)	Emotional disclosure	Decrease*	Decrease
Salomons et al. (2014)	Cognitive behavioural therapy	Decrease	Decrease
Matre et al. (2006)	Placebo analgesia	Decrease	Decrease
Meeker et al. (2019)	Transcranial direct current stimulation	Decrease	Anodal: decrease[†] Cathodal: no effect
Mohammadian et al. (2004)	Spinal manipulation therapy	Decrease	Decrease
Rebhorn et al. (2012)	Acupuncture	Decrease*	No effect
Ditre et al. (2018)	Nicotine deprivation	Increase	Increase
Helfert et al. (2018)	Verbal suggestion	Increase*	No effect
Smith et al. (2018)	Sleep disruption	Increase*	Increase (in male participants only)

*Not all studies reported a clear hypothesised direction of effect. In these cases, this author hypothesised the direction of effect based on published literature. Studies that failed to report a clear hypothesised direction of the effect and where this author hypothesised the direction of the effect have been denoted with an asterisk.

[†]Anodal transcranial direct current stimulation was no better than sham transcranial direct current stimulation.

Baseline data (n=3)

Only three studies (Mohammadian et al., 2004, Matre et al., 2006, Salomons et al., 2014) reported baseline data after induction and before manipulation. Therefore, baseline data was available for topical capsaicin inductions (Mohammadian et al., 2004) and burn injury inductions (Salomons et al., 2014, Meeker et al., 2019) only. Mohammadian et al. (2004) conducted a crossover study in which participants received both spinal therapy manipulation and a non-spinal manipulation sham at separate sessions, in a randomised order. Sessions were separated by a washout period of at least seven days. The baseline surface area of SH was assessed after a topical capsaicin application induction and prior to manipulation, and the data were pooled by manipulation (spinal manipulation therapy or sham) across the two sessions. There was a discrepancy in the pooled baseline surface area ratings: 46cm^2 prior to receiving the spinal manipulation therapy and 34cm^2 prior to receiving the non-spinal manipulation therapy (extracted from a bar graph).

Matre et al. (2006) conducted a between-group comparison study in which participants were randomised to either placebo analgesic manipulation (experimental) group or sham manipulation group. The baseline surface area of SH was assessed after inducing a burn injury at two independent induction sessions. These surface area ratings were assessed prior to participants receiving either placebo analgesic manipulation (experimental) or sham manipulation because the manipulation occurred only at a third independent session (sessions separated by four to seven days). The participants in the experimentally group were informed at the beginning of the study that the researchers were investigating "the analgesic effectiveness of a magnet against heat pain". The magnet (placebo analgesia) was introduced to the participants in the first session but it was removed and used 'as an analgesic' in the third session only. Participants in the sham manipulation (control) group were provided with completely different study information – they were informed that the researchers were investigating "the hypersensitivity of the skin after a heat stimulus". Researchers aimed to make the physical environment identical for participants in both groups. Therefore, a magnet was also used for participants in the sham manipulation group, but they were informed that it was a thermometer. The magnet (sham thermometer) was introduced to the participants at the first session and used in the third session only. The median (IQR) surface area for the experimental group was 75 cm^2 (45- 95) (session one) and 49 cm^2 (40 - 80) (session two) (data extracted from box and whisker plot). The median (IQR) surface area for the control group was 50 cm^2 (40 - 75) (session one) and 55 cm^2 (35 - 45) (session two) (data extracted from box and whisker plot).

Salomons et al. (2014) conducted a between-group comparison study in which participants were randomised to receiving either cognitive behavioural therapy (experimental) or sham manipulation (control). The baseline surface area of SH was assessed after inducing a burn injury, prior commencement of the cognitive behavioural therapy and sham manipulation sessions. The cognitive behavioural therapy was approximately five minutes long and focused on reducing participants' negative thoughts and emotions towards painful stimuli. The sham manipulation was also approximately five minutes long and focused on teaching participants how to balance goals, expectations and communicate assertively to manage external pressures (interpersonal effectiveness skills). The baseline means (SEM) area of SH was 48.0 cm^2 (7.01) for the experimental group and 45.0 cm^2 (6.29) for the control group. Overall, participants receiving a burn injury induction displayed the largest surface area of SH. However, Matre et al. (2006) reported a larger area than Salomons et al. (2014). Topical capsaicin application induced a similar area of SH to that of the burn injury reported by Salomons et al. (2014). This may indicate a similar potency between burn injury inductions and topical capsaicin induction.

Manipulations hypothesised to decrease the area of secondary hyperalgesia (n=14)

Fourteen studies used manipulations hypothesised to decrease area of secondary hyperalgesia. Seven studies successfully decreased the area of SH with hyperbaric oxygen therapy (Wahl et al., 2019, Rasmussen et al., 2015), emotional disclosure (You et al., 2014), cognitive behavioural therapy (Salomons et al., 2014), placebo analgesia (Matre et al., 2006), transcranial direct current stimulation (Meeker et al., 2019) and spinal manipulation therapy (Mohammadian et al., 2004). Four of the five studies that used localised application of cold reported no effect (Werner et al., 2002, Yucel et al., 2001) and the remaining one reported an increase in the area of SH (Pud et al., 2006). However, the latter study had a high risk of reporting bias, as discussed in the *Risk of bias* section above. Whole-body cooling and heating (Baron et al., 1999) and acupuncture (Rebhorn et al., 2012) failed to decrease the area of SH.

The effect of heat or cold stimulation on the surface area of secondary hyperalgesia (n=6)

Six studies used a version of heat and or cold stimulation to manipulation area of SH (Yucel et al., 2001, Pud et al., 2006, Werner et al., 2002, Baron et al., 1999). As discussed above, Baron et al. (1999) used an intradermal capsaicin injection induction and then exposed participants to a whole-body cooling and whole-body heating manipulation. There were negligent differences in the mean (SEM) area of SH between the two manipulations: 88.1 cm^2 (13.1) with whole-body cooling (high sympathetic activity) and 86.4 cm^2 (13.1) with whole-body heating (low sympathetic activity). These data suggest that high and low sympathetic activity did not influence the area of SH induced by an intradermal capsaicin injection.

Pud et al. (2006) and Werner et al. (2002) both used localised application of cold to manipulate secondary hyperalgesia, however, their methods differed slightly. Pud et al. (2006) used capsaicin injection induction and then exposed participants to cold stimulations of 20°, 10° and 0°, in randomised orders for 30 seconds each, eight minutes after induction. Werner et al. (2002) used a burn injury induction with a hot contact thermode, and then applied a cold stimulation of 8 °C (experimental) or non-active sham (control) for 30 minutes from eight minutes after induction. Pud et al. (2006) reported a mean (SEM) area of 21.7 cm^2 (2.7) at 8 minutes (directly after manipulation) and 15.0 cm^2 (3.3) at 30 minutes after induction. The area of SH increased from 5.1 cm^2 at baseline to 21.7 cm^2 8 minutes after a localised cold application. Pud et al. (2006) reported that localised application of cold increased the area of SH. The mean (SEM) area of SH at the control site was not reported at 8 minutes or 30 minutes after induction. Therefore, it is plausible that the area of SH at the control site followed the same trajectory as that at the experimental site, despite the localised cold application at the experimental site.

Werner et al. (2002) reported localised application of cold did not influence the area of secSH. The median (IQR) area for experimental and control site were measured at 10, 40, 80, 120 and 160 minutes after the 30 minutes cooling period (Appendix 6, Section 6.4). In this current study, the median area over the entire 160-minute time period was calculated to facilitate comparison with surface area data from other studies. The median area was calculated to be 50 cm^2 for the experimental site and 54 cm^2 for the control site. There was not a statistically significant difference ($p > 0.4$. The exact p-value was not reported.) difference in the area of SH between the two groups. These data indicate that localised application of cold did not influence the area of SH induced by burn injury.

Yucel et al. (2001) conducted three experiments using differing induction methods (intradermal capsaicin injection, topical capsaicin, and burn injury) after exposing participants to a five-minute heating manipulation at a temperature of 45 °C. After induction, participants were exposed to another heating manipulation for two minutes at a temperature 2°C above their individual heat pain threshold. Yucel et al. (2001) reported assessing area of SH. However, these data were not reported. The author of this current study attempted to contact the corresponding author, requesting missing data, but their email address was no longer active. Therefore, these data were considered unavailable for this current review.

The effect of hyperbaric oxygen therapy on the surface area of secondary hyperalgesia (n=2)

Rasmussen et al. (2015) and Wahl et al. (2019) conducted crossover studies to assess the influence of hyperbaric oxygen therapy on SH. They both used a contact thermode burn injury induction and directly after, exposed participants to hyperbaric oxygen therapy (manipulation condition) and to ambient pressure and room air (control condition) at two separate sessions. In fact, the latter study is a replication of the former study. Rasmussen et al. (2015) reported an average of 37 days and Wahl et al. (2019) 28 days or more washout period between each session.

Rasmussen et al. (2015) pooled the surface area data by manipulation (hyperbaric oxygen therapy vs ambient conditions) across both sessions and reported median (95% CI) area of SH at 45, 85, 125, 175, 235 minutes after induction as well as the overall median (95% CI) over the entire testing period (Appendix 6, Section 6.5). Although, Wahl et al. (2019) was a replication study, they did not report surface area data at each time point. Instead, they reported the mean (95% CI) area of SH over the entire 120-minute period.

Rasmussen et al. (2015) the median (95% CI) area of SH were 42.0 cm^2 (31.1 – 71.4) when participants received the hyperbaric oxygen therapy manipulation and 34.6 cm^2 (22.9 – 39.8) when participants received the induction under ambient conditions (data extracted from plot). There was a significant (p = 0.011) difference in the area of SH produced after each of these conditions. These data suggest that hyperbaric oxygen therapy is effective in manipulating the area of SH induced by burn injury.

Wahl et al. (2019) reported the mean (95% CI) area of SH to be 18.8 cm^2 (10.5– 27.0) when participants received the hyperbaric oxygen therapy manipulation and 32.0 cm^2 (20.1–43.9) when participants received the induction under ambient conditions. There was a significant (p = 0.021) difference in the area of SH produced after each of these conditions. These data suggest that hyperbaric oxygen therapy is effective in manipulating the area of SH induced by burn injury.

The effect of emotional disclosure on the surface area of secondary hyperalgesia (n=1)

As discussed above, You et al. (2014) used a topical capsaicin induction four days and one month after exposing participants to once-off emotional disclosure (experimental) or sham intervention (control group). At four days after the manipulation procedure the mean (SEM) area of SH for participants in the experimental group 132 cm^2 (105 – 159) for those with a history of trauma and 70 cm^2 (38 – 98) for those without a history of trauma. Area of SH for participants in the control group was 75 cm^2 (61 – 90) for those with a history of trauma and 65 cm^2 (50 – 81) for those without a history of trauma. The difference in area between the experimental and control group was statistically significant (p <0.050. The exact p-value was not reported), suggesting that the emotional disclosure was effective in reducing the area of SH at four days. In the experimental group, there was also a statistically significant difference (p < 0.025. The exact p-value was not reported) in the area of SH between those with a history of trauma and those without a history of trauma, indicating that emotional disclosure was more effective in reducing the area of SH in participants with a history of trauma at four days.

At one month after the manipulation procedure the mean (SEM) area of SH for participants in the experimental group was 38 cm^2 (22 – 56) for those with a history of trauma and 98 cm^2 (70 – 120) for those without a history of trauma. Area for participants in the control group was 90 cm^2 (59 – 120) for those with a history of trauma were and 50 cm^2 (21 – 70) for those without a history of trauma (all data extracted from graph). The difference in intensity ratings between the experimental and control group was statistically significant (p <0.050. The exact p-value was not reported), suggesting that the emotional disclosure was effective in reducing the area of SH at one month. In the experimental group, there was also a statistically significant difference (p < 0.025. The exact p-value was not reported) in the area of SH between those with a history of trauma and those without a history of trauma, indicating that emotional disclosure was more effective in reducing the area of SH in participants with a history of trauma at one month.

The effect of cognitive behavioural therapy on the surface area of secondary hyperalgesia (n=1)

Salomons et al. (2014) used a contact thermode burn injury induction and exposed participants to approximately five minutes of cognitive behavioural therapy. Induction and manipulation were performed over eight sessions. There were initial and final induction sessions (without the manipulation) where baseline and final sensory ratings, respectively, were recorded. The cognitive behavioural therapy was focused on reducing participants' negative thoughts and emotions towards painful stimuli. Salomons et al. (2014) reported the mean (SEM) area of SH to be 29.8 cm^2 (7.31) for the experimental and 48.5 cm^2 (8.80) for the control groups. There was a statistically significant difference ($p < 0.05$. The exact p-value was not reported.) between the two groups, suggesting that altering pain-related thoughts with cognitive behavioural therapy, is effective influence reducing the area of SH induced by burn injury.

The effect of placebo analgesia on the surface area of secondary hyperalgesia (n=1)

Matre et al. (2006) used a contact thermode burn injury induction over three experimental sessions, separated by four to seven days apart. Placebo analgesia manipulation (experimental group) was only used in the third session and was compared with control conditioned. The placebo analgesia manipulation consisted of participants in the experimental group being informed that "the aim of the study was to test the analgesic effectiveness of a magnet against heat pain". Participants in the control group were inform that "aim of the study was to investigate hypersensitivity of the skin after a heat stimulus". The median (IQR) (on a box and whisker plot) area of SH was 40 cm^2 (30 – 65) in the experimental group and 50 cm^2 (35 – 75) in the control group. A statistically significant difference ($p = 0.002$) was found between baseline area measurements and area measurements after manipulation in the experimental group, indicating the placebo analgesia to be effective.

The effect of transcranial direct current stimulation on the surface area of secondary hyperalgesia (n=1)

Meeker et al. (2019) conducted a crossover study, using a topical capsaicin and heat induction and then exposed participants to transcranial direct current stimulation (experimental condition) and sham transcranial direct current stimulation (control condition) in separate sessions. Sessions were separated by a minimum of two weeks to allow for a washout period. Meeker et al. (2019) reported a mean (SEM) area of 20 cm^2 (16 – 26) after participants received anodal transcranial direct current stimulation, 12 cm^2 (6 – 18) after participants received cathodal transcranial direct current stimulation, and 11 cm^2 (4 – 19) after participants received sham transcranial direct current stimulation (data extracted from a plot). There was a significantly greater reduction (p = 0.075) in the area of SH after anodal transcranial direct current stimulation as compared to cathodal transcranial direct current stimulation. However, the difference in the reduction in area between the anodal transcranial direct current stimulation and the sham transcranial direct current stimulation was not significant (p = 0.91). These data indicate that anodal transcranial direct current is effective in reducing the area of SH; however, it is no better than sham transcranial direct current.

The effect of spinal manipulation therapy on the surface area of secondary hyperalgesia (n=1)

Mohammadian et al. (2004) conducted a crossover study and used a topical capsaicin induction and then exposed participants to spinal manipulation (experimental manipulation) and non-spinal manipulation (sham manipulation) at two separate sessions. Sessions where separated by at least seven days to allow for a washout period. Mohammadian et al. (2004) reported the mean (SEM) area after spinal manipulation treatment (experimental) and non-spinal manipulation treatment (control) and displayed results on a 3-D bar graph; however, the SEM data were not visible due to dark shadings on the graph. These data were requested from the corresponding author, but they did not respond. The mean area of SH was 27 cm^2 after the experimental manipulation and 45 cm^2 after the sham manipulation. There was a statistically significant difference (p < 0.007. The exact p-value was not reported.) between the two groups, suggesting that spinal manipulation therapy, was effective in reducing the area of SH induced by topical capsaicin.

The effect of acupuncture on the surface area of secondary hyperalgesia (n=1)

Rebhorn et al. (2012) used an intradermal capsaicin injection induction twenty minutes after exposing participants to traditional Chinese Medicine acupuncture (experimental group) or sham acupuncture (control group). Area was measured at 15 minutes and 30 minutes after induction. The mean area (data extracted from graph) was 55 cm^2 at 15 minutes and 89 cm^2 at 30 minutes after induction for the experimental group and the mean area was 60 cm^2 at 15 minutes and 91 cm^2 at 30 minutes after the induction for the control group. Rebhorn et al. (2012) reported that the mean area for the experimental group was comparable to the mean area for the control group. However, no statistics were reported. These data suggest that acupuncture did not influence the area of SH induced by intradermal capsaicin injection.

Manipulations hypothesised to increase the area of secondary hyperalgesia (n=3)

Three studies used manipulations hypothesised to increase the area of secondary hyperalgesia. Nicotine deprivation (Ditre et al., 2018) and sleep deprivation (Smith et al., 2018) successfully increased area of secondary hyperalgesia. Importantly, sleep deprivation was successful in increasing area of SH in male participants only. There was no effect on area of SH in female participants. Verbal suggestion (Helfert et al., 2018) failed to increased area of SH.

The effect of nicotine deprivation on the surface area of secondary hyperalgesia (n=1)

As discussed above, Ditre et al. (2018) used a topical capsaicin induction in a cohort of smokers after exposing them to 12 – 24 hours of nicotine deprivation (experimental group) and compared it to participants who were allowed to continuing smoking (control group). The mean (SD) area of SH was 71.98 cm^2 ± 55.17 for the experimental group and 45.07 cm^2 ± 37.14 for the control group. There was a statistically significant difference ($p < 0.05$. The exact p-value was not reported.) in the area between the experimental and control groups. These data suggest that acute nicotine withdrawal effectively influenced the area of SH induced by topical capsaicin.

The effect of sleep disruption on the surface area of secondary hyperalgesia (n=1)

Smith et al. (2018) conducted a crossover study and used a topical capsaicin application induction after exposing participants to two consecutive nights of sleep disruption (experimental condition) or two nights of undisturbed sleep (control condition). The sleep disruption consisted of participants having an eight-hour sleep opportunity; however, participants were woken for one of the eight hours and a further three 20-minute periods (randomly assigned). The control condition consisted of eight full hours of undisturbed sleep. There was a minimum of two-weeks between each condition, to allow for participants to return to their habitual sleep behaviours. The mean (SEM) area was reported for male and female participants separately (data extracted from a plot) (area was measured in mm^2. In this current study, area measurements were converted to cm^2 to allow for comparison across all included studies). For male participants, the mean area of SH was 18 cm^2 after the sleep disruption and 11 cm^2 after the undisturbed sleep. For female participants, the mean (SEM) area of SH was 12.5 cm^2 after the experimental condition and 15.1 cm^2 after the control condition. Post-hoc tests confirmed a significant (p = 0.008) increase in the area of SH after the sleep disruption in male participants only. Sleep disruption had no statistically significant effect on the area of SH in female participants. These data suggest sleep disruption effectively influenced the area of SH induced by topical capsaicin in male participants only.

The effect of verbal suggestion on the surface area of secondary hyperalgesia (n=1)

As discussed above, Helfert et al. (2018) used a high-concentration topical menthol induction after exposing them to verbal suggestion. No individual nor group data on the effects of the manipulation on area of SH were reported. These missing data were requested from the corresponding author; however, there was no response. Although it was reported that there was no significant change in the area of SH after manipulation, these data could not be verified and were considered unavailable.

Pooling of data

Studies were pooled by class of manipulation. Two subgroups of classes of manipulation were identified for pooling: 1) localised application of cold, and 2) hyperbaric oxygen therapy. Two studies were included in Subgroup 1 (Pud et al., 2006, Werner et al., 2002) and two studies were included in Subgroup 2 (Wahl et al., 2019, Werner et al., 2002). No other studies could be pooled by class of manipulation. However, pooling of data was not possible for both subgroups because in Subgroup 1, Pud et al. (2006), did not report area measurements for the control group, and in Subgroup 2, two different statistical measures were used: median (95% CI) (Rasmussen et al., 2015) and mean (95% CI) (Werner et al., 2002).

Relative ranking of manipulations

It was not possible to pool studies that investigated the effect of manipulations on area of secondary hyperalgesia, owing to the high clinical heterogeneity among the studies and unavailable data. Therefore, the quantity and quality of data did not allow for determining the pooled effect sizes relative ranking of the different manipulations in the order of potency and risk.

Publication bias

It was outlined in the protocol that funnel plots will be examined for publication bias. However, there was vast clinical heterogeneity among the studies. Therefore, it was not possible to pool more than 10 studies and assess for publication bias (Higgins and Green, 2011). As discussed above, no other appropriate methods of measuring publication bias were identified.

Measures of manipulation effect

Studies were subgrouped by the hypothesised direction (i.e. decrease or increase) of manipulation effect on intensity of secondary hyperalgesia. There was high clinical heterogeneity among the studies in each group; therefore, it was not possible to measure the manipulation effect and estimate the potencies of the manipulation methods. Appendix 6, Section 6.6 describes the differences in the reporting of the area ratings across the studies in such subgroup.

Assessment of the quality of body of evidence

Only two types of manipulation were used by more than one study: hyperbaric oxygen therapy was used by two studies (Rasmussen et al., 2015, Wahl et al., 2019), and cold application was used by three studies (Werner et al., 2002, Pud et al., 2006, Yucel et al., 2001). Tables 9 summarises the assessment of the quality of body of evidence for the effect of hyperbaric oxygen therapy compared to ambient conditions on experimentally induced SH in healthy humans. There were serious limitations in the designs of these studies resulting in the overall risk of bias being determined as unclear (Wahl et al., 2019) and high (Rasmussen et al., 2015). Therefore, the quality of evidence was downgraded by 1 level. The accuracy of the certainty of the evidence was graded as *moderate,* meaning that "further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate" (Guyatt et al., 2008) because of the serious limitations of the risk of bias and the small sample size in the current body of evidence.

Table 10 summarises the assessment of the quality of body of evidence for the effect of cold application compared to sham on experimentally induced SH in healthy humans. There were *very serious* limitations in the designs of these studies, resulting in a judgment of high risk of bias for both studies. Additionally, Pud et al. (2006) scored high for risk of reporting bias. Therefore, the quality of evidence was downgraded by 2 levels. There were differences in the induction methods and outcome measurements methods (see Table 3). Therefore, the quality of evidence was downgraded

by 1 level. There were missing data in that measurements of the area of SH were not reported for all time points for both the experimental and control groups (Pud et al., 2006). Specifically, the mean (SEM) area of SH was not reported at 8 minutes or 30 minutes after induction at the control site. Therefore, it is plausible that the size of the surface area at the control site followed the same trajectory as the size of the surface area at the experimental site. The localised cold application may not have increased in area at the experimental site, but rather the increase in the area at 8 minutes may have been the normal trajectory of the effect of the induction procedure. The accuracy of the certainty of the evidence was graded as *very low, meaning* "Any estimate of effect is very uncertain" (Guyatt et al., 2008) because of the very serious limitations in the risk of bias and imprecision and the serious limitations in the studies' consistency.

Table 9 Assessment of the quality of body of evidence for the effect of hyperbaric oxygen therapy compared to ambient conditions on area of secondary hyperalgesia

Population: healthy human adults (>18 years old) **Setting:** experimental laboratory **Intervention (manipulation):** hyperbaric oxygen therapy **Comparison (control):** ambient conditions						
Outcome measurement: area of secondary hyperalgesia						Test accuracy of certainty of evidence
Number of studies (number of participants)	Study design	Factors that may decrease certainty of evidence				
		Risk of bias	Indirectness	Inconsistency	Imprecision	
2 (36 participants) (Rasmussen et al., 2015, Wahl et al., 2019)	Crossover, within-subject comparison, experimental	Serious limitations	No	No	No	Moderate

Table 10 Assessment of the quality of body of evidence for the effect of cold application compared to sham on area of secondary hyperalgesia

Population: healthy human adults (>18 years old) **Setting:** experimental laboratory **Intervention (manipulation):** cold application **Comparison (control):** sham						
Outcome measurement: area of secondary hyperalgesia						Test accuracy of certainty of evidence
Number of studies (number of participants)	Study design	Factors that may decrease certainty of evidence				
		Risk of bias	Indirectness	Inconsistency	Imprecision	
2 (38 participants) (Pud et al., 2006, Werner et al., 2002)	Within-subject comparison, experimental	Very serious	No	Serious	Very serious	Very low

Pain ratings during the induction of secondary hyperalgesia (n=7)

Seven studies (Mohammadian et al., 2004, Yucel et al., 2001, Wahl et al., 2019, Werner et al., 2002, Pud et al., 2006) assessed pain elicited by the induction, prior to the manipulation. Yucel et al. (2001) (Experiment 2) and Pud et al. (2006) reported similar pain ratings during induction with intradermal capsaicin injection. Mean (SD) pain ratings during intradermal capsaicin injection were 7.8 (2.0) (0 – 10 VAS) (Yucel et al., 2001) and 7.5 (0.38) (originally reported on 0 – 100 VAS, rescaled to 0 – 10) (Pud et al., 2006). Yucel et al. (2001) (experiment 1) and Mohammadian et al. (2004) reported similar pain ratings during application of topical capsaicin. The mean pain ratings during topical capsaicin application were 4.05 (Yucel et al., 2001) (Experiment 1) and 4.0 (Mohammadian et al., 2004). Yucel et al. (2001) (Experiment 1 and 2) also pre-heated the experimental site for five minutes at 45°C prior to intradermal capsaicin injection and topical application, whereas Pud et al. (2006) and Mohammadian et al. (2004) did not. These data suggest that pre-heating may not influence pain ratings during induction of SH by intradermal or topical capsaicin.

Wahl et al. (2019) and Werner et al. (2002) reported pain during a contact thermode burn injury induction prior to their manipulation procedures. Wahl et al. (2019) reported the mean pain intensity (0 – 10 VAS) during the burn injury before for each manipulation (hyperbaric oxygen therapy or ambient conditions) separately. This was a crossover study (i.e. participants received both the hyperbaric oxygen therapy manipulation and the ambient conditions on separate occasions. Data were reported for all participants before both hyperbaric oxygen therapy manipulation and the ambient conditions), therefore, there should be no difference in pain ratings during induction prior to each manipulation. Therefore, in this current study, the combined mean pain ratings during the burn injury induction, before the manipulation, was determined to be 3.15 (view Appendix 6, Section 6.7 for the pain ratings during induction for each group). Werner et al. (2002) reported median (IQR) pain ratings for each group for every minute during the burn injury induction, before the manipulation. In this current study, the overall median pain ratings were determined for each group: 3.39 for the experimental group and 3.33 for the control group (view Appendix 6, Section 6.8 for pain during induction at each time point for each group).

One study assessed pain ratings during topical capsaicin induction, *after* the manipulation with nicotine deprivation (Ditre et al., 2018). After the manipulation, pain ratings (0 – 10 VAS) were reported at 0, 5, 10, 15, 20, 25 and 30 minutes during the 30-minute application of topical capsaicin for the nicotine-deprived (experimental) and continued smoking (control) groups separately (view Appendix 6, Section 6.9 for pain during induction at each time point for each group). This current study determined the mean (SD) pain intensity during the 30-minute application of topical capsaicin to be 3.63 (3.12) for the nicotine deprivation group and 2.52 (2.31) for the control group.

One study performed a low-frequency electrical stimulation induction *simultaneously during* manipulation with cognitive loading in two independent experiments (Torta et al., 2019) (Experimental 1 and 2). The mean (SD) pain ratings (originally reported on 0 – 100 NRS, rescaled to 0 – 10) during low-frequency electrical stimulation were 6.8 (1.9) in Experiment 1 and 6.6 (1.6) in Experiment 2.

These data suggest that intradermal capsaicin injection may be the most painful induction used to induced secondary hyperalgesia, followed by low-frequency electrical stimulation. Similar pain ratings were reported during topical capsaicin application and burn injury.

Pain during manipulation of secondary hyperalgesia (n = 2)

One would suspect that certain manipulations, such as acupuncture, spinal manipulation, and cold application, might be painful. However, no studies reported on pain during those manipulations. Only Pud et al. (2006) and Werner et al. (2002) reported on pain during manipulation procedures, which were both localised application of cold. Both studies used a contact thermode for localised application of cold. Pud et al. (2006) reported pain ratings during the cold stimuli at 20 °C, 10 °C and 0 °C. The combined mean (SD) pain ratings for both experimental and control arms were 0.76 (0.16) for the 20 °C cold stimulus, 1.33 (0.35) for the 10 °C stimulus and 2.2 (0.47) for the 0 °C cold stimulus (reported on 0 – 100, rescaled to 0 – 10). Interestingly, Werner et al. (2002) reported pain to localised application of cold (8°C) to be negligible - reporting a mean of 0 and range 0 – 1 (0 – 100 VAS), rescaled to a range of 0 – 0.1 (0 – 10).

Discussion

The aim of this study was to systematically identify, collate, and describe all the published studies that have applied non-pharmacological manipulations intended to influence experimentally induced SH in healthy human participants. Investigating the effects of these non-pharmacological manipulations is important to improve insight into the mechanisms of SH. Manipulations thought to influence central processes contributing to pain were more likely to be successful in manipulating experimentally induced SH than manipulations thought to influence peripheral processes contributing to pain.

Hypothesised main site of action: peripheral and central action

As discussed in Chapter 1, pain is extremely complex: even the latest theoretic models of pain have limitations (Griffin and Tsao, 2012). Pain is influenced by many physiological processes at the periphery, spinal cord and brain. Nevertheless, certain manipulations will primarily influence afferent sensory signaling (site of principal action: peripheral), whereas others will primarily influence central nervous system activity (site of principal action: central). These sites of action do

not work in isolation; instead, peripheral and central (spinal cord and cortex) mechanisms are interlinked and influence each other (D'Mello and Dickenson, 2008, Stucky et al., 2001). However, for clarity's sake, the results of this review are discussed with manipulations grouped by these presumed sites of principal action: peripheral and central. Some manipulations were not considered to have a principal site of action and target both peripheral and central actions. These manipulations were considered to have a 'controversial' main site of action and have been discussed separately below.

Principal site of action: peripheral

Six manipulations targeted primarily peripheral processes. These were: localised application of cold (Werner et al., 2002, Pud et al., 2006, Yucel et al., 2001)[9] and whole-body cooling and heating (Baron et al., 1999). Localised application of cold and whole body cooling and heating had no effect on the intensity of SH (Werner et al., 2002, Baron et al., 1999). One study reported localised application of cold increased area of SH (Pud et al., 2006), and another reported no effect (Werner et al., 2002). Localised application of cold has previously been tested for reducing pain (Dehghan and Farahbod, 2014, Ni et al., 2015, Thienpont, 2014, Algafly and George, 2007, Nadler et al., 2004, Ivey et al., 1994, Hirvonen et al., 2006) because it is thought to inhibit peripheral inflammation (Werner et al., 2002), thus decreasing the afferent nociception that can induce secondary hyperalgesia. Individual studies have found it to reduce post-operative pain after various orthopaedic procedures, of which SH is a common feature (Quinlan et al., 2017, Cohn et al., 1989, Barber, 2000, Bert et al., 1991, Levy and Marmar, 1993, Scarcella and Cohn, 1995). However, a systematic review of localised application of cold for reducing pain, blood loss and improving function after knee arthroplasty (Adie et al., 2012) judged most of the evidence to be of *'low'* or *'very low'* quality and concluded that any benefit may not be clinically significant. The current results are in line with the findings of Adie et al. (2012): localised cold application and whole-body cooling and heating had no effect on secondary hyperalgesia.

Principal site of action: central

Thirteen manipulations targeted primarily central processes. These manipulations were: cognitive behavioural therapy (Salomons et al., 2014), emotional disclosure (You et al., 2014), hyperbaric oxygen therapy (Rasmussen et al., 2015, Wahl et al., 2019), verbal suggestion (Helfert et al., 2018), negative suggestion (van den Broeke et al., 2014), high attentional and cognitive loading (Kóbor et al., 2009, Torta et al., 2019) placebo analgesia (Matre et al., 2006), sleep deprivation (Smith et al.,

[9] Yucel et al., 2001 reported on three studies but data were unavailable for all three studies.

2018), nicotine deprivation (Ditre et al., 2018) and transcranial direct current stimulation (Meeker et al., 2019). Each manipulation has been discussed separately below.

Cognitive behavioural therapy

Cognitive behavioural therapy manipulation, focused on reducing participants' negative thoughts and emotions towards painful stimuli, successfully reduced the area of SH (Salomons et al., 2014). Cognitive behavioural therapy (CBT) has been previously been tested for reducing pain (Bergeron and Lord, 2003, Evans et al., 2003, Mayou et al., 1997, Monticone et al., 2015, Newton-John et al., 1995, Thomas, 2000, Tichelaar et al., 2007) because it is thought to improve the affective and motivational dimensions of pain by changing unhelpful or harmful patterns of thinking or emotional responses (Sheldon, 2011). Two systematic reviews of CTB for chronic pain reported CBT to be superior to waiting list control conditions in decreasing pain; however, CBT was no better than other active treatments. (exercise and relaxation) (Morley et al., 1999, Monticone et al., 2015). Additionally, CBT in addition to other activity treatments was no better than CBT alone (Monticone et al., 2015). Importantly, the studies included in those reviews were deemed to have *'low'* quality evidence. Another study reported statically significant improvement in self-reported disability and function but not in pain severity in adolescence with chronic pain (Eccleston et al., 2003). An important difference to note is that the clinical studies reported here used pain severity as the outcome, whereas the experimental study in this current review used area of SH as the outcome. Therefore, there are limitations in comparing the experimental study included in this current review with the clinical studies discussed here. Further high-quality research is required to determine and compare the effectiveness of CBT on general pain severity and SH (as assessed by intensity and area of secondary hyperalgesia).

Emotional disclosure

Once-off emotional disclosure successfully decreased the intensity and area of SH in both participants with and without a history of trauma at four days and one month after the manipulation (You et al., 2014). Interestingly, participants with a history of trauma had significantly reduced intensity and area of SH than participants without a history of trauma at both four days and one month after the manipulations. This suggests that the emotional disclosure manipulation was more effective at influencing SH in participants with a history of trauma than those without a history of trauma. A history of traumatic events has been reported to be associated with chronic pain (Goldberg et al., 1999, Nicol et al., 2016). There are conflicting reports on the use of written emotional disclosure of traumatic events for treating chronic pain (Lumley et al., 2012). The mechanisms by which emotional disclosure may decrease pain are unclear but it has been proposed that it may decrease pain through improving mood and decreasing psychological distress (Lumley et

al., 2012). However, emotional disclosure has been reported to improve negative emotions and psychological distress (Ullrich and Lutgendorf, 2002), enhance immune functioning (Pennebaker et al., 1988), and decrease clinic visits six months after the intervention (Pennebaker and Beall, 1986). Emotional disclosure has been reported to have delayed beneficial effects and may decrease mood and increase psychological distress directly after the intervention (Pennebaker and Beall, 1986, Sloan and Marx, 2004, Sloan et al., 2005, Lumley et al., 2012). The results in this current study support these reported long-term benefits, since a reduction in SH was still present one month after the emotional disclosure. You et al. (2014) did not induced and assess the intensity and the area of SH directly after the emotional disclosure. Therefore, it is unclear whether emotional disclosure only has delayed benefits or also reduces the magnitude of SH directly after the manipulation.

Placebo analgesia, negative suggestion and verbal suggestion

Placebo analgesia successfully *decreased* the *area* of SH (Matre et al., 2006). Negative suggestion associated with the HFS induction procedure successfully *increased* the *intensity* of SH (van den Broeke et al., 2014). Verbal suggestion was reported to have failed to increase the intensity and area of SH but data were unavailable, as discussed in the *Results* section (Helfert et al., 2018).

The effect of placebo analgesia is explained by the placebo response, in which positive expectations lead to symptoms improving (Benedetti and Piedimonte, 2019). In contrast, the effect of negative suggestion[10] is explained by the nocebo response, in which negative expectations leads to symptoms worsening (Benedetti et al., 2007). The placebo response has been studied extensively in experimental pain research (Benedetti et al., 2007, Benedetti and Piedimonte, 2019, Colloca and Benedetti, 2007). However, the same can not be said for the nocebo response due to ethical constraints associated with inflicting harm on participants (Colloca and Benedetti, 2007). A decrease or increase in anxiety has been reported to be one of the main mechanisms contributing to the placebo and nocebo responses, respectively, in experimental pain research (Benedetti et al., 2007, Benedetti and Piedimonte, 2019). Increased anxiety has been associated with increased pain severity ratings through upregulation of central processes contributing to pain (Colloca and Benedetti, 2007). Positive expectations reduce anxiety, thus may have an analgesic effect. Negative expectations increase anxiety, thus may have a hyperalgesic effect (Benedetti and Piedimonte, 2019). Interestingly, neither Matre et al. (2006) nor van den Broeke et al. (2014) assessed and compared anxiety levels before and after their manipulations. A recommendation for future

[10] van den Broeke et al. (2013) referred to the manipulation as “negative expectation”. However, as explained in the *Risk of bias* section, van den Broeke et al. (2013) did not specifically assess participants’ expectations and report whether their manipulations indeed did induce negative expectations. Therefore, in this current review, this manipulation was renamed “negative suggestion”.

research on placebo and nocebo responses in experimental pain research would be to assess for a change in anxiety.

This author argues that Matre et al. (2006) did not provide the control participants with neutral information, and instead may have introduced a nocebo response. Participants in the control group were informed "the aim of the study was to investigate hypersensitivity of the skin after heat stimulus". This information may have introduced negative expectations and therefore greater reported pain. Researchers conducting pain studies with the placebo response must ensure to provide control participants with neutral information only, to avoid inducing negative expectations.

An additional recommendation for future research would be for researchers to assess *both* the intensity and area of SH. van den Broeke et al. (2014) assessed intensity of SH only. This is a possible limitation because participants in the experimental group may have felt socially obligated to report increased sensitivity after being informed by an authoritative figure (the researcher) that their skin would "become more sensitive to the pinprick stimulator". van den Broeke et al. (2014) should have also assessed the area of SH, out of participants' view.

The results in this current review suggest the placebo and nocebo responses can manipulate SH through targeting central actions. However, further research is required into the specific mechanisms of the placebo and nocebo responses leading to a change in SH.

Hyperbaric oxygen therapy

Hyperbaric oxygen therapy ($HB0_2$) successfully decreased the area of SH in two experimental studies included in this current review (Rasmussen et al., 2015, Wahl et al., 2019) but, interestingly, failed to influence primary hyperalgesia (Wahl et al., 2019). This may be because primary hyperalgesia is thought to be peripherally mediated and $HB0_2$ primarily influences centrally mediated processes (Casale et al., 2019). A recent narrative review reported $HB0_2$ to be an effective treatment for fibromyalgia, a centrally mediated chronic pain condition (Atzeni et al., 2020). Therefore, $HB0_2$ may be a promising therapeutic modality for neuropathic pain.

Interestingly, both Wahl et al. (2019) and Rasmussen et al. (2015) reported an ordered effect: As described in the *Results* section above, Wahl et al. (2019) and Rasmussen et al. (2015) conducted crossover studies where sessions were separated with a washout period of at least 28 days. Despite this washout period, participants who received the $HB0_2$ manipulation in session one had a smaller area of SH in session two (when receiving the induction under ambient conditions) than those who received the induction under ambient conditions in session one and under $HB0_2$ in session two. proposed that this may be due to "a protective, preconditioning effect of the $HB0_2$", leading to long lasting neuroplastic changes induced by the $HB0_2$ at session one and still present at session two

(under ambient conditions). This author proposes another possible explanation: the placebo response, but only if participants were *not* effectively blinded to the research hypothesis.

In both studies it was unclear whether participants were blinded to the research hypothesis. Participants received "sparse information" about the experimental procedure (Wahl et al., 2019) but the exact details of the information given were not reported. Both studies reported that blinding to group allocation was not possible because of the difficulties of having a reliable sham procedure conducted in an unpressurised hyperbaric chamber. These challenges were not reported in both papers; therefore, this author consulted a research colleague with vast experience in conducting experimentally research with hyperbaric oxygen therapy to discuss potential challenges with blinding. Some of the challenges may be that: 1) blinding would not be compelling as participants would soon realise that the chamber is not pressurised due to the lack of needing to equalise pressure build up between the middle ear and the atmosphere, and 2) even when unpressurised, participants would be exposed to a higher partial pressure of oxygen inside the chamber, therefore not accurately exposing participants to ambient conditions. A blinding assessment to ensure participants were blinded to the research hypothesis was not conducted in either study. Therefore, it is not possible to confidently conclude whether participants were effectively blinded to the research hypothesis. It is plausible that participants that participants knew researchers were investigating the analgesic effect of $HB0_2$. Therefore, participants receiving the $HB0_2$ at session one may have had positive expectations about the analgesic effects of $HB0_2$ that were still present at session two.

There are few randomised controlled trials on the use of $HB0_2$ for treating chronic pain. More clinical research is required on this potentially promising treatment modality to determine its effectiveness for treating neuropathic pain.

High attentional and cognitive loading

High attentional loading successfully decreased the intensity of SH in one of three studies (Torta et al., 2019) (Experiment 2), but had no effect in the remaining two studies (Kóbor et al., 2009, Torta et al., 2019) (experiment 1). There is a large body of evidence reporting that attention is closely associated with reported pain severity: when participants' attention is focused on the painful stimuli they report increased pain severity and when participants' attention is focused away (distracted or cognitively loaded by another task) from the painful stimuli they report decreased pain severity (Bantick et al., 2002, Arntz et al., 1991, de Wied and Verbaten, 2001, Legrain et al., 2009, Wiech et al., 2005, Vlaeyen and Linton, 2000, Rode et al., 2001). However, there is limited research investigating whether high attentional and cognitive loading influences SH. The results of this current review suggest that a difficult, high engaging task can influence the intensity of SH.

Sleep disruption

Sleep disruption successfully increased the area of SH in male participants; there was no effect in female participants (Smith et al., 2018). Sleep disruption is commonly associated with chronic pain; however, the direction of causation is unclear (Lautenbacher et al., 2006). A clinical study including patients with burn injuries reported poor self-reported sleep significantly predicted greater pain the subsequent day, however, greater pain during the data did not significantly predict poor self-reported sleep the subsequent night (Raymond et al., 2001). There are conflicting reports on the effect of sleep deprivation on pain pressure thresholds: some studies reported sleep deprivation reduced pain pressure thresholds (Moldofsky et al., 1975, Moldofsky and Scarisbrick, 1976, Lentz et al., 1999) and some reported no effect (Drewes et al., 1997, Older et al., 1998, Arima et al., 2001); however, there is little research on the effect of sleep deprivation on the area and intensity of secondary hyperalgesia. It has been proposed that frequent sleep disruption, rather than prolonged sleep deprivation, influences endogenous opioid activation, leading to decreased inhibition and thus increased pain (Smith et al., 2007). The results of this current review are in line with reports from Smith et al. (2007): sleep deprivation increased the area of SH in male participants only. It is unclear why there was no effect in female participants. Aside from obvious hormonal differences between the male and female participants, another possible reason for why sleep deprivation effectively manipulated SH in male participants only, is that male participants had approximately 25 minutes less sleep than female participants. Further research is required to investigate physiological and hormonal changes in response to sleep deprivation and how this may influence SH in both males and females.

Nicotine deprivation

Nicotine deprivation successfully increased the intensity and area of SH (Ditre et al., 2018). Nicotine activates alpha-4-beta-2 nicotinic receptors and endogenous opioids, leading to an analgesic effect, thus nicotine deprivation among smoker is thought to increased pain through inactivation of these descending inhibitory mechanisms (LaRowe et al., 2018). An experimental study on a cohort of smokers investigating nicotine deprivation on pain severity reported an increase in pain 12 – 24 hours after the manipulation (LaRowe et al., 2018). Interestingly, participants with no pain at baseline were approximately 3.5 times more likely to report pain after the manipulation. This supports other reports that nicotine has an analgesic effect (Ditre et al., 2016, Cosgrove et al., 2010, Cosgrove et al., 2009). The current results are in line with the findings of LaRowe et al. (2018): nicotine deprivation increases intensity and area of SH within 12 – 24 hours after the manipulation. The duration of increased pain after initiation of nicotine deprivation is unclear. This author recommends that researchers explore the duration increased hyperalgesia to nicotine deprivation.

Transcranial direct current stimulation

Anodal transcranial direct current stimulation targeting the motor cortex successfully decreased the area of SH; however, the effect was no better than sham transcranial direct current stimulation. Cathodal transcranial direct current stimulation had no effect (Meeker et al., 2019). Transcranial direct current stimulation is thought to decrease pain through activation of the anterior cingulate and anterior midcingulate cortex leading to descending inhibition of nociception from the cortex to the spinal cord (Meeker et al., 2019, Fregni et al., 2006). A critical review reported motor cortex stimulation effective in reducing neuropathic pain (Fontaine et al., 2009). However, all studies included in that review did not have effective blinding strategies and control comparisons. The findings in this current study are in line with those of previous reports that anodal transcranial direct current stimulation is more effective in reducing pain than cathodal transcranial direct current stimulation (Mylius et al., 2012, Vaseghi et al., 2015, Vaseghi et al., 2014). Although transcranial direct current stimulation has been reported to be effective in reducing pain, anodal transcranial direct current stimulation was no better than the sham in reducing the area of SH this current review. Therefore, this manipulation may be not be effective in influencing SH.

Principal site of action: controversial

Two manipulations were considered to have a controversial main site of action. These manipulations were acupuncture (Rebhorn et al., 2012) and spinal manipulation therapy (Mohammadian et al., 2004).

Acupuncture

Acupuncture is a form of traditional Chinese medicine which has been used for treating pain for thousands of years (Staud and Price, 2006). The mechanisms by which acupuncture achieves an analgesic effect are unclear (Rebhorn et al., 2012). One proposed mechanism is that acupuncture targets central actions by stimulating the release of endorphins, which in turn inhibits ascending nociception from the spinal cord to the brain (Lin and Chen, 2008). Another proposed theory is that acupuncture targets peripheral actions by stimulating A-beta nerve fibres at the periphery, which results in competing stimuli at the dorsal horn of the spinal cord (Melzack, 1976). These competing stimuli inhibit nociception from the spinal cord to the cortex. This modulation of stimuli at the dorsal horn of the spinal cord is termed The Gate Control Theory (Melzack, 1996), as discussed in Chapter 1.

Acupuncture failed to decrease the area of SH in this current review (Rebhorn et al., 2012). There are conflicting data on the effectiveness of acupuncture for treating pain. Four systematic reviews reported acupuncture to be effective in treating pain associated with peripheral joint arthritis (Manheimer et al., 2010), tension-type headache (Linde et al., 2016), neck disorders (Trinh et al., 2016), and as a migraine prophylaxis (Linde et al., 2009). Three systematic review reported data were inconclusive for the effectiveness of acupuncture for treating shoulder pain (Green et al., 2005), lower back pain (Furlan et al., 2005) and lateral elbow pain (Green et al., 2002). One systematic review reported acupuncture to be ineffective for treating pain associated with rheumatoid arthritis (Casimiro et al., 2005). Acupuncture has been researched extensively: eight systematic review on the effects of acupuncture for different pain conditions were conducted by the Cochrane Group between 2002 – 2016. Despite the vast data, acupuncture is still a controversial treatment for pain. This may indicate that it is not an appropriate treatment managing chronic pain conditions.

Spinal manipulation therapy

Spinal manipulation therapy successfully decreased the area of SH in this current review (Mohammadian et al., 2004). Similarly, to acupuncture, spinal manipulation therapy targets central actions by stimulating the release of endorphins, which in turn inhibit ascending nociception from the spinal cord to the brain (Vernon et al., 1986), and targets peripheral actions through modulation of afferent stimuli at the dorsal horn of the spinal cord is termed The Gate Control Theory (Melzack, 1996). Spinal manipulation therapy has been reported to be inferior to exercise for reducing chronic neck pain (Bronfort et al., 2001). Interestingly, a systematic review reported spinal manipulation therapy to be effective in reducing acute lower back pain but no better than the sham for chronic lower back pain (Bronfort et al., 2004). These findings were interesting since SH is a common feature of chronic pain. Mohammadian et al. (2004) had a high risk of reporting bias and it was unclear whether participants were effectively blinded to the research hypothesis and group allocation. Further research, with effective blinding strategies – including blinding assessments, need to be conducted to confirm the efficacy of spinal manipulation therapy for reducing SH.

Secondary outcome: baseline data

No studies reported baseline data on the intensity of SH after induction and before manipulation of secondary hyperalgesia. Only three of 21 studies reported baseline surface area of SH data. The failure to assess baseline data contributes to measurement bias and the failure to report baseline data contributes to reporting bias. Baseline measurements of intensity and area of SH are essential for establishing the efficacy of the induction method and estimating the potency of the manipulation by comparing the pre-manipulation (baseline) to the post-manipulation assessments (i.e. intensity and/or area of SH).

Two difference induction methods were used by the three studies that reported baseline area of SH before manipulation: burn injury (Salomons et al., 2014, Matre et al., 2006) and topical capsaicin (Mohammadian et al., 2004). Matre et al. (2006) reported an area of 62.5 cm^2 at session one and 52 cm^2 at session two after a burn injury induction. Possibilities for this decrease in area across the two sessions are discussed below. Interestingly, Salomons et al. (2014) reported a slightly a smaller mean area after a burn injury induction: 45.0 cm^2, despite their burn injury being significantly longer (28 minutes vs. five minutes) and being of similar temperatures (mean of 48.3 °C for the experimental group and 47.9 °C for the control group vs. 46 °C for all participants) to that of the burn injury reported by Matre et al. (2006). The mean baseline area of SH induced by topical capsaicin was smaller than that of the area induced by a burn injury: 40 cm^2 (Mohammadian et al., 2004).

Discrepancies in the magnitude of induced surface area of SH were recorded in two studies (Matre et al., 2006, Mohammadian et al., 2004). As explained in the *Results* section, Mohammadian et al. (2004) conducted a crossover study (i.e. participants received both the manipulation and the sham on separate occasions and data were reported for all participants before the manipulation and the sham), therefore, there should be no difference in baseline surface area ratings prior to each manipulation. However, there was a discrepancy in the pooled baseline surface area before the spinal therapy manipulation (46 cm^2) and non-spinal therapy sham manipulation (36 cm^2). Possibilities for this discrepancy in area are: 1) ineffective blinding of participants, 2) ineffective blinding of researchers conducting the outcome measurements, and 3) lack of standardisation of the assessor conducting the outcome measurements. Addressing points 1) and 2), Mohammadian et al. (2004) reported that participants and researchers conducting the outcome measurements were blinded to group allocation; however, blinding was not formally assessed. It is plausible that blinding of participants and outcome assessors could have been broken. Addressing point 3), it was not reported whether the same assessor conducted the outcomes measurements for both groups. It is plausible that two researchers conducted the outcome measures, one for each manipulation, and

did not use a standardised approach, leading to the discrepancy in baseline area data prior to each manipulation.

Matre et al. (2006) assessed surface area of SH after a burn injury induction at two independent testing sessions prior to participants receiving a placebo analgesia manipulation or sham manipulation. The manipulation only occurred at a third independent session (sessions were separated by four to seven days). The median (IQR) for the area of SH was different between participants receiving the placebo analgesia manipulation and those receiving the sham manipulation at both session one and session two. Although participants were randomly assigned to each group those who were to receive the placebo analgesia manipulation at session three had a much larger area of SH at session one than those who were to receive the sham manipulation at session three (75 cm^2 vs 50 cm^2). The area of SH at session two was smaller than at session one for participants who were to receive the placebo analgesia manipulation: 75 cm^2 in session one and 49 cm^2 in session two. Interestingly, the opposite occurred for participants who were to receive the sham manipulation, where the area of SH at session two was larger than at session one: 50 cm^2 at session one and 55 cm^2 session two.

A possible reason for this discrepancy in the trend of surface area measurements across session one and two, could be the recruitment strategy and the different information told to participants in each group (placebo analgesia manipulation vs sham manipulation). Participants in the placebo analgesia group were recruited with a separate advertisement to those in the sham manipulation group. Participants in the placebo analgesia group were informed “the aim of the study was to test the analgesic effectiveness of a magnet against heat pain”. This information was on the advertisement and was repeated at the first session. This information may have been priming participants to expect an analgesic effect at each session, even though the magnet was only present at the third session. Participants in the sham manipulation group were informed “the aim of the study was to investigate hypersensitivity of the skin after a heat stimulus”. This information may have been priming participants to expect pain to increase at each session. This is a hypothesis based on findings reported by van den Broeke et al. (2014) (study included in this review) that negative expectations increase intensity of SH. Therefore, researchers need to be careful of wording on recruitment advertisements, study information sheets and any other verbal instructions or explanations given to participants in experimental pain research. The positive or negative association with the wording may increase or decrease pain ratings.

Secondary outcome: pain during induction

Intensity of pain during induction varied based on induction method. Pain during induction and *before* manipulation of SH were reported for the following three induction procedures: intradermal capsaicin injection (n = 2 studies), topical capsaicin application (n = 2 studies) and thermal injury (n = 2 studies). The reported pain ratings during induction were similar across the two studies for each induction method. The most painful induction method was intradermal capsaicin with mean pain ratings of 7.8 (Yucel et al., 2001) (experiment 2) and 7.5 (Pud et al., 2006) (reported on 0 – 100 ratings scale and rescaled to 0 – 10). The second most painful induction method was topical capsaicin application with mean pain ratings of 4.05 (Yucel et al., 2001) (experiment 1) and 4.0 (Mohammadian et al., 2004). Surprisingly, the least painful induction method was burn injury with pain ratings of 3.15 (Wahl et al., 2019) and 3.35 (Werner et al., 2002).

Low-frequency electrical stimulation induction was performed *during* the manipulation procedure in two experiments reported in a single paper (Torta et al., 2019). The mean pain ratings during induction were 6.8 (experiment 1) and 6.6 (Experiment 2). Therefore, low-frequency electrical stimulation may be the second most painful induction method behind intradermal capsaicin injection if delivered under conditions of high cognitive loading.

There is limited research directly comparing different methods of experimentally inducing sSH. It was out of the scope of this review to directly compare the effectiveness of each induction method, but in fact such a comparison would have been impossible because only two studies reported baseline data from SH assessments. Based on the limited published data available, it is not possible to conclude which induction method induces the most potent model of SH.

Researchers planning experimental pain research should be mindful of these pain ratings during induction when deciding which induction procedure to use. Intradermal capsaicin was reported to be a lot more painful than a burn injury. Researchers must uphold bioethical principles when undertaking experimental research. When deciding which induction procedures to use, researchers must ensure non-maleficence and not put participants through potentially unnecessarily painful experiments when less painful options are available. The current data suggest that a burn injury induction would be less painful to an intradermal capsaicin injection induction. However, further research comparing the effectiveness of induction methods is indicated to allow researchers to make an ethical and informed decision planning induction procedures for experimental pain research.

Limitations of this review

Rescaling of pain rating scales from 0 – 100 to 0 – 10 opens the data to possible inaccuracies. The discrepancies with anchor labelling across the studies and to a greater extent the use of labeling sections of the scales as representing "non-painful" sensations (Torta et al., 2019, Matre et al., 2006) added to the imprecision and limited the reliability of comparing ratings data. Standardisation of anchors for sensation and pain ratings scales ensure is required to allow for accurate comparisons of different induction and manipulation modalities.

Much of the data were extracted from plots, with no raw data being reported. Some plots were not clearly visible. Even though data were extracted in duplicate, independently and discrepancies were compared, there could still be inaccuracies in the data points read from the plots. Additionally, some studies included 3-D bar graphs. Although the evidence is not conclusive, it has been argued that 3-D bar graphs may be more difficult for the reader to examine and produce more inaccuracies in judgement than when examining a 2-D bar graph (Siegrist, 1996). To combat these limitations, researchers need to make their raw data accessible to the public through online platforms such as Open Science Framework, for example (Piedra et al., 2016).

Open access to raw data will also assist with combating high clinical heterogeneity. In this current review, here was high clinical heterogeneity among the study designs, which limited the ability of conducting the meta-analysis. Open access to raw data allows researchers to be able to get unbiased estimates of the effect size when conducting meta-analyses.

Lastly, the blinding strategies were poor in the included studies in this review. None of the studies assessed if blinding was upheld among participants and researchers. This opened studies up to unclear and high risks of performance and measurement bias. Researchers conducting experimental research needed to be mindful of this and ensure to thoroughly report on blinding strategies and always assess if blinding was upheld among both participants and researchers.

Strengths of this review

To this author's best knowledge, this is the first review to systematically identify, appraise and compare non-pharmacological modalities used to manipulate experimentally induced secondary hyperalgesia. This review provides some insight into the mechanisms underlying SH and guides researchers in terms of choices for future research.

Conclusion

In conclusion, 21 non-pharmacological manipulations used to manipulate SH were identified by this review. Nine (of 12) successfully increased or decreased the magnitude of SH, as assessed by the intensity and/or area of SH. Manipulations hypothesised to mainly target central actions were more effective in manipulating SH than manipulations hypothesised to mainly target peripheral actions. This may indicate the SH is centrally, rather than peripherally mediated.

Chapter 3: Experimental study

Introduction

Chronic pain can be partially attributed to structural and functional changes in the central and peripheral nervous systems, referred to as central and peripheral sensitisation, respectively (Sandkühler and Gruber-Schoffnegger, 2012, van Wilgen and Keizer, 2012). Some of these changes cause the nervous system to respond more strongly to input, specifically within the neuronal synapses of the dorsal horn of the spinal cord (Sandkühler and Gruber-Schoffnegger, 2012). This enhanced synaptic activity is termed 'spinal long-term potentiation' (LTP) because it resembles the hippocampal long-term potentiation that is thought to support memory (Ji et al., 2003). Spinal LTP underlies the amplification and prolongation of nociceptive afferent stimuli (Nickel et al., 2012). As such, it is an important physiological mechanism that contributes to central sensitisation and increased pain sensitivity.

Spinal LTP likely contributes to three important clinical phenomena that are commonly found in patients with neuropathic pain: allodynia, primary hyperalgesia and SH (Jensen and Finnerup, 2014). Allodynia is increased sensitivity to a stimulus that is normally non-painful (Merskey and Bogduk, 1994). Hyperalgesia is formally defined as "increased pain from a stimulus that normally provokes pain" (Merskey and Bogduk, 1994) and can occur within the area of tissue damage (primary hyperalgesia) and/or adjacent to the area of tissue damage (secondary hyperalgesia). Although spinal LTP can be assessed using multiple outcomes, this study will focus on these three specific indicators of spinal LTP.

There are many experimental methods to induce SH (refer to the systematic review in Chapter 2 where many methods for inducing SH are discussed), including repeated thermal stimulation (Salomons et al., 2014), high-frequency electrical stimulation (HFS) (Henrich et al., 2015), low-frequency electrical stimulation (Klein et al., 2004), and intradermal or topical application of capsaicin (Serra et al., 2004). High-frequency electrical stimulation (HFS) is a reliable method to induce an experimental (i.e. short-lived) form of SH (Klein et al., 2008, Klein et al., 2004, Vo and Drummond, 2013, van den Broeke and Mouraux, 2014). The half-life of SH after HFS induction was 4.9 hours, in a sample of 55 healthy human subjects (Pfau et al., 2011). This indicates that HFS is a robust method for inducing short-lived SH.

Reports (refer to systematic review in Chapter 2) have shown that experimentally induced SH can be manipulated with pharmacological and non-pharmacological modalities (Andersen et al., 1996, van den Broeke et al., 2014, Ditre et al., 2018). For example, cognitive behavioural therapy intended to reduce negative emotions and thoughts related to pain has been shown to reduce thermally induced SH (Salomons et al., 2014). However, the mechanisms underlying this effect are unclear. The effect could be attributed to a reduction in the threat value of the stimulation, or to other consequences of the cognitive behavioural therapy intervention, such as inducing neuroplastic changes in the regions of the brain associated with descending inhibitory control of nociception, leading to a decrease in central sensitisation at the dorsal horn of the spinal cord. Additionally, inducing negative expectations of pain sensitivity increases the intensity of SH after HFS induction (van den Broeke et al., 2014). These reports indicate that SH can be manipulated with non-pharmacological modalities, via descending cortical mechanisms.

Pain is known to be influenced by the threat value of a stimulus (Arntz and Claassens, 2004, Wiech et al., 2010b). Instructions signifying that a stimulus will be of high intensity appear to increase reported pain intensities of the stimulus, despite the intensity of the stimulus being unchanged (Moseley and Arntz, 2007). The meaning behind a stimulus - for example, the degree to which a person feels threatened that the stimulus can cause tissue damage - is subjective (Arntz and Claassens, 2004). Personal and cultural beliefs, previous experiences, and expectations all play a role in determining the threat value of a stimulus. However, the physiological mechanisms by which threat influences pain are unclear. Specifically, it is not known if threat influences the extent to which synaptic activity is enhanced after sustained or intense neural signalling (spinal LTP). Therefore, the aim of this study was to investigate the effect of a manipulation of the threat value of a stimulus on experimentally induced SH in healthy human volunteers.

The objectives of the study were:

a) to differentially manipulate the threat associated with high-frequency electrical stimulation at two different sites within each participant;

b) to assess and compare (1) intensity ratings (primary outcome) and (2) surface area (secondary outcome) of experimentally induced SH (as assessed using mechanical punctate stimulation) between the sites, and

c) to assess and compare intensity ratings given (exploratory outcomes) for static light touch, dynamic light touch, and a single electrical stimulation, before and after the experimental induction of SH, between the sites.

It was hypothesised that greater threat will be associated with (hypothesis 1) greater intensity and (hypothesis 2) greater surface area of induced SH. Data obtained from assessing static light touch, dynamic light touch and single electrical stimulation will be used for exploratory purposes only.

Methods

Study design

The study was designed as a within-subject, double-blinded, experiment. It was conducted at the University of Cape Town, South Africa. The protocol was approved by the Faculty of Health Sciences Human Research Ethics Committee (REF 498/2019) (Appendix 7), University of Cape Town.

Piloting procedure

An extensive piloting procedure was conducted to ensure the effectiveness of the threat manipulation procedure. This author predicted that it may be difficult to make participants feel threatened due to the controlled environment associated with experimental research paradigms. Throughout the piloting procedures, this author conducted open interviews with participants after each piloting procedure to obtain feedback on the effectiveness of the threat manipulation. Below is a summary of the piloting process including the objectives of each stage, themes arising from the post-procedure interviews, outcomes of each stage and amendments made to the threat manipulation (Tables 11, 12 and 13).

Piloting: stage one

Table 11 Outlining the objectives, outcome, reflections, and strategies used to overcome the challenges of piloting stage one

Number of participants: n = 10	**Objective:** To assess the effectiveness of a visual threat manipulation consisting of images of injured (assumed to be threatening - experimental) and uninjured (control) upper limbs. **Themes arising from post-procedure interviews:** - Difficulty in obtaining images of arms belonging to a variety of skin colours – potentially influencing the relatability of the threat manipulation to participants with difference skin colours to those in the images. - Participants were not concerned about potential tissue damage to their own arm while viewing images of other people's injured arms. - The threat manipulation was not personal enough and therefore did not evoke a sense of threat in the participants. **Outcome:** The visual threat manipulation was not effective in producing a plausible threat of tissue damage in the participants. **Amendments to the threat manipulation:** Goal: to make the threat more personal - the threat manipulation was changed to be an audio voice recording explaining the potential risks of the HFS procedure. - Possessive pronouns, such as '*your* skin', were used to make the threat more personal.

Piloting: stage two

Table 12 Outlining the objectives, outcome, reflections, and strategies used to overcome the challenges of piloting stage two

Number of participants: n = 9	**Objective:** To assess the effectiveness of an audio threat manipulation consisting of threatening (experimental) and non-threatening (control) information about the HFS procedure. This is a similar threat manipulation to that used by Van Damme et al. (2008) in which participants were given either threatening or neutral information about a cold pressor test prior to immersing their hand into the bucket of cold water. Using self-reported anxiety and catastrophic thoughts about the cold pressor test as the manipulation check, Van Damme et al. (2008) concluded that this threat manipulation was effective. **Themes arising from post-procedure interviews:** - Participants trusted that the HFS procedure would not be harmful and assumed the researchers were being excessively cautious. - Participants who were socially acquainted with the researchers were particularly trusting and did not find the threat manipulation compelling. - Again, the threat manipulation was not personal enough to evoke a personal threat of tissue damage. **Outcome:** The audio threat manipulation was not effective in producing a plausible threat of tissue damage in the participants. **Amendments to the threat manipulation:** Goal: To make the threat manipulation even more personal. - The threat manipulation was further adapted to a sham skin examination procedure. In this procedure the integrity of participants' skin around the HFS site was 'assessed' (sham). - Where possible, this author avoiding recruiting people who were socially acquainted with the researchers involved in this project.

Piloting: stage three

Table 13 Outlining the objectives, outcome, reflections, and strategies used to overcome the challenges of piloting stage three

Number of participants: n = 6	**Objective:** To assess the effectiveness of a sham skin examination procedure for the threat manipulation. This is a similar threat manipulation to that used by Wiech et al. (2010). In that study, a sham skin integrity test was conducted in which areas of the skin at the site intended to receive laser stimulation were initially categorised as either "fully approved" for the delivery of laser stimulation or "approved with reservations". Participants were informed that the sites that were "fully approved" were strong enough to withstand the laser stimulus. But the site "approved with reservations" needed close monitoring to prevent tissue damage during the laser stimulation. Using pain ratings from the laser stimulus as a manipulation check, Wiech et al. (2010) concluded this threat manipulation to be effective in their cohort. **Themes arising from post-procedure interviews:** - The researcher conducting the experiment was able to figure out which arm was receiving the HFS under a condition of threat and which was not, thus rendering them unblinded. During this piloting stage, participants were randomised into two groups: Group 1 received the HFS under a condition of threat on the *right* arm and Group 2 received HFS under a condition of threat on the *left* arm. The researcher conducting the experiment knew whether participants were in Group 1 or Group 2 but was naïve to site allocation for each group, i.e. they were not informed which arm received the HFS under a condition of threat in each group. However, after conducting informal interviews with the researcher, it became apparent that the researcher was able to fairly accurately estimate site allocation, based on participants' facial expressions during the HFS procedure and verbal feedback from participants after the procedure. Therefore, rendering them unblinded for the subsequent participant. - This threat manipulation was conducted on a very small cohort. Therefore, it was underpowered to perform statistical comparisons to determine whether there was a significant difference in anxiety ratings and/or threat ratings between site allocation (i.e. which arm received the HFS under a condition of threat). However, based on informal feedback discussions with participants, this threat manipulation was deemed to be the most compelling. **Outcome:** - Threat manipulation deemed compelling, based on informal feedback discussions from participants.

	Amendments to the threat manipulation: Goal: ensure blinding of the researcher conducting the experiment. To achieve this goal, three researchers were involved in running the procedure to ensure blinding of the researcher conducting the outcome measurements. The first, independent researcher randomly allocated participants to site allocation, i.e. which arm would receive the HFS under a condition of threat. A second, independent assistant designated the site allocation in the Affect5 programme. The third researcher conducted the full HFS procedure and outcome measurements without being informed of the site allocation. Additionally, the third researcher (conducting the full HFS procedure and outcome measurements) completed a blinding assessment where they were asked two questions: 1) “Which arm do you think received the HFS under a condition of threat?” and 2) “How confident are you in your answer to the previous questions?” (answered on a on a five-point Likert scale consisting of “not at all confident”, “not confident”, “neutral”, “confident”, “extremely confident”). On completion of piloting of the threat manipulation, the procedures for the full study was initiated.

Recruitment

Volunteers were recruited from the general public using advertisements, social media channels such as Facebook and Twitter, and word of mouth (Appendix 8). Volunteers were sent the study information sheet outlining the details of the procedure via email. Volunteers were screened for exclusion criteria by completing an online eligibility quiz using the Responster platform (https://responster.com) (Appendix 9). After completing the screening quiz, eligible volunteers were contacted via email to organise a booking. Volunteers not eligible for this study were thanked for their interest and given the opportunity to have their details added to the research team's database for notifications about future studies. Participants were able to withdraw from the study at any stage during the procedure or up to 48 hours after the procedure. They were compensated ZAR100[11], in cash, for their time and inconvenience, even if they withdrew from the study. However, no participants withdrew.

Inclusion and exclusion criteria

Volunteers needed to be healthy, pain-free adults between the ages of 18 – 65 (to control for the possible presence of age-related peripheral loss of sensation), be able to consent autonomously, and be fluent in speaking, understanding and reading English (all as per volunteers' self-reports).

Volunteers were excluded from the study if they reported one or more of the following: chronic pain – pain for most days for the past three months (Blyth et al., 2001), pain on the day of testing, self-reported pregnancy, electronic implant (e.g. pacemaker), any kind of heart/cardiovascular problem, diabetes mellitus, neurological problems (e.g. epilepsy), peripheral vascular disease, problems with skin healing, use of analgesics within 24 hours before testing, use of medication that could alter skin sensitivity or healing (analgesic medication, topical medical creams or immune modulators, for example), history of psychiatric problems (fear or anxiety disorder, or clinical depression, for example), and previous participation in this study or a closely related study. Additionally, volunteers with upper limb tattoos distal to the anode were ineligible to participate as some tattoo inks contain metals. Tattoo ink containing iron oxide (usually within black pigmented tattoos) theoretically can conduct an electrical current (Ross and Matava, 2011). Therefore, applying HFS near these tattoos could cause minor skin burns. Due to difficulty identifying the composition of tattoo ink, all volunteers with tattoos at or distal to the intended site of the anode were excluded from participating in this study. Table 14 summarises the inclusion and exclusion criteria.

[11] On 13 March 2020 ZAR100 was equivalent to USD6,21

Table 14 Inclusion and Exclusion Criteria to be eligible to participate in the experimental study

Inclusion criteria	Exclusion criteria
Between the ages of 18 - 65	Chronic pain (pain most days for the past three months)
Provide written informed consent	Pain on the day of testing
	Usage of analgesics within 24 hours of testing
	Sensory impairments
	Mental illnesses (depression and anxiety were considered mental illnesses for this study)
	Self-reported pregnancy
	Electrical or metal implants
	Upper limb tattoos distal to anode.
	Cardiovascular conditions
	Diabetes mellitus
	Neurological problems e.g. epilepsy
	Peripheral vascular disease
	Usage of medication that could alter skin sensitivity or healing (e.g. analgesic medication, topical medical creams or immune modulators)

Roles of researchers involved in this study

Three researchers and one research assistant were involved in this experimental study. Each person had specific roles to ensure blinding, where necessary, and to ensure that the procedure was well-organised and ran smoothly.

Researcher 1:

- Conducted randomisation of participants for site allocation, i.e. which arm (left or right) would receive the HFS under a condition of threat.
- Re-coded the participants' site allocation prior to statistical analysis to ensure blinding of Researcher 3 during analysis.

Researcher 2:

- Conducted the experimental procedure and sensory testing.

Researcher 3:

- Conducted the statistical analysis.

Research assistant:

- Designated the site allocation in the Affect5 programme to ensure blinding of Researcher 2 during the experimental procedure and sensory testing.

Randomisation and blinding

Participants received the HFS on both forearms separately. High-frequency electrical stimulation was delivered to one arm under a condition of threat (experimental) and to the other arm under a neutral condition (control), thus providing a within-subject comparison. An Excel sheet was used to randomise site allocation. First, 13 instances of both Group 1 (receiving the HFS under a condition of threat on the *right* arm) and Group 2 (receiving the HFS under a condition of threat on the *left* arm) were entered into Excel to account for a sample size of 26 (see *Sample size calculations* below). The list was then randomised (Appendix 10). Second, papers stating either 'Group 1' or 'Group 2' (13 for each group) were placed into 26 opaque envelopes. The envelopes were numbered on the outside to match the order specified by the Excel sheet. Third, the envelopes were used in the order specified by the numbers written on them. Researcher 2 (who conducted the assessments) gave the sealed envelope to the research assistant who opened the envelope and designated the site allocation in the Affect5 programme while Researcher 2 was outside the room. Researcher 2 was thus unaware of participants' site allocation.

Blinding of participants

Participants were blinded to the study goals: they were informed that the researchers were investigating how people experience painful and non-painful stimulations.

Blinding of the researchers conducting the experiment

Researcher 2, who conducted the measurement and sensory testing, was blinded to site allocation, i.e. which arm (right or left) received the HFS under a condition of threat, thus mitigating verification bias. Researcher 2 completed a blinding assessment after participants received the HFS and before the sensory testing battery. The blinding assessment consisted of two questions: first, Researcher 2 was asked which arm she thought received the HFS under a condition of threat. If she was uncertain, she was instructed to guess. Second, Researcher 2 rated her confidence in her answer to the first question on a five-point Likert scale consisting of "not at all confident", "not confident", "neutral", "confident", "extremely confident".

Blinding of the researchers undertaking statistical analyses

Researcher 3, who was performing the statistical analyses, was blinded to site allocation while conducting the analyses. Prior to the statistical analysis being performed, Researcher 1 re-coded participants' site allocation. As explained above, participants in Group 1 received the HFS under a condition of threat on their *right* arm (left arm was the control site) and participants in Group 2 received the HFS under a condition of threat on their *left* arm (right arm was the control site). Participants' site allocation was re-coded to "Condition A" or "Condition B", as follows:

Condition A:

- Participants in Group 1 and ratings data from their right arm
- Participants in Group 2 and ratings data from their left arm

Condition B:

- Participants in Group 1 and ratings data from their left arm
- Participants in Group 2 and ratings data from their right arm

Therefore, condition A consisted of outcome measurements on the arm receiving the HFS under a condition of threat and condition B consisted of outcome measurements from the arm not receiving the HFS under a condition of threat. Condition A and B were interpreted by Researcher 3 after all analyses were completed.

Sample size calculation

Pilot data and the GLIMMPSE online calculator (Kreidler et al., 2013) were used to estimate the sample size required to achieve 80% power to detect a minimum 5-point difference on the Sensation and Pain Ratings Scale (SPARS) (see explanation of scale below), which is a scales that has a 100-point range, in SH with alpha set at 0.05. A mixed linear regression was planned, in which the dependent variable was the mean SPARS rating to both pinprick stimulators (128 and 256 mN) at each time point after HFS, minus the equivalent mean rating at the baseline time point (before HFS). The model structure allowed each participant to have their own intercept (i.e. individual participant (ID) was a random factor). The independent variable 'condition' (i.e. experimental or control site) was a fixed factor, and the repeated measures variable 'time' was nested within and fully crossed with ID, because each participant was assessed at each time point. In the lme4 package (Loy and Hofmann, 2014, Bates et al., 2015) of R (version 3.5.3 (2019-03-11)), the model structure would be: lmer(rating ~ condition + (1|ID/time).

This study focused on detecting a main effect of condition. GLIMMPSE estimated that a sample size of 25 participants was required. Therefore, a sample size of 26 was used to allow for counterbalancing for the manipulation site.

Equipment

High-frequency electrical stimulation was provided using a constant current stimulator (DS7A, Digitimer Limited, Hertfordshire, UK). The electrical impulses were controlled by the software programme Affect5 (Spruyt, 2010). Current was directed from the DS7A to a pair of electrodes. The electrodes consisted of two cathodes and two anodes. The cathodes had 10 blunt steel pins arranged in a circle (Fig 2) and were secured to both anterior forearms. The anodes were large surface electrodes (Fig 3), which were secured to both upper arms (Fig 4).

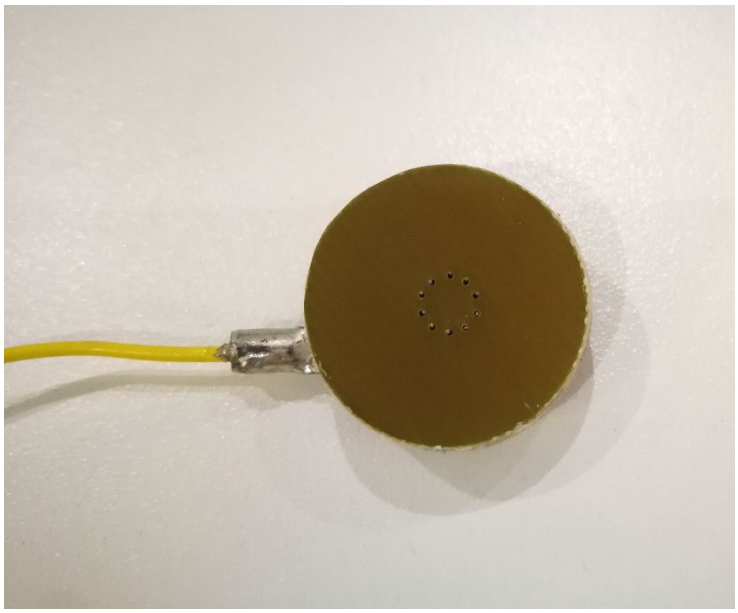


Figure 2 Image of cathode with 10 blunt steel pins arrange in a circle

Figure 3 Image of large surface electrode used as an anode

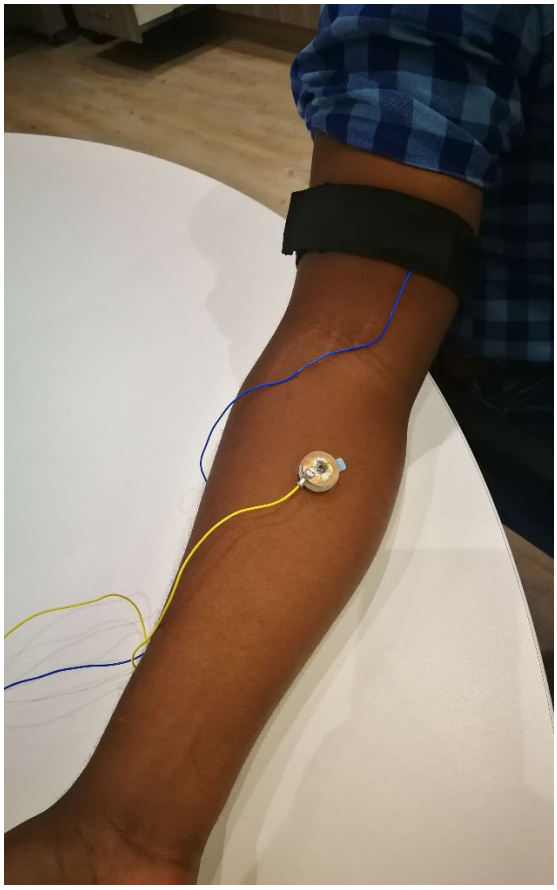


Figure 4 Image of arrangement of cathode secured with double-sided tape to the participant's anterior forearm and anode secured with Velcro to participant's mid-upper arm

Manipulated variables

High-frequency electrical stimulation

The cathodes were secured on the anterior aspects of both the participant's forearms, with a double-sided sticker, approximately eight centimetres distal to the cubital fossa, and avoiding any visibly prominent vasculature. Large surface electrodes were placed around both upper arms and served as the anodes.

The appropriate intensity of the HFS depended on the electrode used and individual's electrical detection threshold. The electrodes in this study most closely resembled those used by Klein et al. (2004), Klein et al. (2008) and Henrich et al. (2015). Their work and pilot work done by this current study's authors have shown robust SH with HFS delivered at 100Hz, at a current of ten times the individual detection threshold.

Participants were orientated to the electrical stimulus (refer to the *Procedure* section) and the stimulus was calibrated to the participant's individual electrical detection threshold. This calibration consisted of single electrical stimuli, with a pulse width of two milliseconds. An adaptive staircase approach was used to determine the individual electrical detection threshold. The electrical detection threshold was used to determine the intensity of the HFS: ten times the electrical

detection threshold. Klein et al. (2004) reported participants' electrical detection threshold to be 0.11 ± 0.06 mA (mean ± SD). Therefore, it was anticipated that the range of currents would be similar in this current study.

The high-frequency electrical stimulation consisted of five one-second trains, two milliseconds pulse width, of 100 Hz frequency, with a nine second break between sequential trains. The intensity of the stimulation was ten times the participant's individual electrical detection threshold.

Threat manipulation

The threat manipulation procedure was similar to that used by Wiech et al. (2010) and consisted of a sham skin examination. In that study a sham skin integrity test was conducted whereby areas of the skin at the site of laser stimulation were initially equally categorised into two categories: "fully approved" and "approved with reservations". Half of the skin sites were graded as strong enough to withstand the laser stimulus (the "approved" category) and the other half were graded as needing close monitoring by the participant during the laser stimulation to prevent tissue damage (the "approved with reservations" category). This sham skin test is presumed to have resulted in a perception that the laser stimulus would be more threatening at certain skin sites compared to others. Participants reported increased pain severity and anxiety when the laser stimulus was applied over the skin sites categorised as "approved with reservations" than when it was applied to the "approved" skin sites. Furthermore, the functional magnetic resonance imaging found that the threat manipulation resulted in increased activity within the mid-cingulate cortex and anterior insula – areas of the brain associated with the perception of threat – during the application of the laser stimuli. Similarly, in this current study, the sham skin examination was conducted after the baseline sensory assessment and before participants received the HFS. Researcher 2 informed the participants that they were examining the robustness of the skin around the electrodes, while using an otoscope to shine light onto the skin and magnify the view of the area. The purpose of the sham skin examination was framed as to determine the risk of skin damage associated with that site undergoing HFS. The (sham) results were shown on a computer screen not visible to the researcher (Fig 5, 6, 7 and 8). For each participant, the experimental site was deemed "approved with reservations" on the screen, with participants instructed to monitor their "fragile" skin closely during the HFS as there was "moderate risk of injury". For the control site, "fully approved" was reported on the screen, with participants informed that the skin is "robust" and there was "low risk of injury".

Figure 8 Sham skin examination results shown on computer screen to each participant. This image was used to induce a context of threat of HFS stimulation at the right forearm

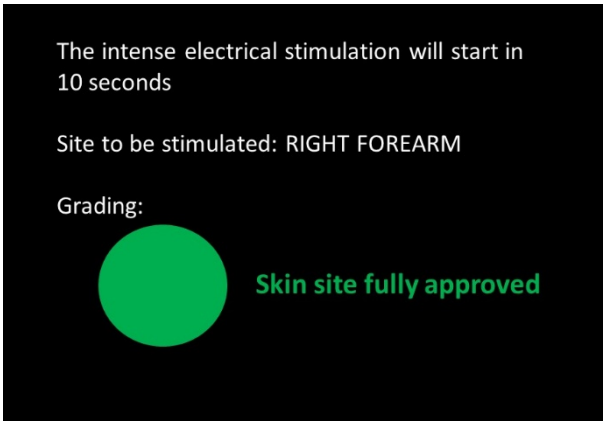


Figure 7 Sham skin examination results shown on computer screen to each participant. This image was used as a control for the right forearm.

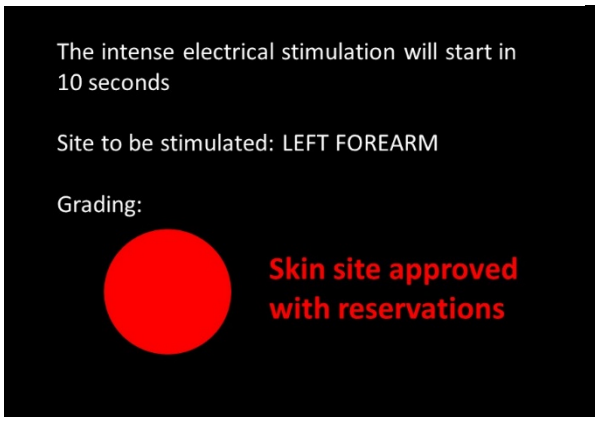


Figure 6 Sham skin examination results shown on computer screen to each participant. This image was used to induce a context of threat of HFS stimulation at the left forearm.

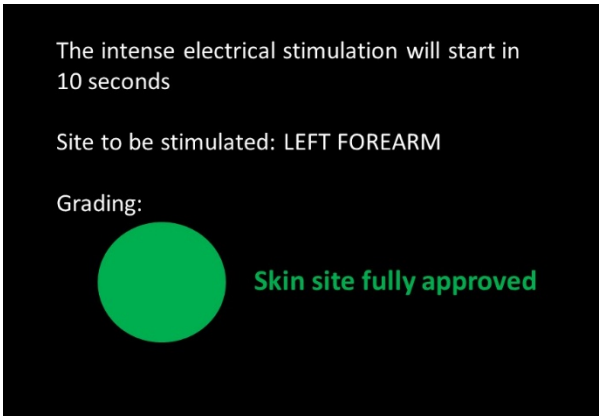


Figure 5 Sham skin examination results shown on computer screen to each participant. This image was used as a control for the left forearm.

Threat manipulation check

The effect of the manipulation was assessed by comparing 1) pain ratings, 2) self-reported anxiety, and 3) self-reported fear of tissue damage between each arm during the HFS induction. A mixed model analysis was used to compare ratings of the HFS trains between each arm. A main effect of condition (receiving the HFS under a condition of threat or neutral conditions) on ratings was tested, whilst allowing for a random intercept for each participant.

Measured variables

Participants verbally reported sensation or pain ratings using the SPARS (Fig 9) (Madden et al., 2016, Madden et al., 2019b, Madden et al., 2019a). This scale is useful as it provides for a range of non-painful and painful sensory experiences. The non-painful range, on the left-hand side of the scale, ranges from -50 – "no sensation" – to 0 – "the exact point at which what you feel transitions to pain". The SPARS is sensitive to change in both painful and non-painful sensory experiences (Madden et al., 2019a).

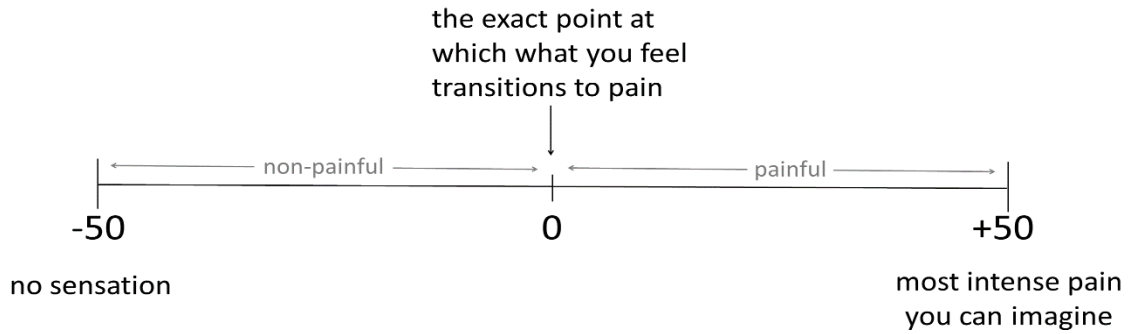


Figure 9 Sensation and Pain Rating Scale (SPARS)

Outcome measurements

Primary outcome

Mechanical punctate stimulation

Mechanical punctate stimulation was provided with two pinprick stimulators (MRS Systems, Heidelberg, Germany), exerting forces of 128mN and 256mN. Increased SPARS ratings to these modalities in the region surrounding the distal electrode, after the HFS, indicated the presence of SH. The mean SPARS ratings of the two different pinprick weights were used to determine the overall mechanical punctate stimulation rating.

Secondary outcome

Mapping surface area of secondary hyperalgesia

The area of SH was mapped using the eight-radial-lines approach (Fig 10 and 11) and a pinprick stimulator exerting a force of 128mN (You et al., 2014).

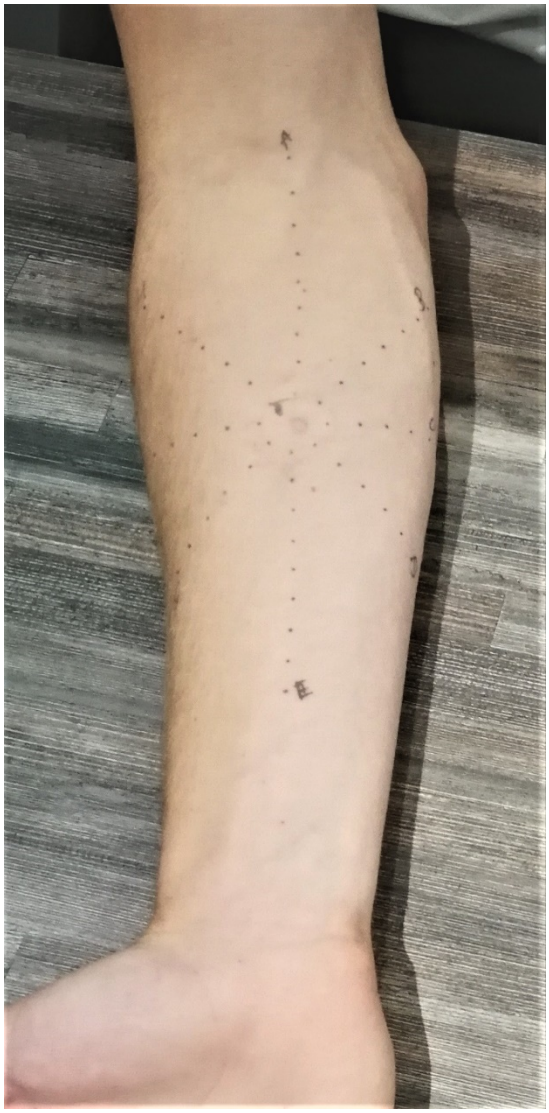


Figure 11 Image showing the mapping of the 8 radial lines at 45° angles on a participant's forearm. Each dot was separated by 1 centimeter.

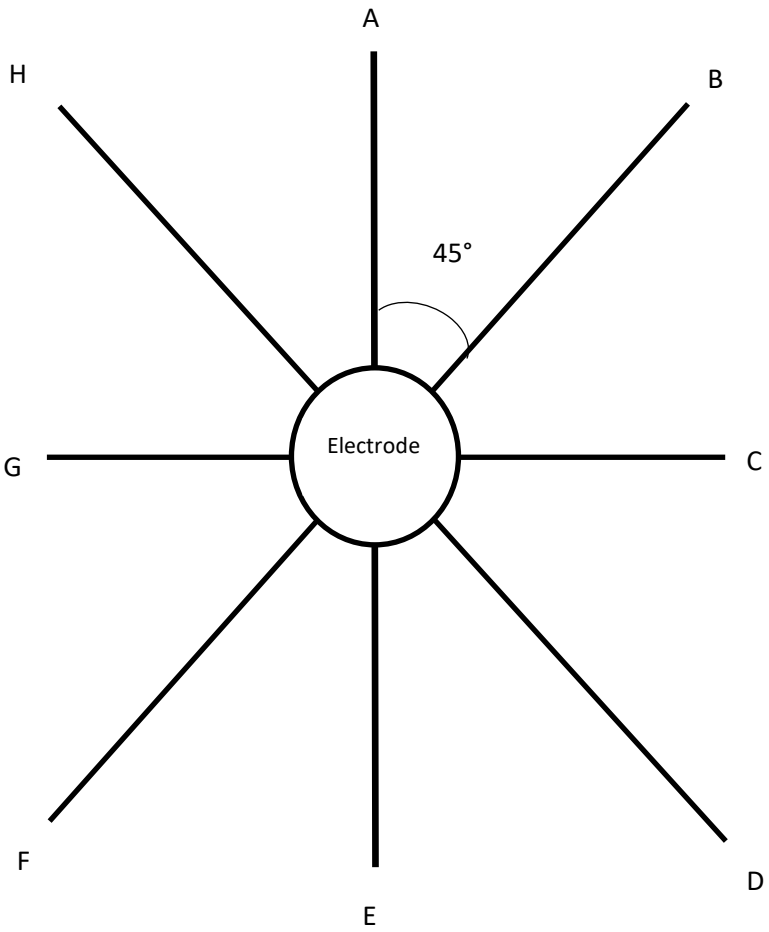


Figure 10 Graphical representation of the 8 radial lines, intersecting at 45° angles, labelled A – H.

Exploratory outcomes

Static light touch

Static light touch sensation was assessed with application of a von Frey filament (MARSTOCK, Schriesheim, Germany) that exerted a force of 32mN upon bending (Rolke et al., 2006).

Dynamic light touch

Dynamic light touch was measured using a cotton wisp and soft brush stroke. Henrich et al. (2015) reported increased non-painful dynamic light touch sensation using cotton wool and a soft brush stroke one to two centimetres over the skin surrounding the area where the stimulus was provided. In that study participants were asked to report pain (with the instruction that any sensation felt as pricking or burning should be reported as painful) on a numerical rating scale, where "0 = no pain and 100 = most intense pain imaginable". This approach was adapted slightly in this current study by asking participants to report their sensation or pain ratings using the SPARS. Dynamic light touch stimulations are typically perceived as non-painful in unsensitised areas as they only activate low-threshold mechanoreceptors (Leem et al., 1993). Therefore, allodynia was deemed present if participants indicated pain to dynamic light touch after HFS.

Single electrical stimulation

A single electrical stimulation was used to assess primary hyperalgesia. The electrical stimulus was two milliseconds long with an intensity of ten times the individual's electrical detection threshold (Henrich et al., 2015).

Questionnaires

A history of previous trauma has been associated with increased area of SH (You et al., 2016). Women reporting childhood trauma and/or recent trauma displayed a larger surface area of SH after application of topical capsaicin than women without a history of trauma (You et al., 2016). Therefore, data from the Childhood Trauma Questionnaire short form (CTQ-SF) and a modified version of the World Health Organisation Composite International Diagnostic Interview for post-traumatic stress disorder (WMH-CIDI) were used in a secondary analysis, to investigate the relationship between a history of trauma and experimentally induced secondary hyperalgesia, in an attempt to replicate the work by You et al. (2016a). Participants also completed several other questionnaires: 10-item Connor-Davidson Resilience Scale, Cohen's Perceived Stress Scale, Pain Catastrophising Scale, Multidimensional Scale of Perceived Social Support, and 16-item Pain Vigilance and Awareness Questionnaire, which were used for exploratory analyses to inform the development of future research questions only. Therefore, data from all those questionnaires have not been reported in this current study. The full list of questionnaires includes:

1. Basic demographic and participant information questionnaire, which included the following items: participant ID code, group allocation, age, sex, ethnicity, medication use, caffeine intake, hand dominance, detection threshold on each arm, and time and day of procedure (Appendix 11).

2. Childhood trauma and adult trauma were screened for using the Childhood Trauma Questionnaire (CTQ-SF) (Bernstein et al., 2003) and a modified version of the World Mental Health Survey Initiative version of the World Health Organisation Composite International Diagnostic Interview for post-traumatic stress disorder (WMH-CIDI) (Kessler et al., 2004), respectively. The CTQ-SF focuses on 5 criteria: emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect (Appendix 12). The modified version of the WMH-CIDI has questions drawn directly from the WMH-CIDI (Kessler et al., 2004). The modified version aims to determine whether participants have been through specific traumatic events. It was used as an inventory and the researcher did not investigate the details of the traumatic event (Appendix 13).
3. A customised questionnaire was used to check the threat manipulation. After the procedure, participants were asked to indicate on a five-point Likert scale the extent to which they agreed or disagreed with the following statements: "At the time of receiving the intense electrical stimulation, I was concerned that it would cause damage to my skin on my right/left arm" and "At the time of receiving the intense electrical stimulation on my right/left arm, I felt anxious". Participants completed these questions with reference to each arm separately (Appendix 14).
4. Resilience was assessed using the 10-item Connor-Davidson Resilience Scale (CD-RISC-10) (Campbell-Sills and Stein, 2007), which is a validated, shortened version of the 25-item Connor-Davidson Resilience Scale (CD-RISC) (Appendix 15).
5. Stress was assessed using Cohen's Perceived Stress Scale (PSS) (Lee, 2012, Cohen, 1988) (Appendix 16).
6. Pain catastrophising is associated with increased pain sensitivity (Sullivan et al., 2005). Therefore, the Pain Catastrophising Scale (PCS) – a valid and reliable outcome measure – was used to assess participants' catastrophic thoughts about pain (Sullivan et al., 1995). The instructions and text of this scale were modified to prompt participants to respond with reference to their thoughts during the HFS (Appendix 17).
7. Pain can be influenced by perceived social support. Social support has been shown to decrease pain intensity (López-Martínez et al., 2008). Therefore, participants' perceived social support was assessed using the Multidimensional Scale of Perceived Social Support (MSPSS) (Zimet et al., 1990). The MSPSS is a 12-item scale on which participants respond to certain statements about the support they receive from family, friends and a significant other using a 7-point Likert-type scale ranging from "very strongly disagree"

to “very strongly agree”. It has been shown to be valid with a South African cohort (Bruwer et al., 2008) (Appendix 18).

8. Attention to pain and changes in pain were assessed using the 16-item Pain Vigilance and Awareness Questionnaire (PVAQ) (McCracken, 1997). The PVAQ is a valid questionnaire for assessing awareness, intrusion and monitoring of pain in non-clinical samples (McWilliams and Asmundson, 2001) (Appendix 29).

These questionnaires do not require expert training to administer. They were all administered via a computer. To ensure confidentiality, the researcher did not have viewing access to the relevant computer screen while the participant completed the questionnaires.

Semi-structured interview

After the threat manipulation check, Researcher 2 asked participants why they did/did not feel anxious of fearful about the HFS induction. Researcher 2 wrote down direct quotes of participants’ responses. These responses were used to gain further insight into the effectiveness of the threat manipulation.

Post-hoc analyses

Post-hoc analyses were performed on the mean (range) of the Pain Catastrophising Scale (Sullivan et al., 1995) and the 10-item Connor-Davidson Resilience Scale (Campbell-Sills and Stein, 2007). These analyses were not initially planned nor described in this study’s protocol. These analyses were performed for exploratory purposes to inform the development of future research questions.

Procedure

Overview of procedure

The procedure was conducted in a small and quiet room in the Research Laboratory in the Department of Anaesthesia and Perioperative Medicine, Groote Schuur Hospital, Cape Town. The procedure lasted approximately two hours. An overview of the procedure is outlined in Figure 12.

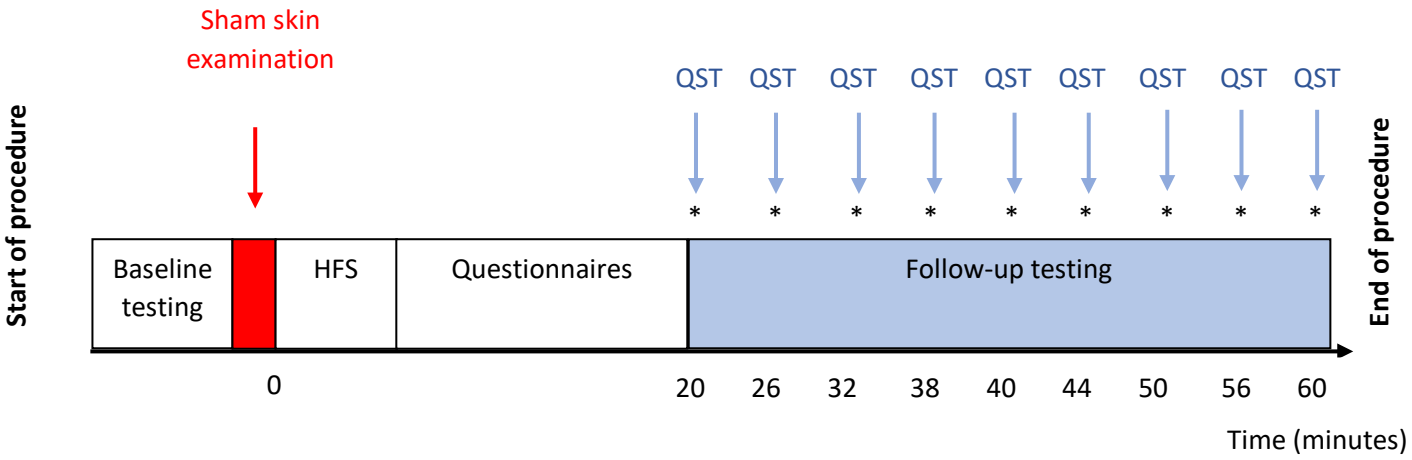


Figure 12 An overview of the experimental procedure. Quantitative sensory testing (QST) occurred at 20, 26, 32, 38, 40, 50, 56 and 60 minutes after the HFS and has been represented at each timepoint by an asterisk.

Researcher 2

A postgraduate student was hired and trained to conduct the experimental procedures (Researcher 2). Researcher 2 underwent extensive training to learn how to perform the HFS procedure safely and conduct the sensory measurements accurately. She used a formal script (Appendix 20) during the procedure to standardise all the information presented to participants. She conducted all the formal data collection.

Preparation

Before the experimental procedure, Researcher 2 used a formal checklist to ensure accurate preparation of the procedure (Appendix 21). Participants were asked to re-read the study information sheet (Appendix 22), confirm that none of the exclusion criteria (Appendix 24) applied, and sign the document of informed consent (Appendix 24). Thereafter, participants were asked to remove any jewellery (rings, watch or bracelets) from their arms and to turn off mobile phones or smart watches so that they would not be distracted by any alerts during the procedure. Participants were clearly briefed about the HFS procedure by Researcher 2.

Researcher 2 used a stencil to mark locations for the electrodes and to mark eight surrounding radial lines, arranged at 45-degree angles to one another, on the participant's skin. The anodes and cathodes were secured to both of the participants' forearms, as described above in the *Manipulated variables* section.

Participants were orientated to the SPARS and the sensory testing battery. This orientation consisted of an explanation of how to use the SPARS and a brief demonstration of each of the sensory tests on Researcher 2's arm (Refer to the script in Appendix 20). Participants had an opportunity to practice using the SPARS while Researcher 2 ran through a practice round of the sensory testing battery.

Individual electrical detection threshold

Participants were orientated to the electrical stimulus and the stimulus was calibrated to the participant's individual electrical detection threshold. This calibration consisted of single electrical stimuli, with a pulse width of two milliseconds. The intensity started at zero and slowly increased until the participants reported that they could feel it. Participants were asked to 'say *yes'* if they felt it, even a little bit. They were informed that the electrical stimulus would feel like a very tiny pinprick. This adaptive staircase approach was used to determine the individual electrical detection threshold. The electrical detection threshold was used to determine the intensity of the HFS: ten times the electrical detection threshold.

Baseline testing

Once the participants were comfortable with using the SPARS, the sensory testing battery consisting of punctate mechanical stimulation, static light touch, and dynamic light touch (refer to O*utcome measurements* above) was conducted six times (three times on each arm) to obtain baseline sensory ratings. Initially, the protocol outlined that primary hyperalgesia will not be assessed at this time point, as the electrical stimulation would not yet be calibrated to the participant. This was an error in the protocol and therefore, there was a deviation from the protocol and baseline primary hyperalgesia was assessed. The area of SH was not mapped at this point as SH had not yet been induced by the HFS.

Sham skin examination

The sham skin examination was performed by Researcher 2 after the baseline testing and before the HFS procedure (see *Threat Manipulation* above). As detailed above, Researcher 2 was blinded to site allocation. A comparison of the HFS SPARS ratings, self-reported anxiety and self-reported fear of tissue damage at each arm served as a manipulation check (see *Threat manipulation check* above).

High-frequency electrical stimulation

Participants were thoroughly briefed on what to expect from the HFS. Participants were informed that most people find the HFS "moderately painful" and they may withdraw with immediate effect at any point during the procedure. They were instructed to say "STOP" if they wished to withdraw, in which case Researcher 2 would have flicked the safety switch on the stimulator to deactivate the stimulator immediately.

Waiting period

There was a waiting period of approximately 20 minutes after the HFS to allow time for the SH to develop. To optimise time, this period was used to administer the questionnaires.

Follow-up testing

The battery of sensory testing was conducted at the following time points after HFS: 20 minutes, 26 minutes, 32 minutes, 38 minutes, 40 minutes, 44 minutes, 50 minutes and 56 minutes. Importantly, the order of the sensory testing modalities was randomised within each time point, for each participant, to decrease predictability and ensure accurate ratings (with the same order used for both arms, within each time point). The surface area of SH was mapped at 20-minute intervals after receiving the HFS, namely: at 20 minutes, 40 minutes and 60 minutes after the HFS.

Post-experiment questionnaire and debriefing

After the follow-up testing, the electrodes were removed, and participants were asked to complete the post-experiment questionnaire. Finally, participants were debriefed on the threat manipulation and reassured about the safety of the procedure by Researcher 2.

Participants completed all the questionnaires in privacy, and on a computer. Details of any traumatic events was not requested. For these reasons, together with the strict eligibility criteria, it was unlikely the questionnaires would have evoked strong emotional responses at the time of testing. Nevertheless, after the procedure, participants were provided with an information pamphlet (Appendix 25) outlining the local non-profit organisations where they could access psychological assistance, if they wished to do so. Additionally, all participants received a list of the community health care centres in Cape Town that provide psychological counselling (Appendix 26) as well as a list of a couple of private practice psychologists within the University of Cape Town's neighbouring communities (Appendix 27).

Statistical analysis

Primary analysis

Data were analysed using a linear mixed modelling approach, so as to account for individual variability in responses whilst still testing for a between-site effect at the group level. The study was designed to have within-subject controls of both pre- and post-induction measurements and control site measurements. Therefore, the change in sensitivity (pre-induction measurements subtracted from post-induction measurements) was compared between arms (within subjects). The exact parameters for the analysis were chosen on the basis of visual inspection of the data (including an assessment of distribution), and the appropriate tests to confirm or refute any assumptions of the analytical strategy. As specified above, the primary outcome was the magnitude of secondary hyperalgesia.

The planned data analysis using the full pilot study data (Appendix 28) was finalised. The protocol (Appendix 29) and the pilot analysis were preregistered with Open Science Framework at https://osf.io/. Preregistration ensures detailed documentation of the research process, therefore supporting accountability and study replication (Lindsay et al., 2016). Initial processing of the formal data had commenced at the time of this protocol being locked online. However, the analysis of the formal data had not been completed. The pilot analysis was not substantively changed after initial processing of the formal data commenced, except that the assessment of the model fit was added, having been omitted from the pilot data analysis.

A mixed linear modelling approach, using the robust 'lmer' option within the lme4 package (Loy and Hofmann, 2014, Bates et al., 2015) was used for the formal data analysis. It allows for both random effects (participant) and fixed effects (threat condition), as used in our sample size calculation. Two models were tried for this analysis: the first was a fully crossed model with ID (rating_controlled ~condition + (1|id/time)). The second model was one in which that assumption was not made (rating_controlled ~ condition + (1|id)). 'Fully crossed with' means that every time point was assessed for every ID. This was indeed the case in this present study's design. Therefore, the fully crossed model most closely represents the design of this experiment. The fully crossed model was compared to the null version of the model (rating_controlled ~ (1|id)) (which does not include condition as a predictor variable). If the ANOVA that compares two models (fully crossed and null version) yields a significant *p*-value then it was concluded that the fully crossed model fits the data better than the null.

It was plausible that the individual calibration approach could have confounded the results, because HFS delivered at a higher current could result in greater SH. This was tested for in the analysis, although a previous, well powered (n = 170, unpublished) investigation of this relationship had found no association.

Researcher 2, who conducted the experiment, was blinded to site allocation. A blinding assessment was performed (as explained above). Cohen's kappa coefficient was determined to ensure blinding was successful. Cohen's kappa ranges from -1 to +1, where '0' represents "random change" and '1' represents "perfect agreement" (McHugh, 2012). Blinding was determined to be successful if Cohen kappa's coefficient was ≤ 0, indicating no agreement between Researcher 2's estimation of site allocation and actual site allocation.

Assessment of model fit for the primary analysis

An assessment of model fit was conducted for both the intensity and surface area analyses. The model assessed for intensity of SH was called model_pre_condition_crossed and had the structure: rating_controlled ~ condition + (1|id/time). The model assessed for area of SH was called model_pre_condition_crossed_sa and had the structure: SA ~ condition + (1|id/time). Four assumptions were assessed. If any of the assumptions was violated, the model was deemed unfit. The four assumptions were: 1) linearity, 2) absence of collinearity, 3) homoscedasticity, and 4) normally distributed residuals (Winter, 2013).

Secondary analysis

A secondary analysis investigated the relationship between a history of trauma and the surface area showing experimentally induced secondary hyperalgesia, replicating the work by You et al. (2016a). In their study, they summed the results of participants' individual scores from the Childhood Trauma Events Scale and the Recent Traumatic Events Scale to obtain an individual stressful life events score. Similarly, in this current study the results of the CTQ-SF and WMH-CIDI were summed. You et al. (2016a) reported a larger surface area of capsaicin-induced SH in participants with a history of trauma than participants without a history of trauma. However, there were no differences in the intensity of SH between those with and those without a history of trauma. In this current study, a univariate regression analysis was conducted to examine whether stressful life events correlate with the area of SH in this sample.

Results

Appendix 30 includes the full data analysis and results.

Participants

Forty people volunteered to participate in this study and completed the eligibility quiz. Fourteen volunteers were excluded for having tattoos distal to the anode (n = 5), chronic pain (n = 5), history of mental illness (n = 3), and unavailable for testing (n = 1). A sample of 26 participants was used for this study. The median age was 21 (range 18 – 55) years old. Of the 26 participants, 16 were female. None of the participants had taken any medication prior to the procedure.

Manipulation checks

HFS intensity ratings

All HFS trains were rated as *'painful',* using the SPARS. The mean (± SD) was 38.77 (± 11.34) at the threatened site and 39.07 (± 11.31) at the control site (Fig 13). These SPARS ratings were not predicted by condition (p = 0.64).

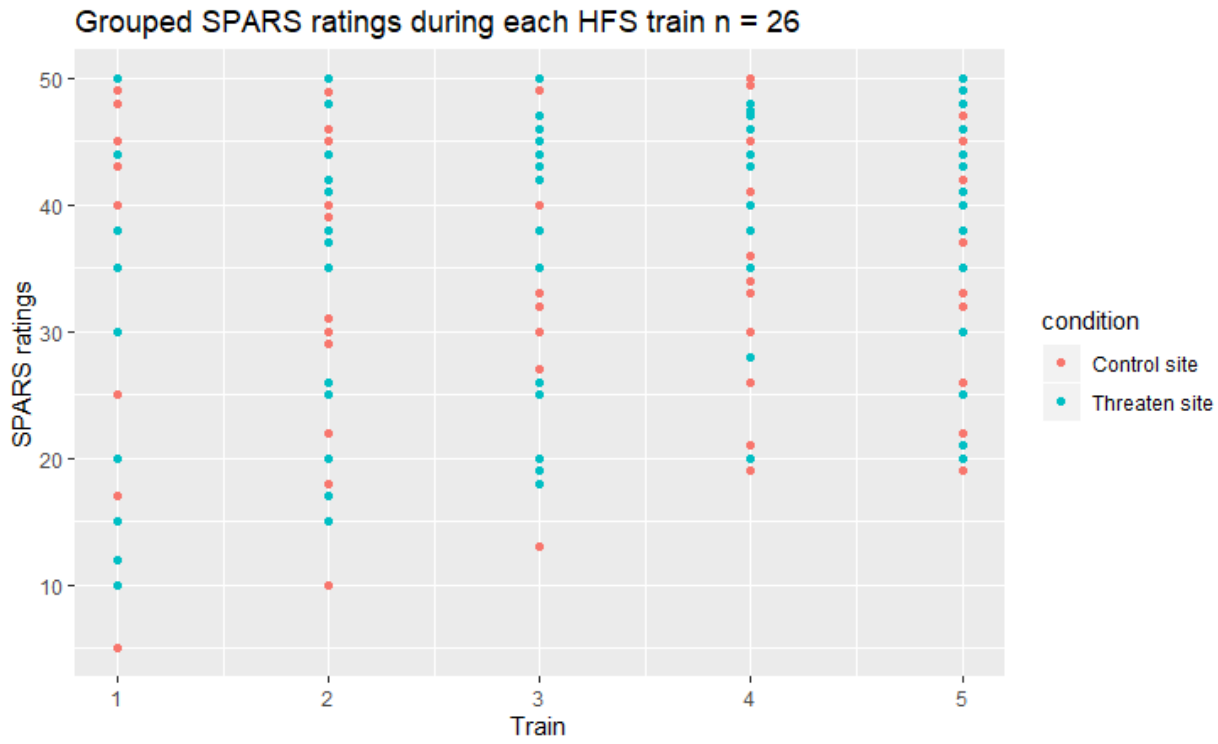


Figure 13 Grouped SPARS ratings during each train

Self-reported anxiety during HFS

The mean (± SD) anxiety ratings were 3.31 (± 1.12) for the threatened site and 3.42 (± 1.14) for the control site out of a maximum of five. The grouped effect of condition was not significantly (p = 0.31) predictive of anxiety ratings (Fig 14).

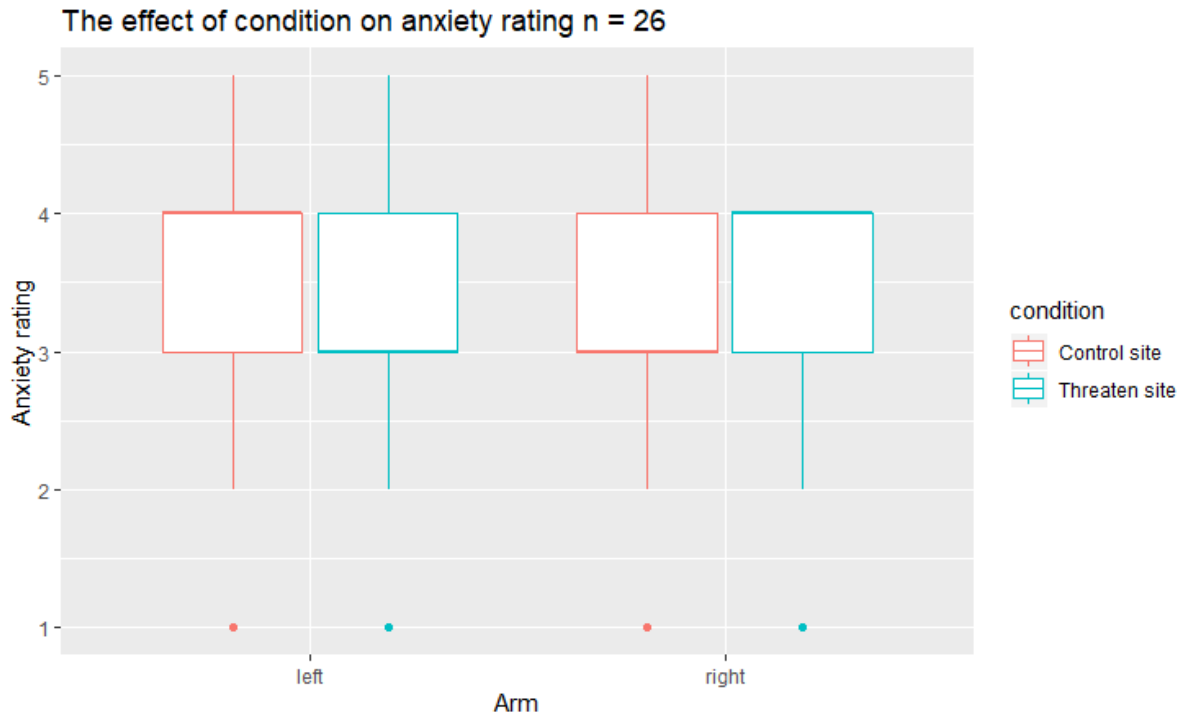


Figure 14 The grouped effect of condition on anxiety ratings

There is no upper line on the box and whisker plot on the far right because the upper quartile value was equal to the maximum value. Additionally, the median anxiety ratings for the control site on the left arm (first box on the left) was the same at the upper quartile. The median anxiety ratings for the threatened site on the left arm was the same as the lower quartile. The median anxiety ratings for the control site on the right arm was the same as the lower quartile. The median anxiety ratings for the threatened site on the right arm was the same as the upper quartile.

Self-reported fear of tissue damage during HFS

The mean (± SD) fear of tissue damage ratings were 2.81 (± 1.30) for the threatened site and 2.50 (± 1.14) for the control site. The grouped effect of condition was not significantly (p = 0.11) predictive of threat ratings (Fig 15).

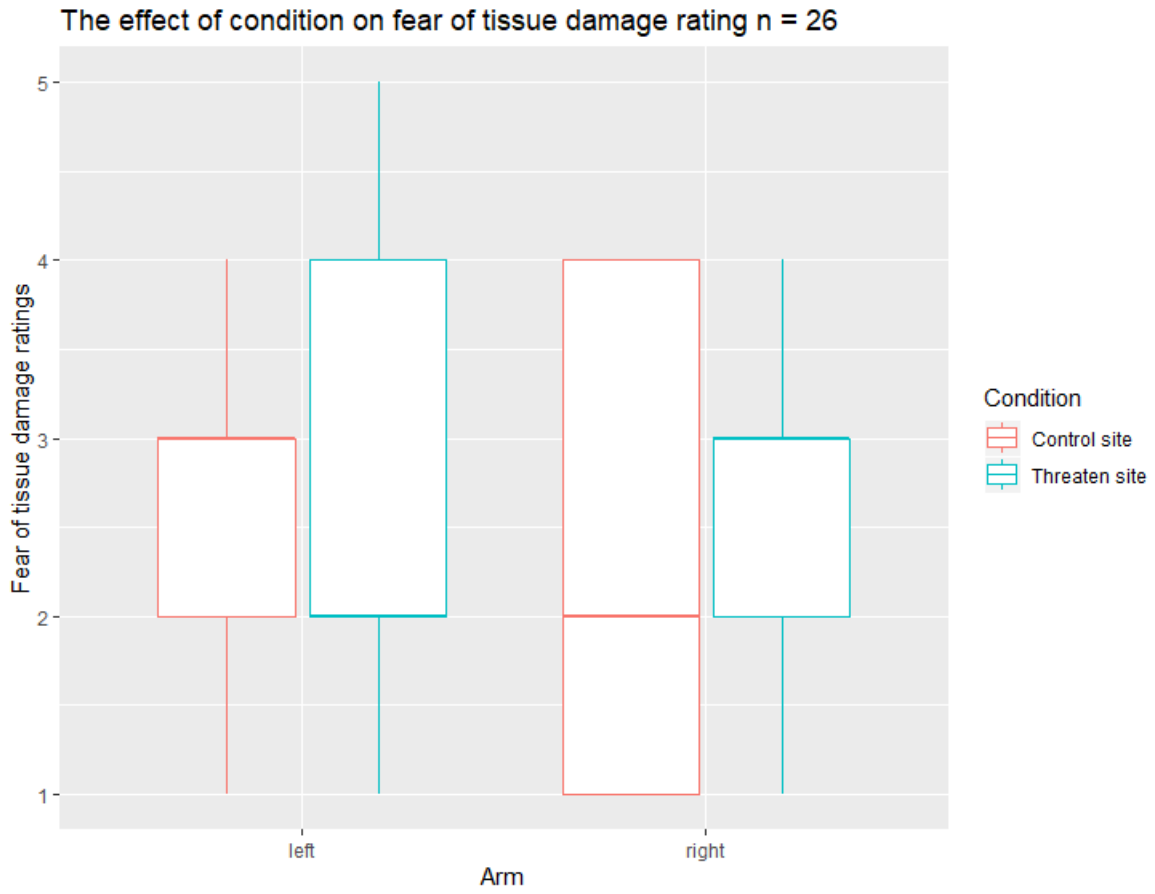


Figure 15 The grouped effect of condition on fear of tissue damage ratings

There are no whiskers on the box and whisker plot on the far right because the upper quartile value was equal to the maximum value and the lower quartile was equal to the minimum value. Additionally, the median threat ratings for the control site on the left arm (first box on the left) was the same at the upper quartile. The median threat ratings for the threatened site on the left arm was the same as the lower quartile. The median threat ratings for the threatened site on the right arm was the same as the upper quartile.

Blinding assessment of the researchers conducting the experiment

A blinding assessment (see section on *Randomisation and blinding* above) was used to determine whether Researcher 2 reliably predicted site allocation (i.e. which arm was receiving the HFS under a condition of threat). Researcher 2 guessed site allocation correctly 42.31% of the time. Cohen's kappa coefficient was calculated to be 0.23, indicating weak agreement between Researcher 2's guess of site allocation and actual site allocation (Calculations in Appendix 30). Therefore, it was confidently concluded that Researcher 2 was effectively blinded to site allocation. Additionally, Figure 16 shows that Researcher 2 made confident assertions that were incorrect. This further indicates that she was effectively blinded.

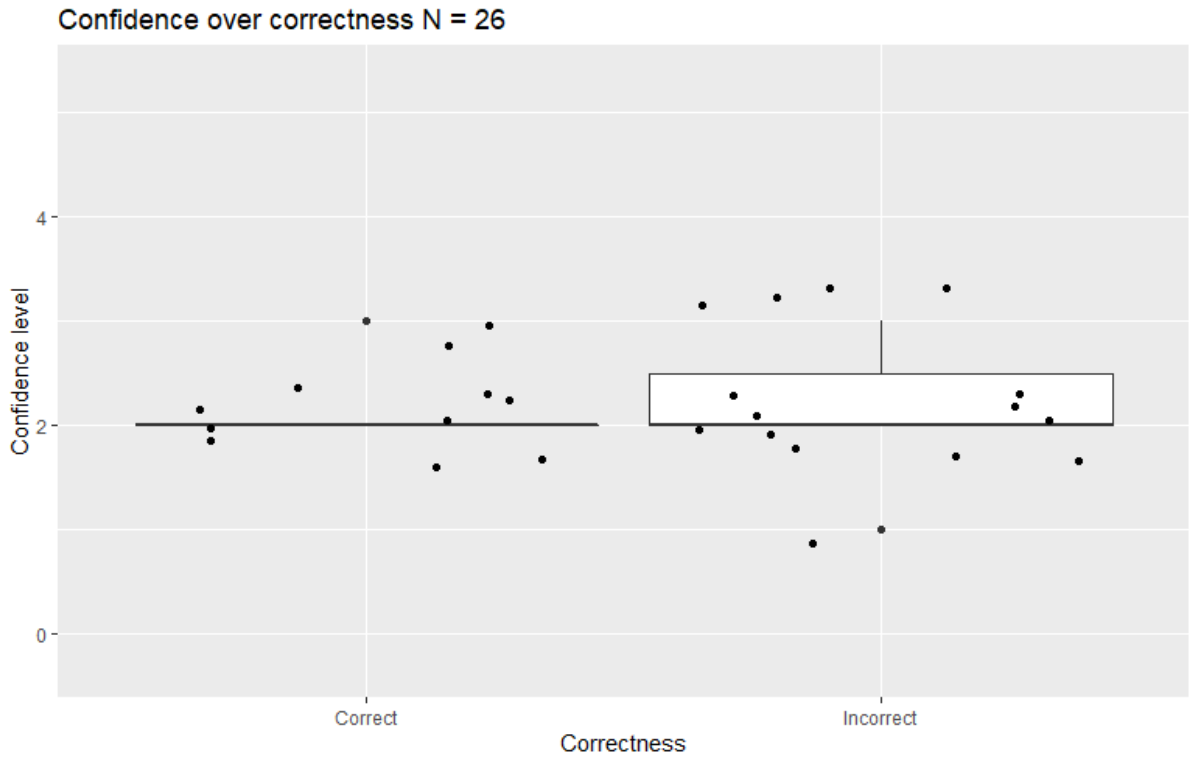


Figure 16 Boxplots displaying the researcher's confidence level over their frequency of correctness

Semi-structured interview

In general, participants reported being more anxious about anticipating the pain associated with the HFS induction rather than the results of the (sham) skin examination. Participants also reported trusting that enough precautions had been taken to ensure the safety of the procedure. Appendix 31 includes quoted responses from participants explaining why they were/were not anxious and/or fearful of tissue damage during the HFS.

Primary analysis

Primary outcome: Mechanical punctate stimulation

The primary aim was to determine the intensity of SH at each site and compare between conditions (site under a condition of threat: threaten site and site not under a condition of threat: control site). Figure 17 displays the intensity of SH over time, grouped by condition. Figure 18 displays the grouped mean intensity of SH over time. There was no clear difference in the intensity of SH between conditions. Intensity of SH was not predicted by condition (p = 0.73) (Fig 19).

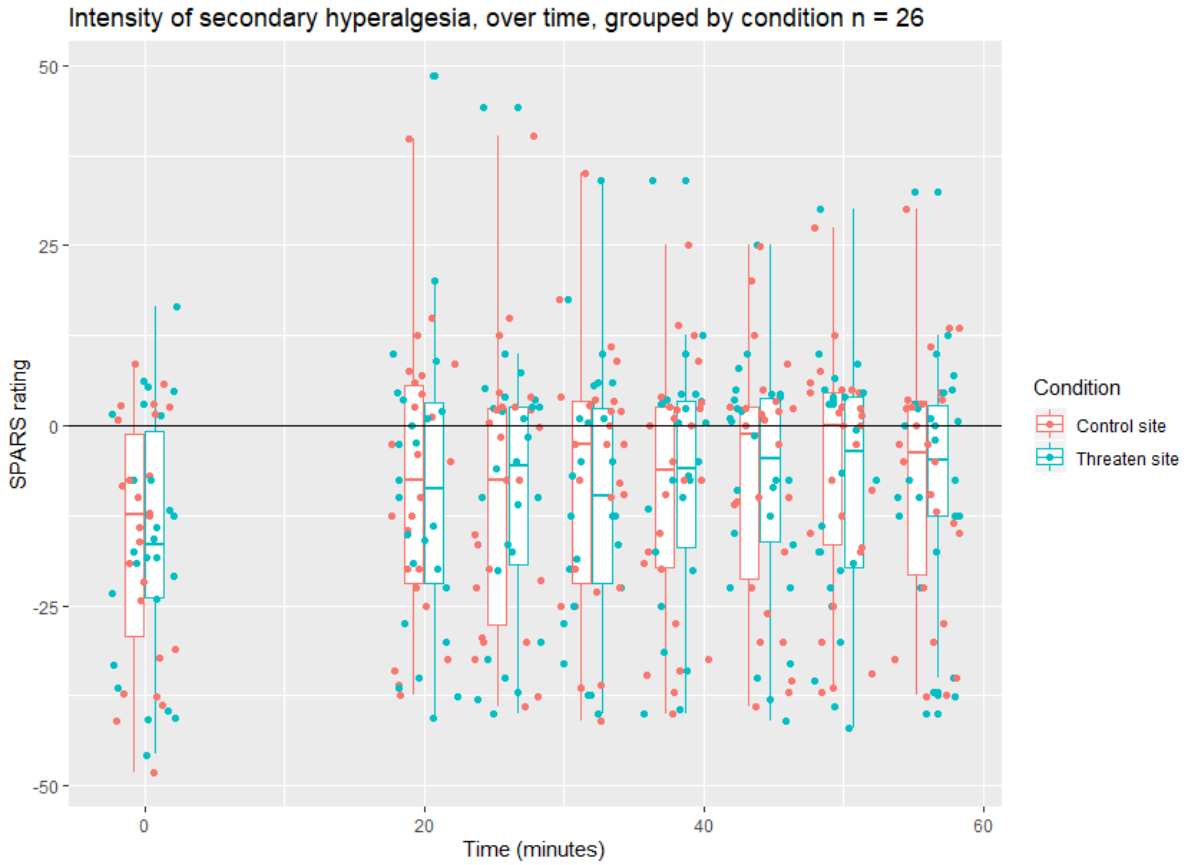


Figure 17 Boxplot displaying the intensity of secondary hyperalgesia over time, grouped by condition.

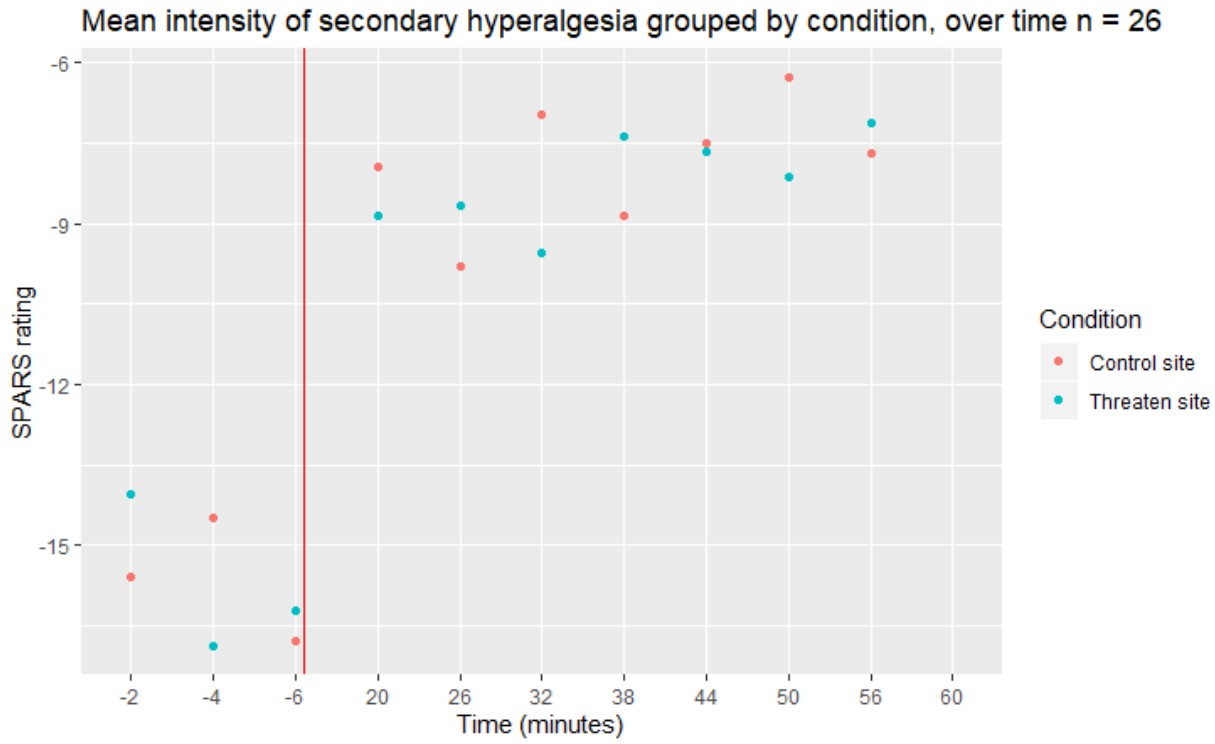


Figure 18 plot describing the grouped mean intensity of secondary hyperalgesia over time. The red vertical line indicates time of HFS induction

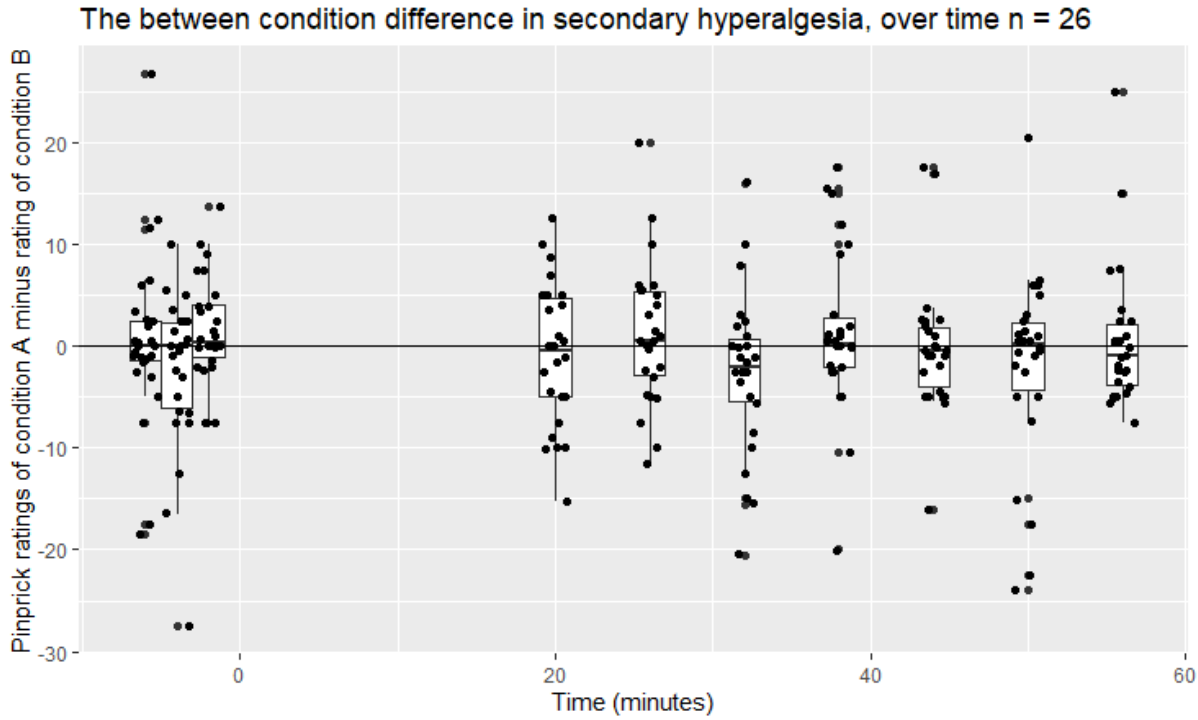


Figure 19 Boxplot describing the difference between condition over time

Assessment of model fit

The model is called model_pre_condition_crossed and has the structure: rating_controlled ~ (1|id/time). All plots can be viewed in Appendix 30, section title: *SH: Assessment of model fit*. First, linearity was assumed by plotting fitted values against residual values, for the fixed factor 'condition'. There was increased density in a blob on the left, but no obvious linear or curvilinear pattern. The assumption deemed to have been upheld. Second, the assumption of absence of collinearity was assessed by checking correlation between time and condition. There was no collinearity observed in the plot and it was deemed that the assumption of absence of collinearity was upheld. Third, assumption of homoscedasticity was assessed, i.e. assessment for equal variance across the range of predicted values. There was increased density in a blob to the left, but the range of the maximum and minimum values seemed consistent across the x-axis. Therefore, the assumption of homoscedasticity was deemed to have been upheld. Fourth, the assumption that residuals were normally distributed was assessed. The Q-Q plot showed extremely minor deviations from the diagonal reference line and the histogram showed acceptable distribution. Therefore, the assumption that residuals were normally distributed was deemed to have been upheld. In conclusion, all four assumptions were upheld by the data, rendering the model suitable.

Assessing for confounding of the intensity of secondary hyperalgesia from individual calibration approach

The mean (± SD) detection threshold and intensity used for the HFS procedure was 1.60 mA (± 0.64 mA). It is plausible that the individual calibration approach could confound the intensity of SH results. This is because HFS delivered at a higher current could result in greater secondary hyperalgesia. Therefore, each participants' maximum recorded intensity of SH was assessed for correlation with their individual current used for the HFS induction procedure. The Shapiro-Wilk Test showed that the data were not normally distributed (p = 0.011). Therefore, a Spearman rank-order correlation test was used to check for a relationship between the calibration current and the peak intensity of secondary hyperalgesia. The peak intensity of SH was determined for each participant individually, i.e. the maximum SPARS rating for each ID code was used. There was no significant correlation between the calibration current and the peak intensity of SH (rho = 0.040; p = 0.78).

Secondary outcome

Mapping surface area of secondary hyperalgesia

The secondary aim was to determine the surface area of SH on each arm and compare between arms. Figure 20 displays the grouped area of SH over time. Secondary hyperalgesia surface area was not predicted by condition (p = 0.16).

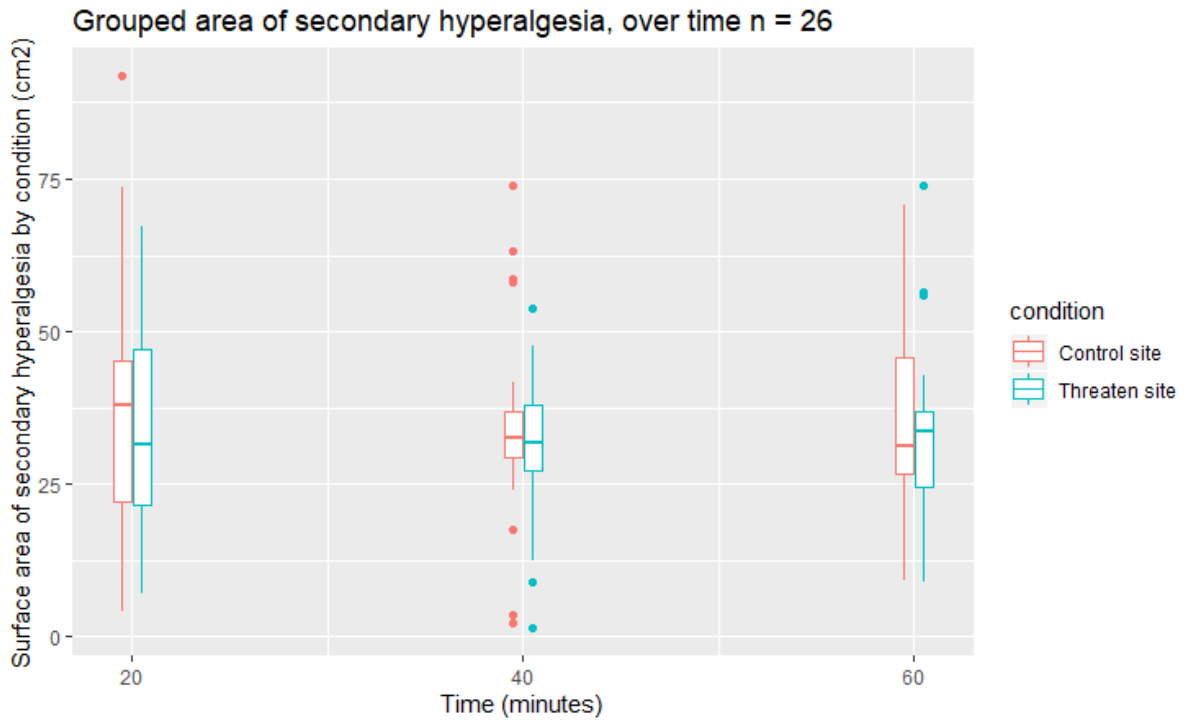


Figure 20 Boxplot describing the grouped area of secondary hyperalgesia over time

Assessment of model fit

The model is called model_pre-condition_crossed_sa and has the structure: SA ~ condition + (1|id/time All plots can be viewed in Appendix 30, section title: *SA: Assessment of model fit*. First, linearity was assumed by plotting fitted values against residual values, for the fixed factor 'condition'. There was no obvious linear or curvilinear pattern; therefore, the assumption was deemed to have been upheld. Second, the assumption of absence of collinearity was assessed by checking correlation between time and condition. There was no collinearity observed in the plot; therefore, the assumption of absence of collinearity was deemed to have been upheld. Third, assumption of homoscedasticity was assessed, i.e. assessment for equal variance across the range of predicted values. There was a slightly increased density in a blob in the middle and the range of the maximum and minimum values seemed slightly lower on the left than on the right. Alternative methods of assessing homoscedasticity were explored. However, the Imer package has been reported to be a robust in fitting linear mixed-effect model to data (Loy and Hofmann, 2014). Therefore, the assumption of homoscedasticity was deemed to have been upheld. Fourth, the assumption that residuals were normally distributed was assessed. The Q-Q plot and the histogram shows normal distribution.

Exploratory outcomes

Static light touch

There was a wide variability in the intensity of static light touch after induction. The mean (± SD) SPARS ratings over the entire sensory testing period were -29.27 (± 20.41) at the threatened site and -29.58 (± 20.79) at the control site. Static light touch intensities were not predicted by condition (p = 0.34) (All plots can be viewed in Appendix 30, section title: *Exploratory outcomes*).

Dynamic light touch

There was a wide variability in the intensity of dynamic light touch after induction. The mean (± SD) SPARS ratings over the entire sensory testing period were -41.71 (± 8.40) at the threatened site and -41.42 (± 8.31) at the control site. Dynamic light touch intensities were not predicted by condition (p = 0.20) (All plots can be viewed in Appendix 30, section title: *Exploratory outcomes*).

Single electrical stimulation

The was a wide variability in the intensity of single electrical stimulation after induction. The mean (± SD) SPARS ratings over the entire sensory testing period were -29.27 (± 20.41) at the threatened site and -29.58 (± 20.79) at the control site. Single electrical stimulation intensities were not predicted by condition (p = 0.34) (All plots can be viewed in Appendix 30, section title: *Exploratory outcomes*).

Secondary analysis

Summed trauma scores and surface area of secondary hyperalgesia

A Shapiro-Wilk test showed that the data were normally distributed (p = 0.48), therefore a Pearson's correlation test was used. There was no statistically significant correlation between summed trauma score and surface area of SH (p = 0.16) (Appendix 30, section title: *Childhood and recent trauma scores*).

Post-hoc analyses

There were technical issues with the computer resulting in data from Question 8 of the Pain Catastrophising Scale (PCS) being missing for the first two participants. Therefore, PCS data from these two participants were excluded in this post-hoc analysis. The mean (range) PCS scores 31 (14 – 50). The mean (range) of resilience, as measured on the 10-item Connor-Davidson Resilience Scale (CD-RISC-10) was 40.81 (32 – 48) (Appendix 30, section title: *Post-hoc analysis*).

Discussion

The aim of this study was to investigate the effect of a manipulation of the threat value of a stimulus on experimentally induced SH in healthy human volunteers. It was hypothesised that greater threat – as indicated by greater self-reported anxiety, fear of tissue damage and HFS intensity ratings at the arm receiving the HFS under a condition of threat - would be associated with (hypothesis 1) greater intensity and (hypothesis 2) greater surface area of secondary hyperalgesia. Despite a careful and thorough piloting procedure to develop and pre-test the threat manipulation, the manipulation checks showed that the threat manipulation (a sham skin examination) was not effective in eliciting a threat of tissue damage. Therefore, one would anticipate that intensity and area of SH would not be predicted by site allocation. The results in this current study confirmed that neither intensity nor area of SH was predicted by site allocation (i.e. which arm received the HFS under a condition of threat).

Threat manipulation

Although a similar sham skin examination has been used effectively as a threat manipulation before (Wiech et al., 2010), sham skin examination did not affect self-reported anxiety or fear of tissue damage in this current study. Additionally, there was no difference in HFS ratings between arms. There are many possibilities as to why this threat manipulation was not effective. These possibilities have been broadly summarised under the following subheadings and explained below: 1) Safety requirements, 2) Trust, 3) Safety cues, 4) Competing threats, 5) Sampling bias, and 6) South African context.

First possibility: Safety requirements

The Human Research Ethics Committee at the University of Cape Town, Department of Health Sciences required that the safety of the HFS procedure was to be explicitly stated in the informed consent form and study information sheet. Therefore, the informed consent document stated, "This procedure involves some pain; however, it is a well-established procedure and is known not to cause any skin damage". Explicitly and repeatedly reassuring participants of the safely of the HFS procedure could have reduced the threat value of the HFS, thus reducing participants' self-reported anxiety, fear of tissue damage and pain ratings during the HFS induction.

Second possibility: Trust

Trust between friends and family and even more importantly between strangers is essential for cooperation and societal development (Balliet and Van Lange, 2013, Dunning et al., 2014). Trust allows for cohesion within social/cultural groups and across different social/cultural groups. Furthermore, trust is essential when conducting human research. The importance of trust between participants and researchers is explicit in the guidelines of many national human research committees, including those of Australia, New Zealand, Canada and America (Guillemin et al., 2018). Results from the semi-structured interview indicated evidence of trust from the participants of the researchers in this current study: One participant reported that they trusted that enough precautions had been taken to ensure the safety of the procedure. Another participant reported that they trusted the Human Research Ethics Committee would not approve an experiment that could cause damage to participants' skin. The threat manipulation was not compelling enough to overcome participants' trust in the researchers and the safe of the research procedure. This could explain why participants did not report greater anxiety and fear of tissue damage at the site receiving the HFS under a condition of threat than at the control site.

Third possibility: safety cues

Certain social situations have been reported to provide people with safety cues, thus decreasing the threat value of the situation (Tang et al., 2007, Lohr et al., 2007). A study investigated the influence of the presence of an observer and perceived threat on reported pain during a cold pressor task (Vlaeyen et al., 2009). Under neutral conditions, i.e. when no threatening information was given to participants about the cold pressor task, there was no influence of the presence of an observer on reported pain. However, under a condition of threat, i.e. when participants were told threatening information about the cold pressor task, participants reported greater pain severity when no observer was present than when an observer was present during the procedure. This suggests that the observer acted as a safety cue in the presence of a threat manipulation. Perhaps the researcher

conducting the experiment, acted as a safety cue for the participants in this current study, thus decreasing the threat value and reported pain during the HFS procedure.

Fourth possibility: competing threats

The threat of the pain associated with the HFS may have competed with the threat of tissue damage. Researcher 2 was instructed to ask participants for feedback on why they agreed or disagreed to feeling anxious or fearful of tissue damage during the HFS procedure. Almost all the participants reported feeling more anxious about anticipating the pain associated with the HFS than about possible tissue damage. The anticipation of the pain associated with the HFS may have been a more powerful threat than the threat of tissue damage; therefore, distracting participants away from the imposed threat of tissue damage.

Fifth possibility: Sampling bias

As discussed in Chapter 1, people that volunteer for pain studies are not representative of the general population. A recent study compared the characteristics of people that volunteered for a pain-related study to those of people that volunteered for a non-pain related study (Karos et al., 2018). Low fear of pain and older age predicted the likelihood of someone volunteering for a pain-related study. Karos et al. (2018) proposed that individuals who display protective behaviours may be less likely to participate in pain-related studies. However, they did not formally assess this. Fear of pain was not assessed in this current study but pain catastrophising was assessed using the Pain Catastrophising Scale (PCS) (Sullivan et al., 1995). A post-hoc analysis of the participants' PCS scores was conducted in this current study as a proxy for fear of pain. In this current study the wording in the PCS was slightly modified to be relevant to the pain induced by the HFS procedure. The mean (range) PCS score was 31 (14 – 50). Catastrophising is considered clinically significant with a score of 30 (Sullivan et al., 1995). Therefore, the mean score of 31 in this current study suggests that some participants had clinically relevant levels of pain catastrophising about the HFS procedure.

This proxy for fear of pain conflicts with the finding of Karos et al. (2018), where it was reported that participants who volunteer for pain-related studies display low fear of pain. This conflict may be due to a discrepancy in the focus of the PCS used in this current study and the Fear of Pain Questionnaire used in Karos et al. (2018). Specifically, the PCS was focused on pain relating to participants' experience of the HFS procedure. The Fear of Pain Questionnaire asked participants to imagine different situations, (e.g. a vehicle accident) and report how fearful they are about the potential pain associated with that situation.

An additional limitation for using PCS as a proxy for fear of pain, is that Karos et al. (2018) did not find a significant correlation between pain catastrophising and willingness to participate in a pain-related study. Additionally, in this current study the PCS was administered *after* the threat manipulation. It is plausible that the threat manipulation induced catastrophic thinking about the HFS procedure, rather than participants displaying clinically relevant levels of catastrophising prior to the procedure, i.e. at baseline. Participants in Karos et al. (2018) were undergraduate students from universities in Belgium and the Netherlands. To this author's knowledge, there are no published data on the characteristics of individuals willing to participate and those who do participate in experimental pain research in South Africa. Therefore, further investigations into the characteristics of South African volunteers willing and not willing to participate in pain-related studies are required. This may provide more information on potential sampling bias in experimental pain studies.

Sixth possibility: South African context

It is important for researchers to reflect on the specific context in which their experiments are conducted and what contextual backgrounds their participants come from. Social, environmental, political and cultural contexts all play a role in influencing pain (Keefe and France, 1999, Turk and Okifuji, 2002). Participants bring their individual biases from previous life experiences, which may influence results experimental pain studies.

This current study was conducted in a South African context, with participants ranging between 18 and 55 years of age. Many South Africans live under permanent threat (Hinsberger et al., 2016). There are daily media reports of murders, sexual assaults, robberies and gang violence. In certain areas in South Africa many people experience emotional and/or physical and/or psychological abuse daily, either endured themselves or by others. There is very little research investigating the influence of this repetitive exposure to threat on pain in the South African context. Perhaps regular exposure to threat habituates people to threat and may raise their 'threat tolerance/threshold'. Perhaps this could be why participants in this present study were not easily threatened by a relatively minor threat, particularly when they were in a relatively safe environment. Importantly, this is speculation and substantially more investigation into these ideas is needed before these conclusions can be confidently drawn.

This researcher proposes further experimental studies in the South African context formally comparing 1) the intensity and magnitude of experimentally induced SH in participants with and without a history of trauma, 2) the effectiveness of different threat manipulations in participants with and without a history of trauma, and 3) the influence of a threat manipulation on the intensity and magnitude of experimentally induced in participants with and without a history of trauma.

Proposed improvements to the threat manipulation

Inducing a threat manipulation in a laboratory setting is known to be difficult. Threat manipulations are known to induce "weak…concerns about the pain stimulus" by participants in experimental pain studies (Vlaeyen et al., 2009). This may be due to participants knowing the pain will be short-lived and assuming the safety of the procedure due to knowledge of strict requirements from human research ethics committees. Although threat manipulations are difficult to conduct in the laboratory setting, there are reports of effective threat manipulations in previous studies (Vlaeyen et al., 2009, Jackson et al., 2005, Van Damme et al., 2008). However, these were not sham skin examinations; these treat manipulations consisted of providing participants with threatening information about the experimental procedure. As discussed in the *Piloting procedure* section, threatening information about the HFS procedure was ineffective in eliciting threat of tissue damage in this current study's piloting sample.

Reflecting on the above described theories for why the threat manipulation was not effective, this author proposes the following modifications to improve the effectiveness of the threat manipulation: 1) removing the statement that HFS is "known not to cause any skin damage" from the informed consent form. This modification would need to be negotiated with the ethical committee to ensure participants are adequately informed prior to participating, 2) Adjusting the social context so that the researcher does not act as a safety cue, thus decreasing the threat value of the situation. 3) Only conducting HFS on one arm rather than two. This modification will remove the competing threat of anxiety about anticipating the HFS on the second arm, after receiving the HFS on the first arm. Further, participants may not have found it compelling that the skin on their one forearm is robust but the skin on the other forearm is fragile. However, this was not assessed in this present study. If this was the case, informing participants that their skin is fragile might be more compelling if the assessment and procedure is only conducted on one arm.

Summed trauma scores and area of secondary hyperalgesia

Summed trauma scores were not correlated with increased area of SH in this current study. This conflicts with published pilot data where summed trauma scores were correlated with increased area but not increased intensity of SH (You et al., 2016).

It is important to note some of the differences between this current study and that conducted by You et al. (2016b) as these differences may account for the conflicting results. The eligibility criteria for inclusion differed between studies. In this present study, volunteers were not eligible to participate if they presented with a history of mental illness; depression and anxiety disorders were considered a mental illness for this study. Additionally, the recruitment poster advertisement outlined that participants needed to complete questionnaires about previous trauma. Therefore, it

was unlikely that the sample in this current study would consist of participants with an extensive history of trauma. You et al. (2016b) specifically recruited female participants with and without a history of trauma. Volunteers were screened using the Childhood Trauma Events Scale and the Recent Traumatic Events Scale. Volunteers reporting no history of trauma and those reporting nine or more traumatic events were invited to participate in their study. Therefore, this author speculates that participants in this current study may have had lower summed trauma scores than participants' summed trauma scores in You et al. (2016b).

Another difference between this current study and You et al. (2016b) may be a difference in participants' characteristics. The mean (range) resilience score of participations in this current study, as reported in the *Post-hoc analysis* section was 40.81 (32 – 48). Interestingly, this is higher than previously reported CD-RISC-10 scores that ranged between 29 – 33.5 in the general population (Campbell-Sills and Stein, 2007, Goins et al., 2013, Levasseur et al., 2017, Lopes and Martins, 2011, Antúnez et al., 2015) and between 20.8 – 33.5 in students and young adults (Hartley, 2012, Campbell-Sills and Stein, 2007, Jones et al., 2017, Reyes et al., 2018, Shlomi, 2010, Rahimi et al., 2014). Data from the second population group were included for the comparison because most participants in this current study were students and young adults. Importantly, none of these studies were conducted on a South African cohort. It would be speculative to conclude that this high resilience score presented in this current study is representative of the general South African population. Therefore, further research is required to investigate whether the high resilience score in this current study is representative of the general South African population.

People with high resilience scores are able to cope with stressful life events better (Connor and Davidson, 2003) and have faster physiological recovery after stressful events (Lü et al., 2016) than people with low resilience scores on the CD-RISC. Further, people with high resilience handle repeated exposure to stressful events better than people with low resilience. Resilience is relevant here, because pain is a considered a stressor (You et al., 2016). Chronic pain can increase the demand of physiological functioning to maintain homeostasis, i.e. chronic pain increases allostatic load (Sturgeon and Zautra, 2010). Physiologically, people with high resilience have better heart rate, respiratory sinus arrhythmia, and systolic and diastolic blood pressure recovery after repeated stressful events than people with low resilience (Lü et al., 2016). Further, people with high resilience are more amenable to adopting strategies to cope with pain than people with low resilience (Sturgeon and Zautra, 2010). High resilience improves coping mechanisms and physiological functioning following stressful events, and therefore may improve pain management outcomes. Unfortunately, You et al. (2016b) did not assess participants' resilience, therefore preventing a comparison between their study and this current study. This author speculates that high resilience

may influence the relationship between trauma history and area of secondary hyperalgesia. It would be beneficial for future research to investigate resilience among South Africans and whether resilience is associated with the repetitive trauma experienced by South Africans. An improved understanding of resilience within the South African context may allow for optimisation of improved treatments for chronic pain.

Strengths

This study provides a comprehensive overview of the challenges associated with conducting a threat manipulation for experimental pain research. This overview will be of benefit to researchers when designing a threat manipulation. This study also highlights the need and provides recommendations for future research investigating the association between threat and chronic pain among South Africans.

Limitations

An obvious limitation of this study is that the threat manipulation was ineffective. Therefore, it is still unclear whether threat of tissue damage is associated with greater intensity and area of SH remains unanswered.

Conclusion

In summary, despite a careful and thorough piloting procedure, the threat manipulation (sham skin examination) used in the current study was not effective in eliciting a threat of tissue damage. The results in this current study confirmed that neither intensity nor area of SH was predicted by site allocation (i.e. which arm received the HFS under a condition of threat), which would be anticipated, given that the threat manipulation was not effective. Further investigations are required to develop effective threat manipulations for experimental pain research. Summed trauma scores were not correlated with increased area of SH in this current study. This conflicts with published pilot data in which summed trauma scores were correlated with increased area but not increased intensity of SH (You et al., 2016). Further research is required in the South African context to investigate the can you be a bit more general and say the potential relationship? between trauma history and the magnitude (both intensity and area) of SH.

Chapter 4: Conclusion

Research objectives

The two main objectives of this research were: 1) to conduct a systematic review identifying and describing non-pharmacological modalities used to manipulate (i.e. increase or decrease) experimentally induced secondary hyperalgesia (SH), and 2) to conduct an experimental study investigating the effect of a threat manipulation on experimentally induced SH. The purpose of the systematic review was to identify and describe which non-pharmacological manipulations have an influence on experimentally induced SH and whether these manipulations increase or decrease the magnitude of SH. A better understanding of the effects of these non-pharmacological manipulations will provide insight into the mechanisms associated with SH. The purpose of the experimental study was to determine whether greater threat was associated with (hypothesis 1) greater intensity and (hypothesis 2) greater surface area of experimentally induced SH. This concluding chapter will summarise the main findings, the strengths, and the limitations of both the systematic review and experimental study, and provide recommendations for future research.

Summary of main findings

Systematic review

Of the 21 studies included in the systematic review, there were six different methods used to experimentally induce SH, with the most common methods being topical capsaicin (seven of 21) and burn injury (six of 21). Interestingly, 15 different non-pharmacological manipulations were used. Most studies (12 of 21) assessed the effect of a manipulation on the area of SH only (Matre et al., 2006, Mohammadian et al., 2004, Meeker et al., 2019, Pud et al., 2006, Rasmussen et al., 2015, Salomons et al., 2014, Wahl et al., 2019, Yucel et al., 2001, Smith et al., 2018, Rebhorn et al., 2012) (Yucel et al. (2001) reported on three studies, all of which were included in this review). Four studies assessed the effect of a manipulation on the intensity of SH only (Kóbor et al., 2009, Torta et al., 2019, van den Broeke et al., 2014) (Torta et al. (2019) reported on three studies, of which two were included in this review), and five studies assessed the effect of a manipulation on both the area and the intensity of SH (Baron et al., 1999, Ditre et al., 2018, Helfert et al., 2018, Werner et al., 2002, You et al., 2014).

Manipulations were categorised and reported based on the hypothesised direction of effect (i.e. increase or decrease) on the magnitude of SH. Most manipulations were hypothesised to decrease the intensity (six of nine) (Baron et al., 1999, Werner et al., 2002, Kóbor et al., 2009, Torta et al., 2019, You et al., 2014) and the area (14 of 17) (Baron et al., 1999, Werner et al., 2002, Pud et al., 2006, Yucel et al., 2001, Rasmussen et al., 2015, Wahl et al., 2019, You et al., 2014, Salomons et al.,

2014, Matre et al., 2006, Meeker et al., 2019, Mohammadian et al., 2004, Rebhorn et al., 2012) of secondary hyperalgesia. The remaining manipulations were hypothesised to increase the intensity (three of nine) (Ditre et al., 2018, Helfert et al., 2018, van den Broeke et al., 2014) and the area (three of 17) (Ditre et al., 2018, Helfert et al., 2018, Smith et al., 2018) of SH.

Only two studies were successful in decreasing the intensity of SH. The studies used an emotional disclosure manipulation (You et al., 2014) and high cognitive loading with a modified version of the N-Back task (Torta et al., 2019) (Experiment 2). Four studies failed to decrease the intensity of SH. These studies used whole-body cooling and heating (Baron et al., 1999), localised cold application (Werner et al., 2002), high attentional loading (Kóbor et al., 2009), and high cognitive loading using the Eriksen Flanker Task (Torta et al., 2019) (Experiment 1). Two studies successfully increased the intensity of SH. These studies used 24 hours of nicotine deprivation (Ditre et al., 2018) and negative suggestion (van den Broeke et al., 2014). One study failed to increase the intensity of SH. This study used verbal suggestion by reporting correct or incorrect information about the contents of the substance used for the induction (Helfert et al., 2018).

Seven studies were successful in decreasing the area of SH. These studies used hyperbaric oxygen therapy (Rasmussen et al., 2015, Wahl et al., 2019), emotional disclosure (You et al., 2014), cognitive behavioural therapy (Salomons et al., 2014), placebo analgesia (Matre et al., 2006), transcranial direct current stimulation applied to target the motor cortex (Meeker et al., 2019) and spinal manipulation therapy applied to the thoracic spine (Mohammadian et al., 2004). Three studies failed to decrease the area of SH. These studies used whole-body cooling and heating (Baron et al., 1999), localised cold application (Pud et al., 2006, Werner et al., 2002) and intradermal capsaicin (Rebhorn et al., 2012). Interestingly, although localised cold application was hypothesised to *decrease* the area of SH, one study reported that it *increased* the area of SH (Pud et al., 2006). However, this study had a high risk of reporting bias. Another study with a low risk of reporting bias reported that localised cold application had no effect on the area of SH (Werner et al., 2002). Two studies successfully increased the area of SH. These studies used nicotine deprivation (Ditre et al., 2018) and sleep deprivation (Smith et al., 2018). Interestingly, sleep deprivation was effective in increasing the area of SH in male participants only; there was no effect in the area of SH in female participants. One study failed to increase the area of SH. This study used verbal suggestion by reporting correct or incorrect information about the contents of the substance used for induction (Helfert et al., 2018).

Most of the manipulations – except for acupuncture, low cognitive loading and negative suggestion – that were thought to influence central processes contributing to pain were successful in either increasing or decreasing the intensity and/or area of SH. Conversely, manipulations that influence

peripheral processes contributing to pain, such as localised cold application, failed to manipulate the intensity and area of SH. These findings support the theory that SH is modulated by central processes. It is possible that the manipulations that were successful in increasing or decreasing the magnitude of SH achieved this by facilitating or inhibiting spinal LTP at the dorsal horn of the spinal cord. Alternatively, these manipulations may have influenced cortical processes contributing to pain resulting in an increase or decrease in SH. Further research is needed to gain insight into how each of these manipulations was or was not successful in manipulating experimentally induced SH. This will provide clarity of the mechanisms of SH. Clarification of these mechanisms will inform development of non-pharmacological therapies that directly target the mechanisms of SH.

Experimental study

The aim of this within-subject, double-blinded experimental study was to investigate the effect of a threat manipulation – brought about by a sham skin examination – on experimentally induced secondary hyperalgesia, using HFS. The effect of the threat manipulation was expected to be indicated by greater self-reported anxiety, fear of tissue damage and HFS intensity ratings for the arm receiving the HFS under a condition of threat. It was anticipated that inducing threat of tissue damage in an experimental pain study would be difficult given the strict ethical requirements and controlled environment associated with experimental studies. A careful and thorough piloting procedure was conducted to develop and pre-test the threat manipulation. Additionally, the chosen threat manipulation procedure (sham skin examination) had been used and reported successful in eliciting threat of tissue damage in a previously published study (Wiech et al., 2010). Despite this, the threat manipulation was ineffective in eliciting a threat of tissue damage in this current study. Therefore, in accordance with this lack of difference in threat value between sites, there was no difference in the intensity and area of SH at the experimental site (i.e. the site received the HFS under a condition of threat) and the control site. Six possible reasons were identified to explain why the threat manipulation was ineffective in this current study's sample population: 1) Safety requirements, 2) Trust, 3) Safety cues, 4) Competing threats, 5) Sampling bias, and 6) South African context. Researchers should consider these challenges to ensure that the threat manipulation is effective for future research.

Chronic life stress has been reported to influence central processes contributing to increased pain severity (Kehlet et al., 2006). However, the mechanisms by which chronic life stress achieves this are unclear. A secondary analysis was conducted to assess for a correlation between each participant's summed trauma score and their mean area of SH across both sites. A previously published pilot study reported a history of trauma correlated with an increased area of SH, following the application of topical capsaicin (You et al., 2016). Interestingly, the results of this current study did not support those findings – there was no correlation between summed trauma scores and the area of SH in this current study. To this author's best knowledge, there are no other published studies reporting on the correlation (or lack thereof) between trauma history and area of SH.

These conflicting results may be due to differing characteristics between participants in You et al. (2016b) and those in this current study. A post-hoc analysis of participants in this current study's resilience scores, as measured on the CD-RISC-10, showed that they had higher resilience scores than those from the general population (Campbell-Sills and Stein, 2007, Goins et al., 2013, Levasseur et al., 2017, Lopes and Martins, 2011, Antúnez et al., 2015), and in a population of students and young adults (Hartley, 2012, Campbell-Sills and Stein, 2007, Jones et al., 2017, Reyes et al., 2018, Shlomi, 2010, Rahimi et al., 2014). High levels of resilience have been associated with improved management of stressful life events and faster physiological recovery after stressful life events. Participants in You et al. (2016b) may have had low levels of resilience – this was not formally assessed and is therefore a speculation. However, the high resilience score in this current study may account for the lack of correlation found between trauma history and area of secondary hyperalgesia, because resilience is associated with improvement management of stressful life events. None of those published studies on resilience scores included a cohort of South Africans. Therefore, it is unclear whether the high resilience scores in this current study are specific to this sample or similar to that of the general South African population. Therefore, it would be interesting and useful for future research to investigate the relationship between trauma history and the area of SH and how resilience may or may not influence this relationship.

Strengths of research

Systematic review

This is the first systematic review collating and describing non-pharmacological modalities used to manipulate experimentally induced SH, to this author's best knowledge. Secondary hyperalgesia is widely considered to be a centrally mediated process and the findings of this review support this. Therefore, to effectively treat SH therapeutic modalities may need to influence central, rather than peripheral processes thought to contribute to pain. This review is beneficial in providing insight into the mechanisms of SH.

Experimental study

There is limited published research on the effects of threat of tissue damage on experimentally induced secondary hyperalgesia. To this author's best knowledge, there are no published experimental studies investigating threat and SH within the South African context. Data obtained from national World Mental Health Surveys support that chronic pain is highly prevalent in South Africa, with and approximately half (48.3%) of South Africans experiencing chronic pain within the 12 months prior to the survey (Tsang et al., 2008). Many South Africans also live under constant threat (Scorgie et al., 2017). Therefore, there is a clear need for further research investigating the association between threat and chronic pain among South Africans. This thesis describes specific challenges to inducing a threat of tissue damage within a controlled experimental environment and important factors for researchers to consider when designing a threat manipulation.

Limitations of research

Systematic review

A considerable limitation of this review was that a meta-analysis could not be conducted - there was high clinical heterogeneity among the studies and unavailable data, rendering pooling of studies' results impossible. Therefore, it was not possible to generate stable effect sizes of the potencies of the manipulations. Additionally, it was not possible to assess for publication bias across the included studies.

Experimental study

An obvious limitation of the experimental study is that the threat manipulation was ineffective in eliciting a threat of tissue damage. Therefore, the question of whether threat of tissue damage is associated with greater intensity and area of SH remains unanswered.

This experimental study was conducted on healthy humans, i.e. humans without chronic pain. Experimental pain research on healthy humans is useful for bridging the gap between animal studies and research on patients with chronic pain. However, there are limitations when extrapolating findings from experimental pain studies with healthy human participants and applying them to patients with chronic pain. A specific limitation is that experimentally inducing short-lived SH may not reflect the exact physiological changes associated with clinical SH. Therefore, to thoroughly investigate the mechanisms of SH associated with neuropathic pain, further research is required in patients with chronic pain using credible threat manipulations.

Recommendations for future research

This author proposes open access to raw data on online platforms and the standardisation of rating scales in experimental pain research. This will improve the quality of the published experimental pain research and allow for statistically sound comparisons across different studies.

Additional research is required to investigate the specific physiological effects of non-pharmacological modalities reported to manipulate experimentally induced SH. This will provide an improved understanding of the mechanisms of SH and pave the way for the development of improved treatment modalities.

There is limited research into the influence of threat of tissue damage on SH. Further research is required to develop a robust threat manipulation model for experimental pain research. Additionally, the effect of threat on SH in patients with chronic pain needs to be investigated.

There is limited research into the relationship between trauma history and area of secondary hyperalgesia. The results of this study conflicted with those of a previously published pilot study. Further research is required to investigate whether there is a relationship between a history of trauma and area of secondary hyperalgesia. Finally, it was out of the scope of this current study to investigate the relationship between resilience and SH. As discussed in the *Summary of findings* above, high resilience was speculated to be a possible reason for the lack of correlation between trauma history and area of SH. Further research is required to investigate if resilience could influence a relationship between trauma history and secondary hyperalgesia. This may be useful for developing further research investigating whether treatments aimed at improving resilience would also reduce the magnitude of SH.

To conclude, the findings described in this thesis support recent theoretical models of pain that propose that pain is not merely a biological process. Rather, pain is a biopsychosocial sensory and emotional experience. These findings (specifically, those of the systematic review) support that pain can be modulated by non-pharmacological modalities. Further research into specific variables that modulate pain is required for the development of improved treatment modalities, specifically targeting pain mechanisms.

References

ADIE, S., KWAN, A., NAYLOR, J. M., HARRIS, I. A. & MITTAL, R. 2012. Cryotherapy following total knee replacement. *Cochrane Database of Systematic Reviews*.

ALGAFLY, A. A. & GEORGE, K. P. 2007. The effect of cryotherapy on nerve conduction velocity, pain threshold and pain tolerance. *British journal of sports medicine,* 41**,** 365-369.

ANDERSEN, O. K., FELSBY, S., NICOLAISEN, L., BJERRING, P., JENSEN, T. S. & ARENDT-NIELSEN, L. 1996. The effect of Ketamine on stimulation of primary and secondary hyperalgesic areas induced by capsaicin--a double-blind, placebo-controlled, human experimental study. *Pain,* 66**,** 51-62.

ANTÚNEZ, J. M., NAVARRO, J. F. & ADAN, A. 2015. Circadian typology is related to resilience and optimism in healthy adults. *Chronobiology international,* 32**,** 524-530.

ARENDT-NIELSEN, L., CURATOLO, M. & DREWES, A. 2007. Human experimental pain models in drug development: translational pain research. *Current opinion in investigational drugs (London, England : 2000),* 8**,** 41-53.

ARIMA, T., SVENSSON, P., RASMUSSEN, C., DREMSTRUP, K., DREWES, A. & ARENDT-NIELSEN, L. 2001. The relationship between selective sleep deprivation, nocturnal jaw-muscle activity and pain in healthy men. *Journal of oral rehabilitation,* 28**,** 140-8.

ARNTZ, A. & CLAASSENS, L. 2004. The meaning of pain influences its experienced intensity. *Pain,* 109**,** 20-25.

ARNTZ, A., DREESSEN, L. & MERCKELBACH, H. 1991. Attention, not anxiety, influences pain. *Behaviour Research and Therapy,* 29**,** 41-50.

ATZENI, F., MASALA, I., CIRILLO, M., BOCCASSINI, L., SORBARA, S. & ALCIATI, A. 2020. Hyperbaric oxygen therapy in fibromyalgia and the diseases involving the central nervous system. *Clinical and experimental rheumatology,* 38**,** 94.

AYDEDE, M. & SHRIVER, A. 2018. Recently introduced definition of "nociplastic pain" by the International Association for the Study of Pain needs better formulation. *PAIN,* 159**,** 1176-1177.

BALLIET, D. & VAN LANGE, P. A. 2013. Trust, conflict, and cooperation: a meta-analysis. *Psychological Bulletin,* 139**,** 1090.

BANTICK, S. J., WISE, R. G., PLOGHAUS, A., CLARE, S., SMITH, S. M. & TRACEY, I. 2002. Imaging how attention modulates pain in humans using functional MRI. *Brain,* 125**,** 310-319.

BARBER, F. A. 2000. A comparison of crushed ice and continuous flow cold therapy. *The American journal of knee surgery,* 13**,** 97-101; discussion 102.

BARON, R., WASNER, G., BORGSTEDT, R., HASTEDT, E., SCHULTE, H., BINDER, A., KOPPER, F., ROWBOTHAM, M., LEVINE, J. & FIELDS, H. 1999. Effect of sympathetic activity on capsaicin-evoked pain, hyperalgesia, and vasodilatation. *Neurology,* 52**,** 923-923.

BATES, D., MAECHLER, M., BOLKER, B. & WALKER, S. 2015. Ime4: Linear mixedeffects models using Eigen and S4. R package version 1.1–9. Available a thttps. *CRAN. R-project. org/package= lme4*.

BEECHER, H. K. 1946. Pain in men wounded in battle. *Annals of surgery,* 123**,** 96.

BENEDETTI, F., LANOTTE, M., LOPIANO, L. & COLLOCA, L. 2007. When words are painful: Unraveling the mechanisms of the nocebo effect. *Neuroscience,* 147**,** 260-271.

BENEDETTI, F. & PIEDIMONTE, A. 2019. The neurobiological underpinnings of placebo and nocebo effects. *Semin Arthritis Rheum,* 49**,** S18-s21.

BERGERON, S. & LORD, M.-J. 2003. The integration of pelvi-perineal re-education and cognitive-behavioural therapy in the multidisciplinary treatment of the sexual pain disorders. *Sexual and Relationship Therapy,* 18**,** 135-141.

BERNSTEIN, D. P., STEIN, J. A., NEWCOMB, M. D., WALKER, E., POGGE, D., AHLUVALIA, T., STOKES, J., HANDELSMAN, L., MEDRANO, M., DESMOND, D. & ZULE, W. 2003. Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse & Neglect,* 27**,** 169-190.

BERT, J., STARK, J., MASCHKA, K. & CHOCK, C. 1991. The effect of cold therapy on morbidity subsequent to arthroscopic lateral retinacular release. *Orthopaedic review,* 20**,** 755-758.
BLYTH, F. M., MARCH, L. M., BRNABIC, A. J., JORM, L. R., WILLIAMSON, M. & COUSINS, M. J. 2001. Chronic pain in Australia: a prevalence study. *Pain,* 89**,** 127-134.
BOWERING, K. J., O'CONNELL, N. E., TABOR, A., CATLEY, M. J., LEAKE, H. B., MOSELEY, G. L. & STANTON, T. R. 2013. The effects of graded motor imagery and its components on chronic pain: a systematic review and meta-analysis. *The Journal of Pain,* 14**,** 3-13.
BREIVIK, H., EISENBERG, E. & O'BRIEN, T. 2013. The individual and societal burden of chronic pain in Europe: the case for strategic prioritisation and action to improve knowledge and availability of appropriate care. *BMC public health,* 13**,** 1229.
BRIGGS, E. V., BATTELLI, D., GORDON, D., KOPF, A., RIBEIRO, S., PUIG, M. M. & KRESS, H. G. 2015. Current pain education within undergraduate medical studies across Europe: Advancing the Provision of Pain Education and Learning (APPEAL) study. *BMJ open,* 5**,** e006984.
BRONFORT, G., EVANS, R., NELSON, B., AKER, P. D., GOLDSMITH, C. H. & VERNON, H. 2001. A randomized clinical trial of exercise and spinal manipulation for patients with chronic neck pain. *Spine,* 26**,** 788-797.
BRONFORT, G., HAAS, M., EVANS, R. L. & BOUTER, L. M. 2004. Efficacy of spinal manipulation and mobilization for low back pain and neck pain: a systematic review and best evidence synthesis. *The spine journal,* 4**,** 335-356.
BRUWER, B., EMSLEY, R., KIDD, M., LOCHNER, C. & SEEDAT, S. 2008. Psychometric properties of the Multidimensional Scale of Perceived Social Support in youth. *Comprehensive psychiatry,* 49**,** 195-201.
BURKE, D., FULLEN, B., STOKES, D. & LENNON, O. 2017. Neuropathic pain prevalence following spinal cord injury: A systematic review and meta-analysis. *European Journal of Pain,* 21**,** 29-44.
CAMPBELL-SILLS, L. & STEIN, M. B. 2007. Psychometric analysis and refinement of the Connor-davidson Resilience Scale (CD-RISC): Validation of a 10-item measure of resilience. *Journal Of Traumatic Stress,* 20**,** 1019-1028.
CAMPBELL-SILLS, L. & STEIN, M. B. 2007. Psychometric analysis and refinement of the connor–davidson resilience scale (CD-RISC): Validation of a 10-item measure of resilience. *Journal of Traumatic Stress: Official Publication of The International Society for Traumatic Stress Studies,* 20**,** 1019-1028.
CASALE, R., BOCCIA, G., SYMEONIDOU, Z., ATZENI, F., BATTICCIOTTO, A., SALAFFI, F., SARZI-PUTTINI, P., BRUSTIO, P. R. & RAINOLDI, A. 2019. Neuromuscular efficiency in fibromyalgia is improved by hyperbaric oxygen therapy: looking inside muscles by means of surface electromyography. *Clin Exp Rheumatol,* 37**,** S75-S80.
CASIMIRO, L., BARNSLEY, L., BROSSEAU, L., MILNE, S., ROBINSON, V. A., TUGWELL, P. & WELLS, G. 2005. Acupuncture and electroacupuncture for the treatment of rheumatoid arthritis. *Cochrane Database Syst Rev***,** Cd003788.
COHEN, S. 1988. Perceived stress in a probability sample of the United States.
COHN, B., DRAEGER, R. & JACKSON, D. 1989. The effects of cold therapy in the postoperative management of pain in patients undergoing anterior cruciate ligament reconstruction. *The American journal of sports medicine,* 17**,** 344-349.
COLLOCA, L. & BENEDETTI, F. 2007. Nocebo hyperalgesia: how anxiety is turned into pain. *Current Opinion in Anesthesiology,* 20**,** 435-439.
CONNOR, K. M. & DAVIDSON, J. R. 2003. Development of a new resilience scale: The Connor-Davidson resilience scale (CD-RISC). *Depression and anxiety,* 18**,** 76-82.
COSGROVE, K. P., BATIS, J., BOIS, F., MACIEJEWSKI, P. K., ESTERLIS, I., KLOCZYNSKI, T., STIKLUS, S., KRISHNAN-SARIN, S., O'MALLEY, S. & PERRY, E. 2009. β2-nicotinic acetylcholine receptor availability during acute and prolonged abstinence from tobacco smoking. *Archives of general psychiatry,* 66**,** 666-676.

COSGROVE, K. P., ESTERLIS, I., MCKEE, S., BOIS, F., ALAGILLE, D., TAMAGNAN, G. D., SEIBYL, J. P., KRISHNAN-SARIN, S. & STALEY, J. K. 2010. Beta2* nicotinic acetylcholine receptors modulate pain sensitivity in acutely abstinent tobacco smokers. *Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco,* 12**,** 535-539.

CRUCCU, G. 2007. Treatment of painful neuropathy. *Current Opinion in Neurology,* 20**,** 531-535.

D'MELLO, R. & DICKENSON, A. H. 2008. Spinal cord mechanisms of pain. *BJA: British Journal of Anaesthesia,* 101**,** 8-16.

DALTON, J. E., BOLEN, S. D. & MASCHA, E. J. 2016. Publication bias: the elephant in the review. *Anesthesia and analgesia,* 123**,** 812.

DE WIED, M. & VERBATEN, M. N. 2001. Affective pictures processing, attention, and pain tolerance. *PAIN,* 90**,** 163-172.

DEHGHAN, M. & FARAHBOD, F. 2014. The efficacy of thermotherapy and cryotherapy on pain relief in patients with acute low back pain, a clinical trial study. *Journal of clinical and diagnostic research: JCDR,* 8**,** LC01.

DIELEMAN, J. P., KERKLAAN, J., HUYGEN, F. J., BOUMA, P. A. & STURKENBOOM, M. C. 2008. Incidence rates and treatment of neuropathic pain conditions in the general population. *PAIN®,* 137**,** 681-688.

DITRE, J. W., HECKMAN, B. W., ZALE, E. L., KOSIBA, J. D. & MAISTO, S. A. 2016. Acute analgesic effects of nicotine and tobacco in humans: a meta-analysis. *Pain,* 157**,** 1373.

DITRE, J. W., ZALE, E. L., LAROWE, L. R., KOSIBA, J. D. & DE VITA, M. J. 2018. Nicotine deprivation increases pain intensity, neurogenic inflammation, and mechanical hyperalgesia among daily tobacco smokers. *Journal of abnormal psychology,* 127**,** 578.

DOBSON, J. L., MCMILLAN, J. & LI, L. 2014. Benefits of exercise intervention in reducing neuropathic pain. *Frontiers in cellular neuroscience,* 8**,** 102.

DREWES, A., RÖSSEL, P., ARENDT-NIELSEN, L., DREMSTRUP, K., HANSEN, L., BIRKET-SMITH, L. & STENGAARD-PEDERSEN, K. 1997. Sleepiness does not Modulate Experimental Joint Pain in Healthy Volunteers. *Scandinavian journal of rheumatology,* 26**,** 399-400.

DUNNING, D., ANDERSON, J. E., SCHLÖSSER, T., EHLEBRACHT, D. & FETCHENHAUER, D. 2014. Trust at zero acquaintance: More a matter of respect than expectation of reward. *Journal of Personality and Social Psychology,* 107**,** 122.

DWORKIN, R. H., PANARITES, C. J., ARMSTRONG, E. P., MALONE, D. C. & PHAM, S. V. 2012. Is treatment of postherpetic neuralgia in the community consistent with evidence-based recommendations? *PAIN®,* 153**,** 869-875.

ECCLESTON, C., MALLESON, P., CLINCH, J., CONNELL, H. & SOURBUT, C. 2003. Chronic pain in adolescents: evaluation of a programme of interdisciplinary cognitive behaviour therapy. *Archives of disease in childhood,* 88**,** 881-885.

EHDE, D. M., CZERNIECKI, J. M., SMITH, D. G., CAMPBELL, K. M., EDWARDS, W. T., JENSEN, M. P. & ROBINSON, L. R. 2000. Chronic phantom sensations, phantom pain, residual limb pain, and other regional pain after lower limb amputation. *Archives of physical medicine and rehabilitation,* 81**,** 1039-1044.

ELLIS, R. J., ROSARIO, D., CLIFFORD, D. B., MCARTHUR, J. C., SIMPSON, D., ALEXANDER, T., GELMAN, B. B., VAIDA, F., COLLIER, A. & MARRA, C. M. 2010. Continued high prevalence and adverse clinical impact of human immunodeficiency virus–associated sensory neuropathy in the era of combination antiretroviral therapy: the CHARTER Study. *Archives of neurology,* 67**,** 552-558.

ERIKSEN, B. A. & ERIKSEN, C. W. 1974. Effects of noise letters upon the identification of a target letter in a nonsearch task. *Perception & psychophysics,* 16**,** 143-149.

EVANS, S., FISHMAN, B., SPIELMAN, L. & HALEY, A. 2003. Randomized trial of cognitive behavior therapy versus supportive psychotherapy for HIV-related peripheral neuropathic pain. *Psychosomatics,* 44**,** 44-50.

FINNERUP, N. B., ATTAL, N., HAROUTOUNIAN, S., MCNICOL, E., BARON, R., DWORKIN, R. H., GILRON, I., HAANPÄÄ, M., HANSSON, P. & JENSEN, T. S. 2015. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *The Lancet Neurology,* 14**,** 162-173.

FINNERUP, N. B., SINDRUP, S. H. & JENSEN, T. S. 2010. The evidence for pharmacological treatment of neuropathic pain. *Pain,* 150**,** 573-581.

FLOR, H. 2002. Phantom-limb pain: characteristics, causes, and treatment. *The Lancet Neurology,* 1**,** 182-189.

FONTAINE, D., HAMANI, C. & LOZANO, A. 2009. Efficacy and safety of motor cortex stimulation for chronic neuropathic pain: critical review of the literature. *Journal of neurosurgery,* 110**,** 251-256.

FREGNI, F., BOGGIO, P. S., LIMA, M. C., FERREIRA, M. J. L., WAGNER, T., RIGONATTI, S. P., CASTRO, A. W., SOUZA, D. R., RIBERTO, M., FREEDMAN, S. D., NITSCHE, M. A. & PASCUAL-LEONE, A. 2006. A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury. *PAIN,* 122**,** 197-209.

FREGNI, F., LIGUORI, P., FECTEAU, S., NITSCHE, M. A., PASCUAL-LEONE, A. & BOGGIO, P. S. 2008. Cortical stimulation of the prefrontal cortex with transcranial direct current stimulation reduces cue-provoked smoking craving: a randomized, sham-controlled study. *Journal of Clinical Psychiatry* 32 - 40.

FURLAN, A. D., VAN TULDER, M., CHERKIN, D., TSUKAYAMA, H., LAO, L., KOES, B. & BERMAN, B. 2005. Acupuncture and dry-needling for low back pain: an updated systematic review within the framework of the cochrane collaboration. *Spine (Phila Pa 1976),* 30**,** 944-63.

GEBER, C., KLEIN, T., AZAD, S., BIRKLEIN, F., GIERTHMÜHLEN, J., HUGE, V., LAUCHART, M., NITZSCHE, D., STENGEL, M., VALET, M., BARON, R., MAIER, C., TÖLLE, T. & TREEDE, R.-D. 2011. Test–retest and interobserver reliability of quantitative sensory testing according to the protocol of the German Research Network on Neuropathic Pain (DFNS): A multi-centre study. *PAIN,* 152**,** 548-556.

GIERTHMÜHLEN, J. & BARON, R. Neuropathic pain. Seminars in neurology, 2016. Thieme Medical Publishers, 462-468.

GOINS, R. T., GREGG, J. J. & FISKE, A. 2013. Psychometric properties of the Connor-Davidson resilience scale with older American Indians: The native elder care study. *Research on Aging,* 35**,** 123-143.

GOLDBERG, R. T., PACHASOE, W. N. & KEITH, D. 1999. Relationship between traumatic events in childhood and chronic pain. *Disability and rehabilitation,* 21**,** 23-30.

GORE, M., BRANDENBURG, N. A., DUKES, E., HOFFMAN, D. L., TAI, K.-S. & STACEY, B. 2005. Pain severity in diabetic peripheral neuropathy is associated with patient functioning, symptom levels of anxiety and depression, and sleep. *Journal of pain and symptom management,* 30**,** 374-385.

GRANAN, L.-P. 2017. We do not need a third mechanistic descriptor for chronic pain states! Not yet. *Pain,* 158**,** 179.

GREEN, S., BUCHBINDER, R., BARNSLEY, L., HALL, S., WHITE, M., SMIDT, N. & ASSENDELFT, W. J. 2002. Acupuncture for lateral elbow pain. *Cochrane Database of Systematic Reviews*.

GREEN, S., BUCHBINDER, R. & HETRICK, S. E. 2005. Acupuncture for shoulder pain. *Cochrane Database of Systematic Reviews*.

GRIFFIN, S. C. & TSAO, J. W. 2012. Phantom Limb Syndrome: A Review of Current Theories. *Pain Syndromes–From Recruitment to Returning Troops: Wounds of War IV,* 91**,** 17.

GUILLEMIN, M., BARNARD, E., ALLEN, A., STEWART, P., WALKER, H., ROSENTHAL, D. & GILLAM, L. 2018. Do research participants trust researchers or their institution? *Journal of Empirical Research on Human Research Ethics,* 13**,** 285-294.

GUYATT, G. H., OXMAN, A. D., VIST, G. E., KUNZ, R., FALCK-YTTER, Y., ALONSO-COELLO, P. & SCHÜNEMANN, H. J. 2008. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *Bmj,* 336**,** 924-926.

HARTLEY, M. T. 2012. Assessing and promoting resilience: An additional tool to address the increasing number of college students with psychological problems. *Journal of College Counseling,* 15**,** 37-51.
HELFERT, S., REIMER, M., BARNSCHEID, L., HÜLLEMANN, P., RENGELSHAUSEN, J., KELLER, T., BARON, R. & BINDER, A. 2018. Impact of suggestion on the human experimental model of cold hyperalgesia after topical application of high-concentration menthol [40%]. *European Journal of Pain,* 22**,** 1517-1527.
HENRICH, F., MAGERL, W., KLEIN, T., GREFFRATH, W. & TREEDE, R. D. 2015. Capsaicin-sensitive C- and A-fibre nociceptors control long-term potentiation-like pain amplification in humans. *Brain,* 138**,** 2505-20.
HIGGINS, J. P., ALTMAN, D. G., GØTZSCHE, P. C., JÜNI, P., MOHER, D., OXMAN, A. D., SAVOVIĆ, J., SCHULZ, K. F., WEEKS, L. & STERNE, J. A. 2011. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *Bmj,* 343**,** d5928.
HIGGINS, J. P. & GREEN, S. 2011. *Cochrane handbook for systematic reviews of interventions*, John Wiley & Sons.
HINSBERGER, M., SOMMER, J., KAMINER, D., HOLTZHAUSEN, L., WEIERSTALL, R., SEEDAT, S., MADIKANE, S. & ELBERT, T. 2016. Perpetuating the cycle of violence in South African low-income communities: attraction to violence in young men exposed to continuous threat. *European Journal of Psychotraumatology,* 7**,** 29099.
HIRVONEN, H., MIKKELSSON, M., KAUTIAINEN, H., POHJOLAINEN, T. & LEIRISALO-REPO, M. 2006. Effectiveness of different cryotherapies on pain and disease activity in active rheumatoid arthritis. A randomised single blinded controlled trial. *Clinical and experimental rheumatology,* 24**,** 295.
INSTITUTE FOR HEALTH METRICS AND EVALUATION. 2017. *GBD Compare Data Visualization* [Online]. Available: http://vizhub.healthdata.org/gbd-compare [Accessed 01 June 2018].
INTERNATIONAL ASSOCIATION FOR THE STUDY OF PAIN. 2017. *IASP Terminology* [Online]. Available: https://www.iasp-pain.org/Education/Content.aspx?ItemNumber=1698#Pain [Accessed 10 August 2019].
IVEY, M., JOHNSTON, R. V. & UCHIDA, T. 1994. Cryotherapy for postoperative pain relief following knee arthroplasty. *The Journal of arthroplasty,* 9**,** 285-290.
JACKSON, T., POPE, L., NAGASAKA, T., FRITCH, A., IEZZI, T. & CHEN, H. 2005. The impact of threatening information about pain on coping and pain tolerance. *British journal of health psychology,* 10**,** 441-451.
JAIN, R., JAIN, S., RAISON, C. L. & MALETIC, V. 2011. Painful diabetic neuropathy is more than pain alone: examining the role of anxiety and depression as mediators and complicators. *Current diabetes reports,* 11**,** 275-284.
JENSEN, T. S. & FINNERUP, N. B. 2014. Allodynia and hyperalgesia in neuropathic pain: clinical manifestations and mechanisms. *The Lancet Neurology,* 13**,** 924-935.
JI, R.-R., KOHNO, T., MOORE, K. A. & WOOLF, C. J. 2003. Central sensitization and LTP: do pain and memory share similar mechanisms? *Trends in neurosciences,* 26**,** 696-705.
JONES, S., JOYAL, C. C., CISLER, J. M. & BAI, S. 2017. Exploring emotion regulation in juveniles who have sexually offended: An fMRI study. *Journal of child sexual abuse,* 26**,** 40-57.
KANE, M. J., CONWAY, A. R., MIURA, T. K. & COLFLESH, G. J. 2007. Working memory, attention control, and the N-back task: a question of construct validity. *Journal of Experimental Psychology: Learning, Memory, and Cognition,* 33**,** 615.
KAROS, K., ALLEVA, J. M. & PETERS, M. L. 2018. Pain, please: An investigation of sampling bias in pain research. *The Journal of Pain,* 19**,** 787-796.
KEEFE, F. J. & FRANCE, C. R. 1999. Pain: biopsychosocial mechanisms and management. *Current Directions in Psychological Science,* 8**,** 137-141.
KEHLET, H., JENSEN, T. S. & WOOLF, C. J. 2006. Persistent postsurgical pain: risk factors and prevention. *The lancet,* 367**,** 1618-1625.

KESSLER, R. C., ÜSTUUML & N, T. B. 2004. The World Mental Health (WMH) Survey Initiative Version of the World Health Organization (WHO) Composite. International Diagnostic Interview (CIDI). *International Journal of Methods in Psychiatric Research,* 13**,** 93-121.

KLEIN, T., MAGERL, W., HOPF, H. C., SANDKUHLER, J. & TREEDE, R. D. 2004. Perceptual correlates of nociceptive long-term potentiation and long-term depression in humans. *J Neurosci,* 24**,** 964-71.

KLEIN, T., MAGERL, W., ROLKE, R. & TREEDE, R.-D. 2005. Human surrogate models of neuropathic pain. *Pain,* 115**,** 227-233.

KLEIN, T., STAHN, S., MAGERL, W. & TREEDE, R. D. 2008. The role of heterosynaptic facilitation in long-term potentiation (LTP) of human pain sensation. *Pain,* 139**,** 507-519.

KÓBOR, I., GÁL, V. & VIDNYÁNSZKY, Z. 2009. Attentional modulation of perceived pain intensity in capsaicin-induced secondary hyperalgesia. *Experimental brain research,* 195**,** 467-472.

KREIDLER, S. M., MULLER, K. E., GRUNWALD, G. K., RINGHAM, B. M., COKER-DUKOWITZ, Z. T., SAKHADEO, U. R., BARÓN, A. E. & GLUECK, D. H. 2013. GLIMMPSE: online power computation for linear models with and without a baseline covariate. *Journal of statistical software,* 54.

LAROWE, L. R., KOSIBA, J. D., ZALE, E. L. & DITRE, J. W. 2018. Effects of nicotine deprivation on current pain intensity among daily cigarette smokers. *Experimental and clinical psychopharmacology,* 26**,** 448-455.

LAUTENBACHER, S., KUNDERMANN, B. & KRIEG, J.-C. 2006. Sleep deprivation and pain perception. *Sleep medicine reviews,* 10**,** 357-369.

LEE, E.-H. 2012. Review of the Psychometric Evidence of the Perceived Stress Scale. *Asian Nursing Research,* 6**,** 121-127.

LEEM, J. W., WILLIS, W. D., WELLER, S. C. & CHUNG, J. M. 1993. Differential activation and classification of cutaneous afferents in the rat. *Journal of Neurophysiology,* 70**,** 2411 - 2424.

LEGRAIN, V., VAN DAMME, S., ECCLESTON, C., DAVIS, K. D., SEMINOWICZ, D. A. & CROMBEZ, G. 2009. A neurocognitive model of attention to pain: behavioral and neuroimaging evidence. *Pain,* 144**,** 230-232.

LENTZ, M., LANDIS, C., ROTHERMEL, J. & SHAVER, J. 1999. Effects of slow wave disruption on musculoskeletal pain and fatigue in middle aged women. *The Journal of rheumatology,* 26**,** 1586-92.

LEVASSEUR, M., ROY, M., MICHALLET, B., ST-HILAIRE, F., MALTAIS, D. & GÉNÉREUX, M. 2017. Associations between resilience, community belonging, and social participation among community-dwelling older adults: Results from the Eastern Townships Population Health Survey. *Archives of physical medicine and rehabilitation,* 98**,** 2422-2432.

LEVY, A. S. & MARMAR, E. 1993. The role of cold compression dressings in the postoperative treatment of total knee arthroplasty. *Clinical orthopaedics and related research***,** 174-178.

LIN, J.-G. & CHEN, W.-L. 2008. Acupuncture analgesia: a review of its mechanisms of actions. *The American journal of Chinese medicine,* 36**,** 635-645.

LINDE, K., ALLAIS, G., BRINKHAUS, B., FEI, Y., MEHRING, M., SHIN, B. C., VICKERS, A. & WHITE, A. R. 2016. Acupuncture for the prevention of tension-type headache. *Cochrane Database of Systematic Reviews*.

LINDE, K., ALLAIS, G., BRINKHAUS, B., MANHEIMER, E., VICKERS, A. & WHITE, A. R. 2009. Acupuncture for migraine prophylaxis. *Cochrane Database of Systematic Reviews*.

LINDSAY, D. S., SIMONS, D. J. & LILIENFELD, S. O. 2016. Research preregistration 101. *APS Observer,* 29.

LOHR, J. M., OLATUNJI, B. O. & SAWCHUK, C. N. 2007. A functional analysis of danger and safety signals in anxiety disorders. *Clinical psychology review,* 27**,** 114-126.

LOPES, V. R. & MARTINS, M. D. C. F. 2011. Validação fatorial da escala de resiliência de Connor-Davidson (CD-RISC-10) para brasileiros. *Revista Psicologia: Organizações e Trabalho,* 11**,** 36-50.

LÓPEZ-MARTÍNEZ, A. E., ESTEVE-ZARAZAGA, R. & RAMÍREZ-MAESTRE, C. 2008. Perceived social support and coping responses are independent variables explaining pain adjustment among chronic pain patients. *The Journal of Pain,* 9**,** 373-379.

LOUW, Q. A., MORRIS, L. D. & GRIMMER-SOMERS, K. 2007. The prevalence of low back pain in Africa: a systematic review. *BMC Musculoskeletal disorders,* 8**,** 105.

LOY, A. & HOFMANN, H. 2014. HLMdiag: A suite of diagnostics for hierarchical linear models in R. *Journal of Statistical Software,* 56**,** 1-28.

LÜ, W., WANG, Z. & YOU, X. 2016. Physiological responses to repeated stress in individuals with high and low trait resilience. *Biological Psychology,* 120**,** 46-52.

LUMLEY, M. A., SKLAR, E. R. & CARTY, J. N. 2012. Emotional disclosure interventions for chronic pain: from the laboratory to the clinic. *Translational behavioral medicine,* 2**,** 73-81.

LUNDH, A. & GØTZSCHE, P. C. 2008. Recommendations by Cochrane Review Groups for assessment of the risk of bias in studies. *BMC medical research methodology,* 8**,** 22.

MADDEN, V., CATLEY, M. J., BELLAN, V., RUSSEK, L. N., CAMFFERMANN, D. & MOSELEY, G. L. 2016. The FESTNRS- a new numerical rating scale to assess non-painful and painful experiences in humans [Poster]. Unpublished.

MADDEN, V., KAMERMAN PR, BELLAN V, CATLEY MJ, RUSSEK LN, CAMFFERMAN D & GL., M. 2019a. Was That Painful or Nonpainful? The Sensation and Pain Rating Scale Performs Well in the Experimental Context. *The Journal of Pain (in press)*.

MADDEN, V., KAMERMAN PR, CATLEY MJ, BELLAN V, RUSSEK LN, CAMFFERMAN D & L., M. 2019b. Rethinking pain threshold as a zone of uncertainty. *bioRxiv,* 521302.

MADDEN, V. J., BEDWELL, G. J., CHIKEZIE, P. C., RICE, A. S. & KAMERMAN, P. R. 2019c. A systematic review of experimental methods to manipulate secondary hyperalgesia in humans: protocol. *Systematic reviews,* 8**,** 1-6.

MANHEIMER, E., CHENG, K., LINDE, K., LAO, L., YOO, J., WIELAND, S., VAN DER WINDT, D. A., BERMAN, B. M. & BOUTER, L. M. 2010. Acupuncture for peripheral joint osteoarthritis. *Cochrane database of systematic reviews*.

MATRE, D., CASEY, K. L. & KNARDAHL, S. 2006. Placebo-induced changes in spinal cord pain processing. *Journal of Neuroscience,* 26**,** 559-563.

MAYOU, R. A., BRYANT, B., SANDERS, D., BASS, C., KLIMES, I. & FORFAR, C. 1997. A controlled trial of cognitive behavioural therapy for non-cardiac chest pain. *Psychological medicine,* 27**,** 1021-1031.

MCCRACKEN, L. M. 1997. "Attention" to pain in persons with chronic pain: a behavioral approach. *Behavior therapy,* 28**,** 271-284.

MCHUGH, M. L. 2012. Interrater reliability: the kappa statistic. *Biochemia medica: Biochemia medica,* 22**,** 276-282.

MCWILLIAMS, L. & ASMUNDSON, G. 2001. Assessing individual differences in attention to pain: psychometric properties of the Pain Vigilance and Awareness Questionnaire modified for a non-clinical pain sample. *Personality and individual differences,* 31**,** 239-246.

MEEKER, T. J., KEASER, M. L., KHAN, S. A., GULLAPALLI, R. P., SEMINOWICZ, D. A. & GREENSPAN, J. D. 2019. Non-invasive Motor Cortex Neuromodulation Reduces Secondary Hyperalgesia and Enhances Activation of the Descending Pain Modulatory Network. *Frontiers in neuroscience,* 13**,** 467.

MELZACK, R. 1976. Acupuncture and pain mechanisms (author's transl). *Der Anaesthesist,* 25**,** 204-207.

MELZACK, R. 1996. Gate control theory: On the evolution of pain concepts. *Pain Forum,* 5**,** 128-138.

MELZACK, R. 2001. Pain and the neuromatrix in the brain. *Journal of dental education,* 65**,** 1378-1382.

MELZACK, R. & WALL, P. 1968. Gate control theory of pain. *Pain***,** 11-31.

MERSKEY, H. & BOGDUK, N. 1994. *Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms:,* Seattle, IASP press

MOAYEDI, M. & DAVIS, K. D. 2013. Theories of pain: from specificity to gate control. *Journal of neurophysiology,* 109**,** 5-12.
MOHAMMADIAN, P., GONSALVES, A., TSAI, C., HUMMEL, T. & CARPENTER, T. 2004. Areas of capsaicin-induced secondary hyperalgesia and allodynia are reduced by a single chiropractic adjustment: a preliminary study. *Journal of manipulative and physiological therapeutics,* 27**,** 381-387.
MOHER, D., HOPEWELL, S., SCHULZ, K. F., MONTORI, V., GOTZSCHE, P., DEVEREAUX, P., ELBOURNE, D., EGGER, M. & ALTMAN, D. 2010. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ (Clinical research ed),* 340**,** c332.
MOISSET, X. & LEFAUCHEUR, J. P. 2019. Non pharmacological treatment for neuropathic pain: Invasive and non-invasive cortical stimulation. *Revue Neurologique,* 175**,** 51-58.
MOLDOFSKY, H. & SCARISBRICK, P. 1976. Induction of Neurasthenic Musculoskeletal Pain Syndrome by Selective Sleep Stage Deprivation. *Psychosomatic medicine,* 38**,** 35-44.
MOLDOFSKY, H., SCARISBRICK, P., ENGLAND, R. & SMYTHE, H. 1975. Musculoskeletal symptoms and non-REM sleep disturbance in patients with" fibrositis syndrome" and healthy subjects. *Psychosomatic medicine*.
MONTICONE, M., CEDRASCHI, C., AMBROSINI, E., ROCCA, B., FIORENTINI, R., RESTELLI, M., GIANOLA, S., FERRANTE, S., ZANOLI, G. & MOJA, L. 2015. Cognitive-behavioural treatment for subacute and chronic neck pain. *Cochrane Database of Systematic Reviews*.
MORLEY, S., ECCLESTON, C. & WILLIAMS, A. 1999. Systematic review and meta-analysis of randomized controlled trials of cognitive behaviour therapy and behaviour therapy for chronic pain in adults, excluding headache. *Pain,* 80**,** 1-13.
MOSELEY, G. L. & ARNTZ, A. 2007. The context of a noxious stimulus affects the pain it evokes. *PAIN®,* 133**,** 64-71.
MYLIUS, V., BORCKARDT, J. J. & LEFAUCHEUR, J. P. 2012. Noninvasive cortical modulation of experimental pain. *Pain,* 153**,** 1350-63.
NADLER, S. F., WEINGAND, K. & KRUSE, R. J. 2004. The physiologic basis and clinical applications of cryotherapy and thermotherapy for the pain practitioner. *Pain physician,* 7**,** 395-400.
NEWTON-JOHN, T. R., SPENCE, S. H. & SCHOTTE, D. 1995. Cognitive-behavioural therapy versus EMG biofeedback in the treatment of chronic low back pain. *Behaviour research and therapy,* 33**,** 691-697.
NI, S.-H., JIANG, W.-T., GUO, L., JIN, Y.-H., JIANG, T.-L., ZHAO, Y. & ZHAO, J. 2015. Cryotherapy on postoperative rehabilitation of joint arthroplasty. *Knee Surgery, Sports Traumatology, Arthroscopy,* 23**,** 3354-3361.
NICKEL, F. T., SEIFERT, F., LANZ, S. & MAIHÖFNER, C. 2012. Mechanisms of neuropathic pain. *European Neuropsychopharmacology,* 22**,** 81-91.
NICOL, A. L., SIEBERG, C. B., CLAUW, D. J., HASSETT, A. L., MOSER, S. E. & BRUMMETT, C. M. 2016. The association between a history of lifetime traumatic events and pain severity, physical function, and affective distress in patients with chronic pain. *The Journal of Pain,* 17**,** 1334-1348.
OLDER, S. A., BATTAFARANO, D., DANNING, C., WARD, J. A., GRADY, E., DERMAN, S. & RUSSELL, I. 1998. The effects of delta wave sleep interruption on pain thresholds and fibromyalgia-like symptoms in healthy subjects; Correlations with insulin- like growth factor I. *The Journal of rheumatology,* 25**,** 1180-6.
PARKER, R. & JELSMA, J. 2010. The prevalence and functional impact of musculoskeletal conditions amongst clients of a primary health care facility in an under-resourced area of Cape Town. *BMC musculoskeletal disorders,* 11**,** 2.
PENNEBAKER, J. W. & BEALL, S. K. 1986. Confronting a traumatic event: toward an understanding of inhibition and disease. *Journal of abnormal psychology,* 95**,** 274.

PENNEBAKER, J. W., KIECOLT-GLASER, J. K. & GLASER, R. 1988. Disclosure of traumas and immune function: health implications for psychotherapy. *Journal of consulting and clinical psychology,* 56**,** 239.

PFAU, D. B., KLEIN, T., PUTZER, D., POGATZKI-ZAHN, E. M., TREEDE, R.-D. & MAGERL, W. 2011. Analysis of hyperalgesia time courses in humans after painful electrical high-frequency stimulation identifies a possible transition from early to late LTP-like pain plasticity. *PAIN®,* 152**,** 1532-1539.

PIEDRA, N., CHICAIZA, J., LOPEZ-VARGAS, J. & CARO, E. T. Guidelines to producing structured interoperable data from Open Access Repositories. 2016 IEEE Frontiers in Education Conference (FIE), 2016. IEEE, 1-9.

PORRECA, F., OSSIPOV, M. H. & GEBHART, G. F. 2002. Chronic pain and medullary descending facilitation. *Trends in Neurosciences,* 25**,** 319-325.

PRICE, D. D. 2000. Psychological and neural mechanisms of the affective dimension of pain. *Science,* 288**,** 1769-1772.

PRICE, D. D., HARKINS, S. W. & BAKER, C. 1987. Sensory-affective relationships among different types of clinical and experimental pain. *Pain,* 28**,** 297-307.

PRYSELEY, A., LEDENT, E. Y., DREWES, A. M., STAAHL, C., OLESEN, A. E. & ARENDT-NIELSEN, L. 2009. Applying concepts of generalizability theory on data from experimental pain studies to investigate reliability. *Basic & clinical pharmacology & toxicology,* 105**,** 105-112.

PUD, D., YARNITSKY, D., EISENBERG, E., ANDERSEN, O. K. & ARENDT-NIELSEN, L. 2006. Effects of cold stimulation on secondary hyperalgesia (HA) induced by capsaicin in healthy volunteers. *Experimental brain research,* 170**,** 22-29.

QUINLAN, P., DAVIS, J., FIELDS, K., MADAMBA, P., COLMAN, L., TINCA, D. & CANNON DRAKE, R. 2017. Effects of localized cold therapy on pain in postoperative spinal fusion patients. *Orthopaedic Nursing,* 36**,** 344-349.

RAHIMI, B., BAETZ, M., BOWEN, R. & BALBUENA, L. 2014. Resilience, stress, and coping among Canadian medical students. *Canadian medical education journal,* 5**,** e5.

RASMUSSEN, V., BORGEN, A., JANSEN, E., ROTBØLL NIELSEN, P. & WERNER, M. 2015. Hyperbaric oxygen therapy attenuates central sensitization induced by a thermal injury in humans. *Acta Anaesthesiologica Scandinavica,* 59**,** 749-762.

RAYMOND, I., NIELSEN, T. A., LAVIGNE, G., MANZINI, C. & CHOINIÈRE, M. 2001. Quality of sleep and its daily relationship to pain intensity in hospitalized adult burn patients. *PAIN®,* 92**,** 381-388.

REBHORN, C., BREIMHORST, M., BUNIATYAN, D., VOGEL, C., BIRKLEIN, F. & EBERLE, T. 2012. The efficacy of acupuncture in human pain models: a randomized, controlled, double-blinded study. *PAIN®,* 153**,** 1852-1862.

REN, K. & DUBNER, R. 2002. Descending modulation in persistent pain: an update. *PAIN,* 100**,** 1-6.

REVIEW MANAGER 2014. (RevMan). Version 5.3 ed. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration.

REYES, A., KEARNEY, C., ISLA, K. & BRYANT, R. 2018. Student veterans' construction and enactment of resilience: A constructivist grounded theory study. *Journal of psychiatric and mental health nursing,* 25**,** 37-48.

RODE, S., SALKOVSKIS, P. M. & JACK, T. 2001. An experimental study of attention, labelling and memory in people suffering from chronic pain. *Pain,* 94**,** 193-203.

ROLKE, R., BARON, R., MAIER, C., TÖLLE, T. R., TREEDE, R. D., BEYER, A., BINDER, A., BIRBAUMER, N., BIRKLEIN, F., BÖTEFÜR, I. C., BRAUNE, S., FLOR, H., HUGE, V., KLUG, R., LANDWEHRMEYER, G. B., MAGERL, W., MAIHÖFNER, C., ROLKO, C., SCHAUB, C., SCHERENS, A., SPRENGER, T., VALET, M. & WASSERKA, B. 2006. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): Standardized protocol and reference values. *PAIN,* 123**,** 231-243.

ROSS, J. R. & MATAVA, M. J. 2011. Tattoo-Induced Skin "Burn" During Magnetic Resonance Imaging in a Professional Football Player:A Case Report. *Sports Health,* 3**,** 431-434.

RUSCHEWEYH, R., WILDER-SMITH, O., DRDLA, R., LIU, X.-G. & SANDKÜHLER, J. 2011. Long-term potentiation in spinal nociceptive pathways as a novel target for pain therapy. *Molecular pain,* 7**,** 20.

SALOMONS, T. V., MOAYEDI, M., ERPELDING, N. & DAVIS, K. D. 2014. A brief cognitive-behavioural intervention for pain reduces secondary hyperalgesia. *PAIN®,* 155**,** 1446-1452.

SANDERSON, S., TATT, I. D. & HIGGINS, J. 2007. Tools for assessing quality and susceptibility to bias in observational studies in epidemiology: a systematic review and annotated bibliography. *International journal of epidemiology,* 36**,** 666-676.

SANDKÜHLER, J. & GRUBER-SCHOFFNEGGER, D. 2012. Hyperalgesia by synaptic long-term potentiation (LTP): an update. *Current opinion in pharmacology,* 12**,** 18-27.

SCARCELLA, J. & COHN, B. 1995. The effect of cold therapy on the postoperative course of total hip and knee arthroplasty patients. *American journal of orthopedics (Belle Mead, NJ),* 24**,** 847-852.

SCORGIE, F., BARON, D., STADLER, J., VENABLES, E., BRAHMBHATT, H., MMARI, K. & DELANY-MORETLWE, S. 2017. From fear to resilience: adolescents' experiences of violence in inner-city Johannesburg, South Africa. *BMC public health,* 17**,** 441.

SERRA, J., CAMPERO, M., BOSTOCK, H. & OCHOA, J. 2004. Two types of C nociceptors in human skin and their behavior in areas of capsaicin-induced secondary hyperalgesia. *Journal of neurophysiology,* 91**,** 2770-2781.

SHELDON, B. 2011. *Cognitive-behavioural therapy: research and practice in health and social care*, Routledge.

SHLOMI, H. 2010. The Relationship Between Childhood Family Instability, Secure Attachment, and the Sense of Belonging of Young Adults.

SIEGRIST, M. 1996. The use or misuse of three-dimensional graphs to represent lower-dimensional data. *Behaviour & Information Technology,* 15**,** 96-100.

SLOAN, D. M. & MARX, B. P. 2004. A Closer Examination of the Structured Written Disclosure Procedure. *Journal of Consulting and Clinical Psychology,* 72**,** 165-175.

SLOAN, D. M., MARX, B. P. & EPSTEIN, E. M. 2005. Further Examination of the Exposure Model Underlying the Efficacy of Written Emotional Disclosure. *Journal of Consulting and Clinical Psychology,* 73**,** 549-554.

SMITH, M. T., EDWARDS, R. R., MCCANN, U. D. & HAYTHORNTHWAITE, J. A. 2007. The effects of sleep deprivation on pain inhibition and spontaneous pain in women. *Sleep,* 30**,** 494-505.

SMITH, M. T., REMENIUK, B., FINAN, P. H., SPEED, T. J., TOMPKINS, D. A., ROBINSON, M., GONZALEZ, K., BJURSTROM, M. F. & IRWIN, M. R. 2018. Sex differences in measures of central sensitization and pain sensitivity to experimental sleep disruption: implications for sex differences in chronic pain. *Sleep,* 42**,** zsy209.

SPRUYT, A., CLARYSSE, J., VANSTEENWEGEN, D., BAEYENS, F., & HERMANS, D. 2010. Affect 4.0: A free software package for implementing psychological and psychophysiological experiments. *Experimental Psychology* 57**,** 36 - 45.

STATISTICS SOUTH AFRICA. 2019. *Crime statistics* [Online]. Available: http://www.statssa.gov.za/?page_id=737&id=5=5 [Accessed Jan 2020].

STAUD, R. & PRICE, D. D. 2006. Mechanisms of acupuncture analgesia for clinical and experimental pain. *Expert Review of Neurotherapeutics,* 6**,** 661+.

STUCKY, C. L., GOLD, M. S. & ZHANG, X. 2001. Mechanisms of pain. *Proceedings of the National Academy of Sciences,* 98**,** 11845.

STURGEON, J. A. & ZAUTRA, A. J. 2010. Resilience: a new paradigm for adaptation to chronic pain. *Current pain and headache reports,* 14**,** 105-112.

SULLIVAN, M. J., BISHOP, S. R. & PIVIK, J. 1995. The pain catastrophizing scale: development and validation. *Psychological assessment,* 7**,** 524.

SULLIVAN, M. J. L., LYNCH, M. E. & CLARK, A. J. 2005. Dimensions of catastrophic thinking associated with pain experience and disability in patients with neuropathic pain conditions. *Pain,* 113**,** 310-315.

SUZUKI, R., RYGH, L. J. & DICKENSON, A. H. 2004. Bad news from the brain: descending 5-HT pathways that control spinal pain processing. *Trends in Pharmacological Sciences,* 25**,** 613-617.

TALBOT, K., MADDEN, V. J., JONES, S. L. & MOSELEY, G. L. 2019. The sensory and affective components of pain: are they differentially modifiable dimensions or inseparable aspects of a unitary experience? A systematic review. *British Journal of Anaesthesia,* 123**,** e263-e272.

TANG, N. K., SALKOVSKIS, P. M., POPLAVSKAYA, E., WRIGHT, K. J., HANNA, M. & HESTER, J. 2007. Increased use of safety-seeking behaviors in chronic back pain patients with high health anxiety. *Behaviour Research and Therapy,* 45**,** 2821-2835.

THIENPONT, E. 2014. Does advanced cryotherapy reduce pain and narcotic consumption after knee arthroplasty? *Clinical Orthopaedics and Related Research®,* 472**,** 3417-3423.

THOMAS, V. J. N. 2000. Cognitive behavioural therapy in pain management for sickle cell disease. *International journal of palliative nursing,* 6**,** 434-442.

TICHELAAR, Y. V., GEERTZEN, J. H., KEIZER, D. & VAN WILGEN, C. P. 2007. Mirror box therapy added to cognitive behavioural therapy in three chronic complex regional pain syndrome type I patients: a pilot study. *International journal of rehabilitation research,* 30**,** 181-188.

TORRANCE, N., FERGUSON, J. A., AFOLABI, E., BENNETT, M. I., SERPELL, M. G., DUNN, K. M. & SMITH, B. H. 2013. Neuropathic pain in the community: more under-treated than refractory? *PAIN®,* 154**,** 690-699.

TORTA, D., DE LAURENTIS, M., EICHIN, K. N., VON LEUPOLDT, A., VAN DEN BROEKE, E. & VLAEYEN, J. 2019. High cognitive load may prevent the development of nociceptive hypersensitivity. *PsyArXiv*.

TRINH, K., GRAHAM, N., IRNICH, D., CAMERON, I. D. & FORGET, M. 2016. Acupuncture for neck disorders. *Cochrane Database of Systematic Reviews*.

TSANG, A., VON KORFF, M., LEE, S., ALONSO, J., KARAM, E., ANGERMEYER, M. C., BORGES, G. L. G., BROMET, E. J., DE GIROLAMO, G. & DE GRAAF, R. 2008. Common chronic pain conditions in developed and developing countries: gender and age differences and comorbidity with depression-anxiety disorders. *The journal of pain,* 9**,** 883-891.

TURK, D. C. & OKIFUJI, A. 2002. Psychological factors in chronic pain: Evolution and revolution. *Journal of consulting and clinical psychology,* 70**,** 678.

ULLRICH, P. M. & LUTGENDORF, S. K. 2002. Journaling about stressful events: Effects of cognitive processing and emotional expression. *Annals of Behavioral Medicine,* 24**,** 244-250.

URBAN, M. & GEBHART, G. 1999. Supraspinal contributions to hyperalgesia. *Proceedings of the National Academy of Sciences,* 96**,** 7687-7692.

VAN DAMME, S., CROMBEZ, G., VAN DE WEVER, K. N. & GOUBERT, L. 2008. Is distraction less effective when pain is threatening? An experimental investigation with the cold pressor task. *European Journal of Pain,* 12**,** 60-67.

VAN DEN BROEKE, E., GEENE, N., VAN RIJN, C., WILDER-SMITH, O. & OOSTERMAN, J. 2014. Negative expectations facilitate mechanical hyperalgesia after high-frequency electrical stimulation of human skin. *European journal of pain,* 18**,** 86-91.

VAN DEN BROEKE, E. N. & MOURAUX, A. 2014. High-frequency electrical stimulation of the human skin induces heterotopical mechanical hyperalgesia, heat hyperalgesia, and enhanced responses to nonnociceptive vibrotactile input. *Journal of neurophysiology,* 111**,** 1564-1573.

VAN HECKE, O., AUSTIN, S. K., KHAN, R. A., SMITH, B. & TORRANCE, N. 2014. Neuropathic pain in the general population: a systematic review of epidemiological studies. *PAIN®,* 155**,** 654-662.

VAN WILGEN, C. P. & KEIZER, D. 2012. The sensitization model to explain how chronic pain exists without tissue damage. *Pain Management Nursing,* 13**,** 60-65.

VANDENBROUCKE, J. P., VON ELM, E., ALTMAN, D. G., GØTZSCHE, P. C., MULROW, C. D., POCOCK, S. J., POOLE, C., SCHLESSELMAN, J. J., EGGER, M. & INITIATIVE, S. 2007. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *PLoS medicine,* 4**,** e297.

VASEGHI, B., ZOGHI, M. & JABERZADEH, S. 2014. Does anodal transcranial direct current stimulation modulate sensory perception and pain? A meta-analysis study. *Clin Neurophysiol,* 125**,** 1847-58.

VASEGHI, B., ZOGHI, M. & JABERZADEH, S. 2015. A meta-analysis of site-specific effects of cathodal transcranial direct current stimulation on sensory perception and pain. *PLoS One,* 10**,** e0123873.

VERNON, H. T., DHAMI, M. S., HOWLEY, T. P. & ANNETT, R. 1986. Spinal manipulation and beta-endorphin: a controlled study of the effect of a spinal manipulation on plasma beta-endorphin levels in normal males. *J Manipulative Physiol Ther,* 9**,** 115-23.

VLAEYEN, J. W., HANSSEN, M., GOUBERT, L., VERVOORT, T., PETERS, M., VAN BREUKELEN, G., SULLIVAN, M. J. & MORLEY, S. 2009. Threat of pain influences social context effects on verbal pain report and facial expression. *Behaviour research and therapy,* 47**,** 774-782.

VLAEYEN, J. W. & LINTON, S. J. 2000. Fear-avoidance and its consequences in chronic musculoskeletal pain: a state of the art. *Pain,* 85**,** 317-332.

VO, L. & DRUMMOND, P. D. 2013. High frequency electrical stimulation concurrently induces central sensitization and ipsilateral inhibitory pain modulation. *European Journal of Pain,* 17**,** 357-368.

WAHL, A. M., BIDSTRUP, D., SMIDT-NIELSEN, I. G., WERNER, M. U., HYLDEGAARD, O. & ROTBØLL-NIELSEN, P. 2019. A single session of hyperbaric oxygen therapy demonstrates acute and long-lasting neuroplasticity effects in humans: a replicated, randomized controlled clinical trial. *Journal of pain research,* 12**,** 2337.

WERNER, M. U., LASSEN, B., PEDERSEN, J. L. & KEHLET, H. 2002. Local cooling does not prevent hyperalgesia following burn injury in humans. *Pain,* 98**,** 297-303.

WIECH, K., LIN, C.-S., BRODERSEN, K. H., BINGEL, U., PLONER, M. & TRACEY, I. 2010. Anterior insula integrates information about salience into perceptual decisions about pain. *Journal of Neuroscience,* 30**,** 16324-16331.

WIECH, K., SEYMOUR, B., KALISCH, R., STEPHAN, K. E., KOLTZENBURG, M., DRIVER, J. & DOLAN, R. J. 2005. Modulation of pain processing in hyperalgesia by cognitive demand. *Neuroimage,* 27**,** 59-69.

WINTER, B. 2013. *Linear Models and linear mixed effects models in R with linguistic applications. arXiv:1308.5499* [Online]. Available: http://arxiv.org/pdf/1308.5499.pdf [Accessed].

YOU, D. S., CREECH, S. K. & MEAGHER, M. W. 2016. Enhanced Area of Secondary Hyperalgesia in Women with Multiple Stressful Life Events: A Pilot Study. *Pain Medicine,* 17**,** 1859-1864.

YOU, D. S., CREECH, S. K., VICHAYA, E. G., YOUNG, E. E., SMITH, J. S. & MEAGHER, M. W. 2014. Effect of written emotional disclosure on secondary hyperalgesia in women with trauma history. *Psychosom Med,* 76**,** 337-46.

YUCEL, A., MIYAZAWA, A., ANDERSEN, O. O. & ARENDT-NIELSEN, L. 2001. The effect of heat conditioning of the primary area before and after induction of hyperalgesia by topical/intradermal capsaicin or by controlled heat injury. *Somatosensory & motor research,* 18**,** 295-302.

ZIMET, G. D., POWELL, S. S., FARLEY, G. K., WERKMAN, S. & BERKOFF, K. A. 1990. Psychometric characteristics of the multidimensional scale of perceived social support. *Journal of personality assessment,* 55**,** 610-617.

Appendices

Appendix 1: A systematic review of experimental methods to manipulate secondary hyperalgesia in humans: protocol

Madden *et al. Systematic Reviews* (2019) 8:208
https://doi.org/10.1186/s13643-019-1120-7

Systematic Reviews

PROTOCOL Open Access

A systematic review of experimental methods to manipulate secondary hyperalgesia in humans: protocol

Victoria J. Madden[1,2*], Gillian J. Bedwell[1,3], Prince C. Chikezie[4], Andrew S. C. Rice[5] and Peter R. Kamerman[4,6]

Abstract

Background: Neuropathic pain affects 7–10% of people, but responds poorly to pharmacotherapy, indicating a need for better treatments. Mechanistic research on neuropathic pain frequently uses human surrogate models of the secondary hyperalgesia that is a common feature of neuropathic pain. Experimentally induced secondary hyperalgesia has been manipulated with pharmacological and non-pharmacological methods to clarify the relative contributions of different mechanisms to secondary hyperalgesia. However, this literature has not been systematically synthesised. The aim of this systematic review is to identify, describe, and compare methods that have been used to manipulate experimentally induced secondary hyperalgesia in healthy humans.

Methods: A systematic search strategy will be supplemented by reference list checks and direct contact with identified laboratories to maximise the identification of data reporting the experimental manipulation of experimentally induced secondary hyperalgesia in healthy humans. Duplicated screening, risk of bias assessment, and data extraction procedures will be used. Authors will be asked to provide data as necessary. Data will be pooled and meta-analyses conducted where possible, with subgrouping according to manipulation method. Manipulation methods will be ranked for potency and risk.

Discussion: The results of this review will provide a useful reference for researchers interested in using experimental methods to manipulate secondary hyperalgesia in humans and will help to clarify the relative contributions of different mechanisms to secondary hyperalgesia.

Systematic review registration: This protocol will be registered on PROSPERO before the review begins. Review records will be updated on PROSPERO once the review is complete. This review is intended for publication in a peer-reviewed journal. Analyses and scripts will be made publicly available.

Keywords: Hyperalgesia, Systematic review, Healthy volunteers, Quantitative sensory testing, Behaviour control

Introduction

Rationale

Neuropathic pain (NP) currently affects 6.9–10% of the general population [1] and has severe consequences for the individual and for society. In a multicentre cohort survey, 2 in 3 people with NP had suboptimal sleep, 1 in 3 had a current mood disorder, over 90% reported feeling "sadness most of the time" and "tired most of the time", and 18% had a current risk of suicide [2]. These findings are consistent with other reports that NP is associated with decreased life participation, low health-related quality of life, poor mental health, poor physical function, and higher use of health care services [3, 4]. The problem is only perpetuated by the fact that NP treatments perform poorly: the numbers needed to treat for even the first-line drugs range from 3.6 to 7.7 [5]. Given the severe consequences of NP and the obvious need for better treatments, understanding the mechanisms of NP is an important priority for health care research.

* Correspondence: torymadden@gmail.com
[1]Pain Unit, Department of Anaesthesia and Perioperative Medicine, Neuroscience Institute, University of Cape Town, D23.30 Groote Schuur Hospital, Observatory, Cape Town 7925, South Africa
[2]Department of Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa
Full list of author information is available at the end of the article

Secondary hyperalgesia—increased pain to a stimulus that is normally painful, in the area outside that of tissue damage or stimulation—is a common feature of NP [6]. Although a single model cannot account for all the possible features of clinical NP, experimentally induced secondary hyperalgesia has been widely used as a laboratory-based human surrogate model in studies that aim to elucidate the mechanisms that underlie neuropathic pain [7–11]. Methods that have been used to induce this secondary hyperalgesia in the experimental context include the application of capsaicin (intradermal or topical), topical mustard oil, topical menthol, repetitive heating, and high-frequency electrical stimulation [6, 9]. While secondary hyperalgesia is typically considered the primary outcome of studies that use these inductions, secondary outcomes may include primary hyperalgesia, secondary allodynia, and the surface area of secondary hyperalgesia. These outcomes can be compared to identify the relative contributions of different mechanisms. For example, while capsaicin-induced primary hyperalgesia could be attributed to changes in both peripheral and central nervous system processing, the broader distribution of secondary hyperalgesia suggests that it is mediated by changes in central processing [12].

Individual responses to an induction of secondary hyperalgesia can be useful for identifying certain profiles of sensitivity and factors that may be linked to those profiles. For example, in a small pilot study, women with a history of trauma showed a greater surface area of secondary hyperalgesia than women who did not have a history of trauma, after both groups underwent the same induction procedure [13]. This profiling may also have direct clinical application: the magnitude of induced secondary hyperalgesia could be useful for grouping patients by phenotype in order to better predict their responses to treatment [14].

As a surrogate model for NP, experimentally induced secondary hyperalgesia can be manipulated using pharmacological, psychological, or physical techniques, with a view to identifying potential treatments. The NMDA antagonist, Ketamine, has been found to reduce secondary hyperalgesia induced by capsaicin [15]. Negative expectations have been linked to greater secondary hyperalgesia induced by electrical stimulation [16], and an emotional disclosure intervention reduced the surface area of secondary hyperalgesia induced by capsaicin in women with a prior history of trauma [17]. A potential treatment that shows promise in animal studies can be tested in humans using the secondary hyperalgesia model as a step in the process towards refining and testing the treatment for clinical use.

Manipulating experimentally induced secondary hyperalgesia with interventions that have clinical potential is an important line of inquiry towards developing viable treatments for the relief of NP. However, although numerous studies have manipulated secondary hyperalgesia, there has been no attempt to systematically synthesise and appraise the literature on this topic.

Aims and objectives

Aim

With this study, we aim to systematically identify, collate, and describe all the published studies that have applied manipulations intended to influence experimentally induced secondary hyperalgesia in healthy human participants. In doing so, we hope to provide a resource that will summarise the literature to date, provide pooled effect size estimates where possible, and identify gaps in knowledge and opportunities for further inquiry. Therefore, the primary aim of this systematic review is to identify, describe, and compare methods that have been used to manipulate experimentally induced secondary hyperalgesia in healthy humans.

Objectives

1. To identify methods used to manipulate secondary hyperalgesia
2. Describe each method in terms of procedure, essential equipment required, pain reported by participants during manipulation and harm reported in studies using each method
3. Determine the effect of each manipulation on
   a. The magnitude of static mechanical secondary hyperalgesia
   b. The surface area affected by secondary hyperalgesia

Methods

Eligibility criteria

Types of studies

We will include published and in-press or accepted records for which title, abstract, and full-text versions are available in English. Only prospective experimental studies will be included, that is, studies that attempted to manipulate secondary hyperalgesia for the purpose of studying the effects of the manipulation, and that did so in the context of an experiment, such that the secondary hyperalgesia was not a naturally occurring clinical phenomenon. In other words, participants must have begun the study without the secondary hyperalgesia being studied. To be eligible, studies must have assessed secondary hyperalgesia within 120 min after induction (so as not to miss the anticipated peak of the effect).

Types of study participants

We will include data from healthy human participants only. We place no restriction on the age of participants,

but data from adults will be treated separately from data from children (< 18 years old).

Types of interventions

We will include data from experimental studies that aimed to manipulate secondary hyperalgesia (defined as "increased pain from a stimulus that normally provokes pain" [18] in an area adjacent to the stimulated area). Studies that manipulate secondary hyperalgesia as one step in a larger study will be considered eligible, provided that suitable baseline/control data are available to allow for estimation of the effect of the manipulation on secondary hyperalgesia.

Types of outcome measures

Pain will be defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" [18]. Pain must be measured by subjective self-report. Therefore, studies must have assessed the subjective (participant-reported) intensity of pain or sensation to somatosensory stimulation.

Primary outcome

The primary outcome is secondary hyperalgesia. Studies must have assessed mechanical secondary hyperalgesia (specifically, participant self-report to punctate mechanical stimulation) applied to the area surrounding the induction/manipulation site. Further, in order to qualify as "hyperalgesia", the post-manipulation assessment must be compared to a within-subject control site (e.g. opposite limb on which induction but not manipulation was performed) or time point (e.g. after induction but before manipulation or, in the case of repeated inductions, to the same induction procedure performed without manipulation) or a between-subject control (e.g. group that underwent induction without the manipulation).

Secondary outcomes

- Surface area of secondary hyperalgesia, as measured using reproducible methods (such as a radial lines approach [11, 13, 15])
- Time course of secondary hyperalgesia
- Pain (except to test stimulation) reported during and after manipulation procedure
- Risks of the manipulation, defined as adverse events (e.g. skin damage, other adverse reaction)

Search methods for identification of studies

Electronic searches

We will search the following electronic databases with a strategy that spans the time from their inception to the date of the search:

- Biosis (via Web of Science)
- PubMed (includes MEDLINE)
- Scopus
- ScienceDirect
- PsychArticles
- PsychInfo
- Cochrane library
- Web of Science Core (use to search and then use menu on left to filter for Core option and Biosis)

The search strategy will be:

(("human*" OR "women" or "woman" OR "man" OR "men" OR "participant*" OR "volunteer* OR individual*")

OR

"normal skin" OR "healthy skin")

AND

("secondary hyperalgesia" OR "punctate hyperalgesia" OR "pinprick pain" OR "pinprick hyperalgesia" OR "mechanical hyperalgesia" OR "mechanical pain" OR "heat hyperalgesia" OR "neurogenic hyperalgesia"))

with all terms searched for in the title, keywords, or abstract.

+ limit to humans when possible in each database.

Other sources

We will check reference lists of eligible studies to check for other eligible studies not identified by electronic searching. We will also contact experts in the field and the corresponding authors of the most recent narrative reviews on experimental induction and manipulation of secondary hyperalgesia to ask for their assistance in identifying any missed studies (including Walter Magerl, Rolf Baron, Jürgen Sandkühler, Mark Wallace, Peter Drummond). We will also request any unpublished data from labs that have published extensively on these techniques, including data obtained during model development or optimisation.

Data collection and analysis

Data management

The Systematic Review Facility (http://syrf.org.uk/) will be used to manage the review process.

Study selection

Two of three reviewers (VJM, GJB, and PC) will independently screen each identified record for eligibility in two sequential stages, screening (stage 1) title and abstracts and (stage 2) full texts. We will contact study authors a maximum of three times to obtain additional information that could influence eligibility. A customised eligibility form (see table below) will be used to record decisions in stage 2. Any disagreements about study inclusion will be resolved by discussion or by adjudication from a fourth, independent reviewer (PRK) if necessary. The study selection process will be reported using a PRISMA flow diagram.

Inclusion/exclusion criteria and grouping table

	INCLUSION	EXCLUSION	
Participants	Pain-free, healthy humans	Animals OR people with pain	
Study design	Used an experimental procedure with the aim of inducing AND manipulating secondary hyperalgesia (identifiable goal AND site AND induction procedure AND manipulation procedure)	Review *(set aside for cross-checking)* OR Not an experimental procedure OR No identifiable manipulation procedure	
Outcomes	Pain or sensitivity to provocation assessed subsequent to induction AND manipulation Acceptable: pain yes or no, self-report of intensity, quality, pain threshold.	Subjective ratings not provided Unacceptable: facial expression, physical behaviour measurement, or psychophysiology in absence of self-report	
Include?	Tick in *every* box above: include ☐	OR Tick in *any* box above: exclude ☐	Review ☐

Assessment of risk of bias

A risk of bias assessment will be completed on each study. Two reviewers will independently appraise the risk of bias for the domains of selection, performance, detection, attrition, measurement, reporting, and other sources of bias. The criteria used to rate the risk of bias will be based on recommendations from the Cochrane collaboration [19] known quality instruments (e.g. the CONSORT [20] and STROBE [21] statements as relevant) and on known areas of bias relevant to the study design used [22], and are specified in the risk of bias assessment tool and guide (see Additional file 1). We will pilot the form on 2–3 studies and adapt it prior to formal application to all included studies. The focus of the risk of bias assessment will be on the risk that the data to be extracted to answer the questions of this review could be biased. The appraisals of the two independent reviewers will be compared and any disagreements resolved through discussion and consensus, or by third party adjudication if necessary.

Data extraction and management

Two of reviewers will independently extract data from each included study, using a standardised data extraction form. We will pilot and refine the data extraction form using 2–3 studies beforehand. We will contact study authors a maximum of 3 times to obtain required data that are unavailable or unclear from the published texts. If no reply is received within 6 weeks, we will consider the data unavailable. If the relevant data are not provided within 6 weeks of the first reply, we will consider the data unavailable for this review. Published data that seem implausible will be verified directly with the corresponding author where possible. Reviewers will resolve disagreements by discussion and consensus, or by a third party adjudication if necessary.

Study design We will extract data on the study design, including the location/setting, date, sample size, primary aim, and outcomes measured.

Participants We will extract data on the participants, including number, age, sex, pain status and sites of pain, co-morbid diagnoses, and psychological variables.

Interventions We will extract data on the intervention(s) used to manipulate secondary hyperalgesia, including timing, duration, dosage (e.g. intensity or concentration), method of administration, modality, site, adverse effects, and ease of application where relevant. We will extract data on the equipment used (e.g. electrodes, needles, images, scripts). We will extract data on the control (site and/or time point and/or group). We will extract data on the manipulation check, where relevant (e.g. for psychological manipulations).

Outcomes We will extract data on the test stimulus modalities, the scale or measure used to assess pain or change in perceived intensity, and the timing of assessments, that is, time from induction to first measurements and to each subsequent measurement, in minutes. We are interested in the following outcomes:

1) Secondary hyperalgesia intensity to calibrated punctate mechanical stimulation (e.g. Von Frey filament or calibrated pinprick stimulator)
2) The surface area affected by secondary hyperalgesia as tested in [1]
3) The duration of secondary hyperalgesia as tested in [1]

Where studies have included other outcomes associated with changes in secondary hyperalgesia (e.g. changes in brain activity shown on imaging), and when the secondary hyperalgesia data for that study indicate that the manipulation successfully influenced secondary hyperalgesia, we will discuss the findings as they pertain to the other outcomes.

Results We will extract data on the participants in each study (including age, sex) the baseline and follow-up ratings for experimental and control sites for each participant or group, for each outcome. We will extract the number of participants who reported adverse effects and the number and natures of adverse effects reported.

Measures of intervention effects

When determining the potency of each manipulation method, our goal is to estimate the size of the effect of the manipulation by comparing the post-manipulation outcomes with the pre-manipulation outcomes and/or a control site/condition/group. For manipulations that are thought to have localised effects, a within-subject control site will be considered acceptable. For manipulations that are thought to have time-limited effects, a within-participant control time point will be acceptable. For manipulations that are thought to have systemic effects, a between-subject control or a control time-point (with suitable washout period) will be required. Data will be requested from authors where necessary.

Pooling of data

Data from different studies will be quantitatively pooled where possible and sensible, with subgrouping according to modality of induction/manipulation (e.g. negative expectation manipulation will be grouped separately to ketamine manipulation) and outcome (e.g. secondary hyperalgesia to mechanical punctate stimulation will be grouped separately to secondary allodynia to brush stroke), and with consideration given to measures of statistical heterogeneity (e.g. I^2 statistic) and sensitivity analyses that exclude studies with particularly high risk of bias and studies in which obvious sources of methodological heterogeneity have been identified. This is not an individual patient data meta-analysis. We plan to use the RevMan software [23] or the R software with the package metafor to pool data and plot pooled data. The most up-to-date version of the relevant software package will be used. The outcome measure will be a standardised mean difference. We will use a random effects model to allow for anticipated heterogeneity between studies.

Where data exist but are unavailable, we plan to discuss the information that is available (e.g. number of participants tested, reasons for data unavailability, methods used) in narrative form, so as to mitigate against publication bias and provide a comprehensive summary of the literature available to answer the review question.

Relative ranking of interventions

If the quantity and quality of data allow, we will compare the pooled effect sizes (where available) to rank the different manipulations in the order of potency and risk. If the results reveal different potencies for the different outcomes of interest, then these ranked lists will be compiled separately for the different outcomes.

Publication bias

We will use funnel plots to examine for publication bias.

Assessment of the quality of the body of evidence

We will use the GRADE criteria [24] to assess the quality of the body of evidence for each manipulation, wherever more than one study is available for a certain manipulation.

Publication and dissemination plan

We will update the review records on PROSPERO once the review is complete. We aim to publish this review in a peer-reviewed journal and to make all analyses and scripts publicly available.

Reporting

Please see Additional file 2 PRISMA-P checklist for the elements included in this protocol.

Additional file

Additional file 1: Risk of bias assessment tool. (DOCX 26 kb)
Additional file 2: PRISMA-P form. (DOC 85 kb)

Acknowledgements
Not applicable

Authors' contributions
VJM conceived and developed the protocol; piloted the procedure; refined, finalised, and submitted the protocol, and approved the final version of the protocol. GJB piloted the procedure, refined the protocol, and approved the final version of the protocol. PC refined and approved the final version of the protocol. ASCR approved the final version of the protocol. PRK refined and approved the final version of the protocol.

Funding
VJM was supported by an Innovation Postdoctoral Fellowship from the National Research Foundation of South Africa. GJB is supported by Postgraduate Research Grants from PainSA, the South African Society of Physiotherapy, and the University of Cape Town.

Availability of data and materials
The public release of data used in this systematic review will depend on the ethics permissions to which the original data are subject. It is our preference to make data publicly available, but this may not be feasible due to existing restraints on the data. However, analysis scripts will be made publicly available for verification of the analytical processes.

Ethics approval and consent to participate
This review will gather and appraise existing data from studies for which healthy volunteer participants have previously given consent. We will not include data from any study for which participants did not give informed consent. Where raw data are requested, authors will be asked to verify that their ethical permissions allow them to release the anonymised data for ongoing research.

Consent for publication
Please see previous section.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Pain Unit, Department of Anaesthesia and Perioperative Medicine, Neuroscience Institute, University of Cape Town, D23.30 Groote Schuur Hospital, Observatory, Cape Town 7925, South Africa. [2]Department of Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa. [3]Department of Health and Rehabilitation Sciences, University of Cape Town, Cape Town, South Africa. [4]School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. [5]Pain Research Group, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London, UK. [6]School of Pharmacy and Biomedical Sciences, Faculty of Health Sciences, Curtin University, Perth, Australia.

Received: 24 January 2019 Accepted: 26 July 2019
Published online: 19 August 2019

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. Radat F, Margot-Duclot A, Attal N. Psychiatric co-morbidities in patients with chronic peripheral neuropathic pain: a multicentre cohort study. Eur J Pain. 2013;17(10):1547–57.
3. Attal N, Lanteri-Minet M, Laurent B, Fermanian J, Bouhassira D. The specific disease burden of neuropathic pain: Results of a French nationwide survey. Pain. 2011;152(12):2836–43.
4. Torrance N, Ferguson JA, Afolabi E, Bennett MI, Serpell MG, Dunn KM, et al. Neuropathic pain in the community: More under-treated than refractory? Pain. 2013;154(5):690–9.
5. 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.
6. Magerl W, Klein T. Experimental human models of neuropathic pain. In: Cervero F, Jensen TS, editors. Handbook of Clinical Neurology. 81: Elsevier; 2006. p. 503–16.
7. Randic M, Jiang M, Cerne R. Long-term potentiation and long-term depression of primary afferent neurotransmission in the rat spinal cord. J Neurosci. 1993;13(12):5228–41.
8. Baron R, Maier C, Attal N, Binder A, Bouhassira D, Cruccu G, et al. Peripheral neuropathic pain: a mechanism-related organizing principle based on sensory profiles. Pain. 2017;158(2):261–72.
9. Klein T, Magerl W, Hopf H-C, Sandkühler J, Treede R-D. Perceptual correlates of nociceptive long-term potentiation and long-term depression in humans. J Neurosci. 2004;24(4):964–71.
10. Sandkuhler J. Understanding LTP in pain pathways. Mol Pain. 2007;3:9.
11. Henrich F, Magerl W, Klein T, Greffrath W, Treede R-D. Capsaicin-sensitive C- and A-fibre nociceptors control long-term potentiation-like pain amplification in humans. Brain. 2015;138(9):2505–20.
12. 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.
13. 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.
14. Reimer M, Helfert SM, Baron R. Phenotyping neuropathic pain patients: implications for individual therapy and clinical trials. Curr Opin Support Palliat Care. 2014;8(2):124–9.
15. 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.
16. van den Broeke EN, Geene N, van Rijn CM, Wilder-Smith OH, Oosterman J. Negative expectations facilitate mechanical hyperalgesia after high-frequency electrical stimulation of human skin. Eur J Pain. 2014;18(1):86–91.
17. You DS, Creech SK, Vichaya EG, Young EE, Smith JS, Meagher MW. Effect of written emotional disclosure on secondary hyperalgesia in women with trauma history. Psychosom Med. 2014;76(5):337–46.
18. Merskey H, Bogduk N. Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms: IASP press Seattle; 1994.
19. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343:d5928.
20. Moher D, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, et al. CONSORT 2010 Explanation and Elaboration: updated guidelines for reporting parallel group randomised trials. BMJ. 2010:340.
21. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. PLoS Med. 2007;4(10):e297.
22. Sanderson S, Tatt ID, Higgins JP. Tools for assessing quality and susceptibility to bias in observational studies in epidemiology: a systematic review and annotated bibliography. Int J Epidemiol. 2007;36(3):666–76.
23. The Cochrane Collaboration. Review Manager (RevMan). 5.3 ed. Copenhagen: The Nordic Cochrane Centre; 2014.
24. Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, et al. GRADE guidelines: 1. Introduction—GRADE evidence profiles and summary of findings tables. J Clin Epidemiol. 2011;64(4):383–94.

Publisher's Note

Appendix 2: Risk of bias assessment tool

Article ID:	Reviewer:	
Selection bias		
	Decision	Justification
Was the sampling/recruitment strategy appropriate to minimise bias?	☐ Yes ☐ No ☐ Unclear	
Was it clearly and appropriately determined that participants were pain-free?	☐ Yes ☐ No ☐ Unclear	
[B-G only] Similar baseline demographics among participants (age/sex/medical/psychological state)?	☐ Yes ☐ No ☐ Unclear	
[Psych manip] Neutral psych status?	☐ Yes ☐ No ☐ Unclear	
[B-G only] Random allocation [B-site] Random allocation	☐ Yes ☐ No ☐ Unclear	
Risk of selection bias summary	☐ High (failure to include any of the above probably influenced results FOR THE QUESTION OF THIS REVIEW) ☐ Low (results unlikely to have been influenced) ☐ Unclear (not enough information)	
Performance bias		
Blinding	Decision	Justification
Were participants blinded to the research question and paradigm and [if relevant] group allocation?	☐ Yes ☐ No ☐ Unclear	
Risk of performance bias summary	☐ High ☐ Low ☐ Unclear	

Detection bias		
Were outcome assessors blinded to the research question and paradigm?	☐ Yes ☐ No ☐ Unclear	
Were analysing researchers blinded to the group allocation of participants and/or to site allocation?	☐ Yes ☐ No ☐ Unclear	
Risk of detection bias summary	☐ High ☐ Low ☐ Unclear	
Manipulation veracity		
[Psych] Did a manipulation check confirm the effectiveness of the manipulation?	☐ Yes ☐ No ☐ Unclear	
Risk of manipulation veracity problem	☐ High ☐ Low ☐ Unclear	
Attrition bias		
Incomplete outcome data	Decision	Justification
Have attrition/exclusions/ withdrawals been reported and appropriately dealt with in analysis?	☐ Yes ☐ No ☐ Unclear	
Risk of attrition bias summary	☐ High ☐ Low ☐ Unclear	
Measurement bias		
	Decision	Justification
Were valid and reliable outcome measurements used to assess severity & SA of secondary hyperalgesia?	2H: ☐ Yes ☐ No ☐ Unclear SA: ☐ Yes ☐ No ☐ Unclear	
Were identical equipment items used for measurements between groups/sites/time points?	2H: ☐ Yes ☐ No ☐ Unclear SA: ☐ Yes ☐ No ☐ Unclear	
Did the same assessor conduct assessments between groups/sites/time points?	2H: ☐ Yes ☐ No ☐ Unclear	

	SA: □ Yes □ No □ Unclear	
Risk of measurement bias summary	2H: □ High □ Low □ Unclear SA: □ High □ Low□ Unclear	
Reporting bias		
Selective reporting	Decision	Justification
Were all outcomes for experimental and control groups reported on?	□ Yes □ No	
Were conflicts of interest and funding sources declared?	□ Yes □ No	
Risk of reporting bias summary	□ High □ Low □ Unclear	

Risk of bias summary		
Risk of bias	Description	Study bias outcome
High risk of bias	Plausible bias that seriously weakens confidence in the results.	
Low risk of bias	Plausible bias unlikely to seriously alter or diminish trust in the results.	
Unclear risk of bias	Insufficient information available to make a judgement.	

Comments:

Appendix 3: Risk of bias guide to decision-making for risk of bias

Article ID:	Reviewer:	
Selection bias		
	Decision	Justification
Was the sampling/recruitment strategy appropriate to minimise bias?	☐ Yes ☐ No ☐ Unclear	Yes: general population or subgroup. Convenience sampling is acceptable as long as eligibility criteria do not restrict to a certain group that could plausibly respond differently to the induction. No: group selected on basis of particular feature (e.g. high catastrophising positive affect / athletes in training)
Was it clearly and appropriately determined that participants were pain-free?	☐ Yes ☐ No ☐ Unclear	Yes: participant self-report of no pain at time of testing AND no history of chronic pain (pain on most days for > 3 months) in preceding 2 years. No: reports failure to ask BOTH questions. Unclear: does not report asking both questions.
[B-G only] Similar baseline demographics among participants (age/sex/medical/psychological state)?	☐ Yes ☐ No ☐ Unclear	Yes: Psych (trauma Hx, stress status, general affect, sex, age, medication variables accounted for and similar) No: Psychiatric diagnoses or medication use (esp. analgesics/anti-inflammatories/SNRI, etc) amongst participants. Unclear: not reported *Consider design features, e.g. within-subject control or pre-post design
[Psych manip] Neutral psych status?	☐ Yes ☐ No ☐ Unclear	Yes: Psych variables accounted for and normal No: selected for responses on psych assessment
[B-G only] Random allocation [B-site] Random allocation	☐ Yes ☐ No ☐ Unclear	Yes: random sequence generation / roll of die / other truly random procedure named No: counterbalancing of group size (i.e. pseudo-randomisation) [but consider ROB in context] / sequential allocation Unclear: not reported in enough detail to allow decision
Risk of selection bias summary	☐ High (failure to include any of the above probably influenced results FOR THE QUESTION OF THIS REVIEW) ☐ Low (results unlikely to have been influenced) ☐ Unclear (not enough information)	

assessment

Performance bias		
Blinding	Decision	Justification
Were participants blinded to the research question and paradigm and [if relevant] group allocation?	☐ Yes ☐ No ☐ Unclear	Yes: evidence provided - blinding strategy AND blinding check AND results reported AND analysis done accordingly No: Blinding reported broken

		Unclear: not enough information / failure to report)
Risk of performance bias summary	☐ High ☐ Low ☐ Unclear	High: Plausible doubt that participant blinding was applied and maintained throughout Low: Confident that participant blinding was applied and maintained throughout Unclear: not enough information to make informed judgement (e.g. blinding strategy AND blinding check AND results mentioned BUT not fully reported)
Detection bias		
Were outcome assessors blinded to the research question and paradigm?	☐ Yes ☐ No ☐ Unclear	Yes: evidence provided - blinding strategy AND blinding check AND results reported AND analysis done accordingly No: Blinding reported broken Unclear: not enough information / failure to report)
Were analysing researchers blinded to the group allocation of participants and/or to site allocation?	☐ Yes ☐ No ☐ Unclear	Yes: evidence provided - blinding strategy AND blinding check AND results reported AND analysis done accordingly No: Blinding reported broken Unclear: not enough information / failure to report)
Risk of detection bias summary	☐ High ☐ Low ☐ Unclear	High: Plausible doubt that participant blinding was applied and maintained throughout Low: Confident that participant blinding was applied and maintained throughout Unclear: not enough information to make informed judgement (e.g. blinding strategy AND blinding check AND results mentioned BUT not fully reported)
Risk of manipulation veracity problem		
[Psych] Did a manipulation check confirm the effectiveness of the manipulation?	☐ Yes ☐ No ☐ Unclear	Yes: manip check done and results reported and confirmed effectiveness No: no manipulation check done OR manip check done but results not reported. Unclear: manip check done and results confirmed ineffectiveness or were inconclusive
Risk of manipulation veracity problem	☐ High ☐ Low ☐ Unclear	
Attrition bias		
Incomplete outcome data	Decision	Justification
Have attrition/exclusions/ withdrawals been reported and appropriately dealt with in analysis?	☐ Yes ☐ No ☐ Unclear	Yes: no attrition/withdrawals OR stats handled withdrawals appropriately AND relevant adverse events reported
Risk of attrition bias summary	☐ High ☐ Low	

	□ Unclear

Measurement bias		
	Decision	Justification
Were valid and reliable outcome measurements used to assess severity & SA of secondary hyperalgesia?	2H: □ Yes □ No □ Unclear SA: □ Yes □ No □ Unclear	Yes: Self-report: VAS / NRS / validated scale Surface area: independently duplicated measurements or validated approach Consider test-retest reliability if relevant No: single measurement of distance/SA; un-validated self-report scale
Were identical equipment items used for measurements between groups/sites/time points?	2H: □ Yes □ No □ Unclear SA: □ Yes □ No □ Unclear	
Did the same assessor conduct assessments between groups/sites/time points?	2H: □ Yes □ No □ Unclear SA: □ Yes □ No □ Unclear	
Risk of measurement bias summary	2H: □ Yes □ No □ Unclear SA: □ Yes □ No □ Unclear	

Reporting bias		
Selective reporting	Decision	Justification
Were all outcomes for experimental and control groups reported on?	□ Yes □ No	Check each outcome (compare methods vs results)
Were conflicts of interest and funding sources declared?	□ Yes □ No	Consider relevant conflicts
Risk of reporting bias summary	□ High □ Low □ Unclear	

Risk of bias summary		
Risk of bias	Description	Study bias outcome
High risk of bias	Plausible bias that seriously weakens confidence in the results.	
Low risk of bias	Plausible bias unlikely to seriously alter or diminish trust in the results.	
Unclear risk of bias	Insufficient information available to make a judgement.	

Comments:

Appendix 4: Data extraction form

Study identification

First author	
Year of publication	
First word of title	
Sponsorship source	
Country	
Location/setting	
Comments	

Author's contact details

Author's name	
Institution	
Email	
Address	

Methods

Author's name	
Study design	(RCT / case-control / cross-over / pre-post experimental W-S / pre-post experimental B-G)
Primary aim	
Sample size	

Participants

Inclusion criteria	
Exclusion criteria	
Sample size calculation	

Baseline characteristics

	Induction (experimental)	Control	Overall
Age			
Sex (n male; n female)			
Co-morbid diagnoses			
Psychological variables			

Interventions

	Induction (experimental)	Control
Method/modality:		
Timing		
Duration		
Dosage		
Method of administration		
Equipment required		
Ease of application score		

Outcomes

	Secondary hyperalgesia		**Secondary allodynia**		**Prim hyperalg**	**Prim allodynia**
	intensity (cont)	**surf area (cont)**	**allodynia (dyn)**	**allodynia (stat)**		
Test stimulus modality/ies:						
Report scale used						
Time point(s)						
Level reported	**indiv/ grp**	**indiv/ grp**	**indiv/ grp**	**indiv/ grp**	**indiv/ grp**	**indiv/ grp**

Results

Note: for point estimate, specify mean/median/mode; for variance, specify SD/SE/SEM/CI.

Secondary hyperalgesia intensity

	Baseline				Time point 1:			
	Point est	Variance	Sample size	p-value	Point est	Variance	Sample size	p-value
Measure used								
Experimental 1								
Experimental 2								
Control								

	Time point:				Time point:			
	Point est	Variance	Sample size	p-value	Point est	Variance	Sample size	p-value
Measure used								
Experimental 1								
Experimental 2								
Control								

	Time point:				Time point:			
	Point est	Variance	Sample size	p-value	Point est	Variance	Sample size	p-value
Measure used								
Experimental 1								
Experimental 2								
Control								

Secondary hyperalgesia surface area

	Baseline				Time point 1:			
	Point est	Variance	Sample size	p-value	Point est	Variance	Sample size	p-value
Measure used								
Experimental 1								
Experimental 2								
Control								

	Time point:				Time point:			
	Point est	Variance	Sample size	p-value	Point est	Variance	Sample size	p-value
Measure used								
Experimental 1								
Experimental 2								
Control								

Secondary allodynia (dynamic)

	Baseline				Time point 1:			
	Point est	Variance	Sample size	p-value	Point est	Variance	Sample size	p-value
Measure used								
Experimental 1								
Experimental 2								
Control								

	Time point:				Time point:			
	Point est	Variance	Sample size	p-value	Point est	Variance	Sample size	p-value
Measure used								
Experimental 1								
Experimental 2								
Control								

Secondary allodynia (static)

	Baseline				Time point 1:			
	Point est	Variance	Sample size	p-value	Point est	Variance	Sample size	p-value
Measure used								
Experimental 1								
Experimental 2								
Control								

	Time point:				Time point:			
	Point est	Variance	Sample size	p-value	Point est	Variance	Sample size	p-value
Measure used								
Experimental 1								
Experimental 2								
Control								

Primary hyperalgesia

	Baseline				Time point 1:			
	Point est	Variance	Sample size	p-value	Point est	Variance	Sample size	p-value
Measure used								
Experimental 1								
Experimental 2								
Control								

	Time point:				Time point:			
	Point est	Variance	Sample size	p-value	Point est	Variance	Sample size	p-value
Measure used								
Experimental 1								
Experimental 2								
Control								

Primary allodynia

	Baseline				Time point 1:			
	Point est	Variance	Sample size	p-value	Point est	Variance	Sample size	p-value
Measure used								
Experimental 1								
Experimental 2								
Control								

	Time point:				Time point:			
	Point est	Variance	Sample size	p-value	Point est	Variance	Sample size	p-value
Measure used								
Experimental 1								
Experimental 2								
Control								

Adverse events

Nature(s)	
Number of events	
n affected	

Appendix 5: Cohen's Kappa statistic for measuring agreement

Title and abstract screening:

Step 1: Number in agreement over the total

$$Po = \frac{(157+1983)}{2283}$$

$$\frac{(51+153)}{2283}$$

$$\frac{88+153}{2283}$$

Step 2: Probability that both reviewers said 'yes'

$$\left[\frac{(51+153)}{2283}\right] \times \left[\frac{(88+153)}{2283}\right]$$

Step 3: Probability that both reviewers said 'no'

$$\left[\frac{88+1959}{2283}\right] \times \left[\frac{(51+1959)}{2283}\right]$$

Step 4: Determine the overall probability that the reviewers would randomly agree

$$Pe = \left[\left[\frac{(51+153)}{2283}\right] \times \left[\frac{(88+153)}{2283}\right]\right] \times \left[\left[\frac{88+1959}{2283}\right] \times \left[\frac{(51+1959)}{2283}\right]\right]$$

Step 5: Determine Cohen kappa's coefficient

$$K = \frac{Po - Pe}{1 - Pe}$$

K = 0.47

Full test screening:

Step 1: Number in agreement over the total

$$Po = \frac{124 + 56}{228}$$

$$\frac{24 + 124}{228}$$

$$\frac{15 + 124}{228}$$

Step 2: Probability that both reviewers said 'yes'

$$\left[\frac{24 + 124}{228}\right] \times \left[\frac{15 + 124}{228}\right]$$

Step 3: Probability that both reviewers said 'no'

$$\left[\frac{56 + 15}{228}\right] \times \left[\frac{56 + 24}{228}\right]$$

Step 4: Determine the overall probability that the reviewers would randomly agree

$$Pe = \left[\left[\frac{24 + 124}{228}\right] \times \left[\frac{15 + 124}{228}\right]\right] \times \left[\left[\frac{56 + 15}{228}\right] \times \left[\frac{56 + 24}{228}\right]\right]$$

Step 5: Determine Cohen kappa's coefficient

$$K = \frac{Po - Pe}{1 - Pe}$$

$$K = 0.59$$

Appendix 6: Supplementary data for systematic review

6.1 Intensity of secondary hyperalgesia reported in Werner et al. (2002)

Time point after manipulation (minutes)	Median (IQR) intensity ratings for experimental group	Median (IQR) intensity ratings for control group
10	1.5 (2.3)	1.9 (2.5)
40	1.2 (2.4)	1.3 (2.4)
80	1.1 (2.1)	1.0 (2.7)
120	1.2 (1.7)	1.1 (2.1)
160	1.0 (1.5)	0.5 (1.5)
Overall mean determined for in this current study	1.2	1.16

Intensity ratings were reported on 0 – 100 ratings scale, rescaled to 0 – 10

6.2 Intensity of secondary hyperalgesia reported in Ditre et al. (2018)

Ring number	Mean (SD) intensity ratings for experimental group	Mean (SD) intensity ratings for control group
8th (Outermost)	3.96 (7.08)	1.12 (1.81)
7th	4.73 (7.63)	1.42 (2.35)
6th	6.44 (8.98)	2.05 (2.79)
5th	8.81 (10.43)	3.25 (3.54)
4th	11.51 (11.73)	4.79 (4.96)
3rd	15.03 (13.79)	6.55 (5.88)
2nd (innermost)	18.40 (16.14)	9.10 (7.52)
Overall mean	17, 05 (11.0)	4.04 (4.12)

6.3 Differences in reporting of intensity of secondary hyperalgesia

6.3.1 Manipulations hypothesised to decrease the intensity of secondary hyperalgesia

Study	Statistical measure for intensity	Additional notes
Baron et al. (1999)	Mean (SEM)	No control data
Werner et al. (2002)	Median (IQR)	Range not reported
Kóbor et al. (2009)	Mean (SEM) after being normalized to a range of 0-1	Data unavailable
Torta et al. (2019) Experiment 1	Mean (SD)	
Torta et al. (2019) Experiment 2	Mean (SD)	
You et al. (2014)	Mean (SEM)	Intensity of secondary hyperalgesia was measured four days and one month after the manipulation.

6.3.2 Manipulations hypothesised to increase the intensity of secondary hyperalgesia

Study	Statistical measure for intensity	Additional notes
Ditre et al. (2018)	Mean (SD)	
van den Broeke et al. (2014)	Mean only	
Helfert et al. (2018)		Data unavailable

6.4 Area of secondary hyperalgesia reported in Werner et al. (2002)

Time point after manipulation (minutes)	Median (IQR) area for experimental group (cm^2)	Median (IQR) area for control group (cm^2)
10	50 (30 – 70)	50 (35 – 72)
40	49 (30 – 73)	63 (28 – 75)
80	50 (33 – 70)	52 (30 – 73)
120	44 (27 – 69)	54 (30 – 76)
160	40 (21 – 71)	42 (25 – 72)
Overall median determined in this current study	50	54

6.5 Area of secondary hyperalgesia reported in Rasmussen et al. (2015)

Time point after induction (minutes)	Median (95% CI) area for experimental group (cm^2)	Median (95% CI) area for control group (cm^2)
45	41 (33 – 51)	57 (46 – 67)
85	37 (27-45)	58 (42 – 74
125	34 (25 – 40)	54 (39 – 69)
175	33 (24 – 44)	55 (36 – 70)
235	29 (17 – 40)	35 (29 – 60)
Overall median (95% CI)	42.0 (31.1 – 71.4)	34.6 (22.9 – 39.8)

6.6 Differences in reporting of area of secondary hyperalgesia

6.6.1 Manipulations hypothesised to decrease the area of secondary hyperalgesia

Study	Statistical measure for intensity	Additional notes
Baron et al. (1999)	Mean (SEM)	No control data
Werner et al. (2002)	Median (IQR)	Range not reported
Pud et al. (2006)	Mean (SEM)	
Yucel et al. (2001) Experiment 1		Data unavailable
Yucel et al. (2001) Experiment 2		Data unavailable
Yucel et al. (2001) Experiment 3		Data unavailable
Rasmussen et al. (2015)	Median (95% CI)	
Wahl et al. (2019)	Mean (95% CI)	
You et al. (2014)	Mean (SEM)	Area of secondary hyperalgesia was measured four days and one month after the manipulation.
Salomons et al. (2014)	Mean (SEM)	
Matre et al. (2006)	Median (IQR)	Range not reported
Meeker et al. (2019)	Mean (SEM)	
Mohammadian et al. (2004)	Mean (SEM)	
Rebhorn et al. (2012)	Mean only	

6.6.2 Manipulations hypothesised to increase the area of secondary hyperalgesia

Study	Statistical measure for intensity	Additional notes
Ditre et al. (2018)	Mean (SD)	
Helfert et al. (2018)		Data unavailable
Smith et al. (2018)	Mean (SEM)	

6.7 Pain ratings during induction reported in Wahl et al. (2019)

	Mean intensity during induction in the experimental group	Mean intensity during induction in the control group
	3.0	3.3
The overall combined mean pain ratings during induction across both groups was determined in this current study	3.15	

6.8 Pain during induction reported in Werner et al., 2002

Time point during induction (minutes)	Median (IQR) area for experimental group	Median (IQR) area for control group
0	33 (22 – 60)	34 (18 – 56)
1	34 (14 – 48)	34 (14 – 50)
2	35 (20 – 52)	35 (18 – 54)
3	35 (22 – 50)	33 (19 – 56)
4	34 (24 – 54)	32 (14 – 58)
5	33 (26 – 58)	33 (15 – 53)
6	33 (24 – 58)	33 (13 – 52)
7	34 (25 – 64)	32 (14 – 52)
Overall mean determined in this current study	33.9	33.3
Rescaled to 0 – 10	3.39	3.33

6.9 Pain ratings during induction reported in Ditre et al. (2018)

Time point during induction (minutes)	Mean (SD) area for experimental group	Mean (SD) area for control group
0	1.48 (2.40)	1.03 (1.62)
5	2.48 (2.60)	1.55 (1.75)
10	3.49 (3.24)	2.25 (2.18)
15	4.03 (3.28)	2.70 (2.39)
20	4.49 (3.37)	3.25 (2.67)
25	4.66 (3.45)	3.47 (2.77)
30	4.82 (3.53)	3.38 (2.82)
Overall mean determined for in this current study	3.63 (3.12)	2.52 (2.31)

Appendix 7: Ethical approval by the Faculty of Health Sciences Human Research Ethics Committee, University of Cape Town

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: sumayah.arlefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

14 February 2019

HREC REF: 498/2018

Dr T Madden
Department of Anaesthesia
Ward D23.30
NGSH

Dear Dr Madden

PROJECT TITLE: THE EFFECT OF THREAT MANIPULATION ON EXPERIMENTALLY INDUCED SPINAL LONG-TERM POTENTIATION (MSc Candidate - Ms G Bedwell) Sub-study linked to 012/2018

Thank you for your response letter dated 03 February 2019, addressing the issues raised by the Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 28 February 2020.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student: Ms Gillian Bedwell will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

UBurgess

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Appendix 8: Poster advertisement

Would YOU like to help us understand pain better?

We are researching the physiological mechanisms of chronic pain.

A clear understanding will help us to develop improved treatment interventions in managing patients with chronic pain.

- ⇒ **Are you between the ages of 18—65?**
- ⇒ **Do you <u>not</u> have chronic pain?**
- ⇒ **Do you <u>not</u> have any electrical/mental implants (e.g pace-maker)?**
- ⇒ **Do you <u>not</u> have sensory impairments?**

If you answered **YES** to all the above questions you may be eligible to participate in our study.

<u>What is the procedure?</u>

Assessment of sensation and pain ratings to light touch, slight pinpricks, and single electrical stimulations will be conducted before and after receiving 5 brief high-frequency electrical stimulations. **It may be moderately painful.** It will require some effort to tolerate, but it is short-lived and predictable, so most people find it tolerable.

You will also need to answer a few questionnaires on stress, self-efficacy and optimism, and previous trauma.

<u>How long will it take?</u>

Between 60—90 minutes

<u>Anything else that I need to know?</u>

The experiment will take place in the Department of Anaesthesia and Perioperative Medicine, D23, Groote Schuur Hospital. You will be compensated R100 for your time and inconvenience.

If you are interested please contact us at
research.sense.studies@gmail.com

Appendix 9: Eligibility quiz on the Responster platform

Volunteers were asked to answer the following yes/no questions on the Responster platform:

1. Are you between the ages of 18 – 65 years?
2. Do you have chronic pain (pain on most days for the past three months)?
3. Do you have any sensory impairments (decreased sensation in your skin)?
4. Do you have a mental illness (clinical depression and anxiety counts as a mental illness for this study)?
5. Are you pregnant?
6. Do you have any electrical or metal implants (e.g. a pacemaker)?
7. Do you have any tattoos on your forearms?
8. Do you have a health/cardiovascular condition?
9. Do you have neurological problems (e.g. epilepsy)?
10. Do you have peripheral vascular disease?
11. Do you have problems with skin healing?
12. Do you use medication that could alter you skin sensitivity or healing (e.g. analgesic medication, topical medical creams or immune modulators)?

The Responster platform was programmed to automatically send the results from the questions to the research team's email address. To be eligible to participate, volunteers had to answer 'no' to all questions, except question 1.

Appendix 10: Randomisation of participants' ID code

Allocation Group 1: received the HFS under a condition of threat on their *right* arm (the left arm was the control site).

Allocation Group 2 received the HFS under a condition of threat on their *left* arm (the right arm was the control site).

Envelope letter	Allocation group
A	2
B	2
C	1
D	2
E	2
F	1
G	2
H	1
I	2
J	1
K	1
L	1
M	2
N	2
O	1
P	1
Q	1
R	1
S	2
T	1
U	2
V	2
W	2
X	1
Y	1
Z	2

Appendix 11: Basic demographic and participant information

Participant ID:		Notes:
Age:		
Sex:		
Ethnicity:		
Group:		
Caffeine intake:		
Medication use:		
Detection threshold R:		
Detection threshold L:		
Dominant hand:		
Arm receiving HFS[12]:		
Intensity used for procedure:		
Time & day of participation:		

[12] This was a standardised form used in all HFS studies being conducted by the research team. In some of those studies only one arm received the HFS only. For this current study, participants received the HFS on both arms. Therefore, Researcher 2 wrote "both" for all participants.

Appendix 12: Childhood Trauma Questionnaire

Instructions: These questions ask about some of your experiences growing up **as a child and a teenager**. For each question, circle the number that best describes how you feel. Although some of these questions are of a personal nature, please try to answer as honestly as you can. Your answers will be kept confidential.

When I was growing up, …	Never True	Rarely True	Sometimes True	Often True	Very Often True
1. I didn't have enough to eat.	1	2	3	4	5
2. I knew there was someone to take care of me and protect me	1	2	3	4	5
3. People in my family called me things like "stupid", "lazy", or "ugly".	1	2	3	4	5
4. My parents were too drunk or high to take care of me.	1	2	3	4	5
5. There was someone in my family who helped me feel important or special.	1	2	3	4	5
6. I had to wear dirty clothes.	1	2	3	4	5
7. I felt loved.	1	2	3	4	5
8. I thought that my parents wished I had never been born.	1	2	3	4	5
9. I got hit so hard by someone in my family that I had to see a doctor or go to the hospital.	1	2	3	4	5
10. There was nothing I wanted to change about my family.	1	2	3	4	5
11. People in my family hit me so hard that it left bruises or marks.	1	2	3	4	5
12. I was punished with a belt, a board, a cord, or some hard object.	1	2	3	4	5
13. People in my family looked out for each other.	1	2	3	4	5
14. People in my family said hurtful or insulting things to me.	1	2	3	4	5
15. I believe that I was physically abused.	1	2	3	4	5
16. I had the perfect childhood.	1	2	3	4	5
17. I got hit or beaten so badly that it was noticed by someone like a teacher, neighbour, or doctor.	1	2	3	4	5
18. I felt that someone in my family hated me.	1	2	3	4	5
19. People in my family felt close to each other.	1	2	3	4	5
20. Someone tried to touch me in a sexual way, or tried to make me touch them.	1	2	3	4	5
21. Someone threatened to hurt me or tell lies about me unless I did something sexual with them.	1	2	3	4	5

22. I had the best family in the world.	1	2	3	4	5
23. Someone tried to make me do sexual things or make me watch sexual things.	1	2	3	4	5
24. Someone molested me.	1	2	3	4	5
25. I believe that I was emotionally abused.	1	2	3	4	5
26. There was someone to take me to the doctor if I needed it.	1	2	3	4	5
27. I believe that I was sexually abused	1	2	3	4	5
28. My family was a source of strength and support.	1	2	3	4	5

Appendix 13: Modified version of the World Mental Health Survey Initiative version of the World Health Organisation Composite International Diagnostic Interview for post-traumatic stress disorder

Instructions: The following questions ask about some of your experiences. For each question, indicate "yes" or "no". Although some of these questions are of a personal nature, please try to answer as honestly as you can.

Questions	Yes	No
1. Did you ever participate in combat, either as a member of the military, or as a member of an organised non-military group?		
2. Did you ever serve as a peacekeeper, or relief worker in a war zone or in a place where there was ongoing terror of people because of political, ethnic, religious or other conflicts?		
3. Were you ever an unarmed civilian in a place where there was a war, revolution, military coup or invasion?		
4. Did you ever live as a civilian in a place where there was ongoing terror of civilians for political, ethnic, religious or other reasons?		
5. Were you ever a refugee – that is, did you ever flee from your home to a foreign country or place to escape danger or persecution?		
6. Were you ever kidnapped of help captive?		
7. Were you ever exposed to a toxic chemical or substance that could cause you serious harm?		
8. Were you ever involved in a life-threatening automobile accident?		
9. Did you ever have any other life-threatening accidents, including on your job?		
10. Were you ever involved in a major natural disaster, like a devastating flood, hurricane, or earthquake?		
11. Were you ever in a man-made disaster, like a fire started by a cigarette, or a bomb explosion?		
12. Did you ever have a life-threatening illness?		
13. As a child, were you ever badly beaten up by your parents or the people who raised you?		
14. Were you ever badly beaten up by a spouse or romantic partner?		
15. Were you ever badly beaten up by anyone else?		

The next question is about rape. We define this as an event during which one person has sexual intercourse with or penetrates the body of another person without their consent, or when they were too young to know what was happening. 16. Did you ever experience an event like the one just described?		
The next question is about sexual assault. We define this as an event during which one person touches another person inappropriately, or without that person's consent. 17. Other than rape, did you ever experience an event like the one just described?		
18. Has someone ever stalked you – that is, followed you or kept track of your activities in a way that made you feel you were in serious danger?		
19. Did someone very close to you ever die unexpectedly; for example, they were killed in an accident, murdered, committed suicide, or had a fatal heart attack at a young age?		
20. Did you ever had a son or daughter who had a life-threatening illness or injury?		
21. Did anyone very close to you ever have an extremely traumatic experience, like being kidnapped, tortured or raped?		
22. When you were a child, did you ever witness serious physical fights at home, like when your father beat up your mother?		
23. Did you ever see someone being badly injured or killed, or unexpectedly see a dead body?		
24. Did you ever <u>do</u> anything that <u>accidently</u> led to the serious injury or death of another person?		
25. Did you ever <u>on purpose</u> either seriously injury, torture, or kill another person?		
26. Did you ever see atrocities or carnage such as mutilated bodies or mass killings?		
27. Did you ever experience any <u>other</u> extremely traumatic or life-threatening event that hasn't been asked about yet?		

Appendix 14: Threat manipulation check

Instructions: please indicate to what extent you agree or disagree with the following statements:

	Strongly disagree	**Disagree**	**Neutral**	**Agree**	**Strongly agree**
1. At the time of receiving the intense electrical stimulation, I was concerned that it would cause damage to my skin on my **RIGHT** arm.					
2. At the time of receiving the intense electrical stimulation, I was concerned that it would cause damage to my skin on my **LEFT** arm.					
3. At the time of receiving the intense electrical stimulation on my **RIGHT** arm, I felt anxious.					
4. At the time of receiving the intense electrical stimulation on my **LEFT** arm, I felt anxious.					

Appendix 15: 10-item Connor-Davidson Resilience Scale

Instructions: Please indicate how much you agree with the following statements as they apply to you over the last MONTH.

If a particular situation has not occurred recently, answer according to how you think you would have felt.

	Not true at all	Rarely true	Sometimes true	Often true	True nearly all the time
1. I am able to adapt when changes occur.					
2. I can deal with whatever comes my way.					
3. I try to see the humorous side of things when I am faced with problems.					
4. Having to cope with stress can make me stronger.					
5. I tend to bounce back after illness, injury, or other hardships.					
6. I believe I can achieve my goals, even if there are obstacles.					
7. Under pressure, I stay focused and think clearly.					
8. I am not easily discouraged by failure.					
9. I think of myself as a strong person when dealing with life's challenges and difficulties.					
10. I am able to handle unpleasant or painful feelings like sadness, fear, and anger.					

.

Appendix 16: Perceived Stress Scale

Instructions: The questions in this scale ask you about your feelings and thoughts **during the last month**. In each case, you will be asked to indicate *how often* you felt or thought a certain way.

	Never	Almost never	Sometimes	Fairly often	Very often
1. In the last month, how often have you been upset because of something that happened unexpectedly?	0	1	2	3	4
2. In the last month, how often have you felt that you were unable					
3. In the last month, how often have you been upset to control the important things in your life?	0	1	2	3	4
4. In the last month, how often have you felt nervous and “stressed”?	0	1	2	3	4
5. In the last month, how often have you felt confident about your ability					
6. In the last month, how often have you been able to handle your personal problems?	0	1	2	3	4
7. In the last month, how often have you felt that things					
8. In the last month, how often have you felt that things were going your way?	0	1	2	3	4
9. In the last month, how often have you found that you could not cope with all the things that you had to do?	0	1	2	3	4
10. In the last month, how often have you been able to control irritations in your life?	0	1	2	3	4
11. In the last month, how often have you felt that you were on top of things?	0	1	2	3	4
12. In the last month, how often have you been angered because of things that were outside of your control	0	1	2	3	4
13. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	0	1	2	3	4

Appendix 17: Pain Catastrophising Scale

Instructions: Please think back to how you thought and felt during the intense electrical stimulation. We are interested in the types of thoughts and feelings that you had during that experience. Listed next are thirteen statements describing different thoughts and feelings that may be associated with pain. Please indicate the degree to which you had these thoughts and feelings when you were experiencing the intense electrical stimulation

	Not at all	To a slight degree	To a moderate degree	To a great degree	All the time
1. I worried all the time about whether the pain would end					
2. I felt I couldn't go on					
3. It was terrible and I thought it was never going to get any better					
4. It was awful and I felt that it overwhelmed me					
5. I felt I couldn't stand it anymore					
6. I became afraid that the pain would get worse					
7. I kept thinking of other painful events					
8. I anxiously wanted the pain to go away					
9. I couldn't seem to keep it out of my mind					
10. I kept thinking about how much it hurt					
11. I kept thinking about how badly I wanted the pain to stop					
12. There was nothing I can do to reduce the intensity of the pain					
13. I wondered whether something serious may happen					

Appendix 18: Multidimensional Scale of Perceived Social Support

Instructions: We are interested in how you feel about the following statements. Read each statement carefully. Indicate how you feel about each statement.

	Very strongly disagree	Strongly disagree	Mildly disagree	neutral	Mildly agree	Strongly agree	Very strongly agree
1. There is a special person who is around when I am in need.	1	2	3	4	5	6	7
2. There is a special person with whom I can share my joys and sorrows.	1	2	3	4	5	6	7
3. My family really tries to help me.	1	2	3	4	5	6	7
4. I get the emotional health and support I need from my family.	1	2	3	4	5	6	7
5. I have a special person who is a real source of comfort to me.	1	2	3	4	5	6	7
6. My friends really try to help me	1	2	3	4	5	6	7
7. I can count on my friends when things go wrong.	1	2	3	4	5	6	7
8. I can talk about my problems with my family.	1	2	3	4	5	6	7
9. I have friends with whom I can share my joys and sorrows.	1	2	3	4	5	6	7
10. There is a special person in my life who cares about my feelings.	1	2	3	4	5	6	7
11. My family is willing to help me make decisions.	1	2	3	4	5	6	7
12. I can talk about my problems with my friends	1	2	3	4	5	6	7

Appendix 19: Pain Vigilance and Awareness Questionnaire

Instructions: This questionnaire is not directly related to your experience of the intense electrical stimulation. It is to do with you, as a person, and your experiences in life. Pain is a common experience. Please think about how you usually behave when you have pain as you answer the following questions.

Please rate the following statements between 0 – 5, where 0 = Never and 5 = Always

1. I am very sensitive to pain	0	1	2	3	4	5
2. I am aware of sudden or temporary changes in pain.	0	1	2	3	4	5
3. I am quick to notice changes in pain intensity	0	1	2	3	4	5
4. I am quick to notice effects of medication on pain	0	1	2	3	4	5
5. I am quick to notice changes in location or extent of pain	0	1	2	3	4	5
6. I focus on sensations of pain	0	1	2	3	4	5
7. I notice pain even if I am busy with another activity	0	1	2	3	4	5
8. I find it easy to ignore pain.	0	1	2	3	4	5
9. I know immediately when pain starts or increases	0	1	2	3	4	5
10. When I do something that increases pain, the first thing I do is check to see how much pain was increased	0	1	2	3	4	5
11. I know immediately when pain decreases	0	1	2	3	4	5
12. I seem to be more conscious of pain than others.	0	1	2	3	4	5
13. I pay close attention to pain	0	1	2	3	4	5
14. I keep track of my pain level	0	1	2	3	4	5
15. I become preoccupied with pain	0	1	2	3	4	5
16. I do not dwell on pain	0	1	2	3	4	5

Appendix 20: Script for procedure

Welcome to this experiment and thank you for being willing to participate. This will take about 2 hours. First, I will introduce you to all the procedures. Next, we will do some baseline sensory testing on both of your forearms. Then you receive the intense electrical stimulation **on your right arm and then on your left arm**.

There will be a 20 minute break in testing, and you will complete some questionnaires during that time. Then we will continue the sensory testing for the 40 minutes after that. So, it's sensory testing, intense electrical stimulation, questionnaires, and sensory testing.

---

1. **Demographic information**

(Ask for info for participation record sheet)

Before we start the procedure, please can you turn off your cell phone just so that no alerts or alarms will go off during the procedure. Please also take off your watch/any jewellery.

2. **Introduction to SPARS**

We use this scale in the experiment so it's important that you understand it. Please take your time to read this.

[Show SPARS chart and ask them to read it (alone). Read it to the participant. Answer any questions.]

This scale runs from -50, which is no sensation – you don't feel anything – through 0, the exact point at which what you feel transitions to pain, to +50, which is the most intense pain you can imagine. If the stimulation trial was non-painful, you'll rate it between -50 and 0 - the range that is marked in yellow as 'non-painful'. A rating closer to -50 means the trial was less intense, and closer to 0 means it was more intense and closer to being painful – but not yet painful, because it's still below zero and in the non-painful range of the scale. If the trial is painful then you'll rate it between 0 and +50 - the range that is marked in green as 'painful'. A higher positive number means more painful. 0 is the exact point of transition between not painful and painful.

When you receive a stimulation, first decide if it was painful or non-painful. Then you can work out where in the appropriate range it fell. Sometimes people also use decimals – e.g. minus 22.5, and that's allowed.

3. **Marking radial lines and electrode placement**

Now I'm going to mark up your arms for the testing procedure. Is that ok?

[mark up using foam template, with A towards the cubital fossa; centre of radial lines approx 8cm from cubital fossa but NOT on prominent vein or scarred area.]

These are the electrodes we use. I will place them on your skin like this, and they will stay there for the whole procedure. We put one on each arm. [Strap electrode goes around upper arm. Disc electrode approx 8cm from cubital fossa.]

[Turn on monitor for participant. Click mouse to bring up 'Stop here until baseline testing completed…']

4. **Introduction to the test battery**

Each time we test your skin, we will use 6 different tests. Each test uses a different kind of stimulus: we can

- Touch you lightly with this filament (demo VFF)
- Brush your skin lightly with a cotton wisp (demo)
- Brush your skin clearly with a brush (demo)
- Press a tiny, blunt-ended metal rod against your skin (demo both)
- Or give you a single electrical stimulus - which I won't do now because we haven't attached the electrodes yet.

You can feel that the sensations evoked by the different tests can be quite distinct in nature. We will ask you to report what you feel on the scale. Do not try to rate the different tests relative to one another. Each time we test you with a new modality, don't try to compare it to the previous modality. Just consider each test in isolation, and start afresh with the scale. We are interested in your experience of each stimulation modality separately. You may also use decimals. Remember that -50 means no sensation at all; 0 is the exact point at which what you feel transitions to pain; +50 is the most intense pain you can imagine. Please stay with these reference points during the whole experiment! I will ask you to close your eyes when we test your skin, but in between the test runs you can open your eyes and look at the scale so that you have a visual image of it – most people find that a helpful approach.

Now I'll run you through the test battery so you get a chance to practice giving ratings for each stimulus modality. When we start the experiment, you'll need to give your rating within about 5 seconds, but for now we have more time. I'll test this arm first, then that one. Please put both your arms on the table, turning them upwards for me.

[perform full test battery on each arm]

---

5. **Detection threshold testing**

This next test is not about pain, it is about feeling an electrical stimulus. We start at an intensity of zero. I will gradually increase the intensity until you tell me that you can feel it. Please say "yes" if you feel it, even a little bit. It will feel like a very tiny pinprick.

[find threshold for each arm; choose most sensible approximation, write it down, set DS7A to that level and then flick switch to x10].

HAVE YOU TESTED BOTH ARMS?

Now we can begin. First, we do 3 rounds of the test battery, which usually takes about 6 minutes. And then we give you the intense electrical stimulation on each arm, we will give you a 20-minute break during which you can complete the questionnaires, and then we spend the rest of the next hour repeating the test battery.

6. **Baseline testing**

7. **Skin examination**

Hmm... it seems like the sensitivity of your skin is different on each arm. This is not unusual. However, it is important for me to thoroughly examine your skin to determine your risk of injury during the intense electrical stimulation for each arm. This device magnifies your skin and allows me to examine different features of your skin very clearly. [show otoscope and use it to carefully examine skin around electrodes on both arms.]

**if participant asks more about how you are assessing the skin answer with the following:

Can I tell you after the procedure? I just need to concentrate quite hard to make a good assessment and then hold all the grades in my head to put into the computer [ask as though they are flustering you a bit].

8. Skin examination results

I need to enter your skin examination findings, along with your sensory rating scores into the main computer next door. It uses the results to estimate how strong or fragile your skin is. If your skin is fragile there is a risk of injury: the intense electrical stimulation could burn your skin and the deeper layers underneath your skin. Based on the information I enter, the computer will class each arm into one of three groups: "fully approved" – there is low risk, so stimulating that skin is very safe; "approved with reservations" – there is moderate risk that the stimulation will damage the skin, or "rejected" – there is too much risk of skin damage. The computer will show you the results of your skin sensitivity on the screen in front of you. If it rejects your skin it won't give the stimulation for that arm; if you get either 'fully approved' or 'approved with reservations' then it will continue and give you the stimulation – but it will always show you the risk rating during the stimulation. The way it calculates your risk score is quite complicated, so I can't tell which group it will put you in. Actually, I'm not allowed to know what the computer tells you – because apparently it can influence how I test you if I do know [act a little dismissive of this]. So don't tell me what the computer says about the risk ratings for your arms – if it lets us continue then we continue. [leave room for 2min. Press SPACE on PC outside room to continue – i.e. to start 'calculation' time].

9. Explanation of HFS

The computer is busy calculating your risk score for each arm. While it does that, I will explain the next part of the procedure. The intense electrical stimulation is the part of the procedure that most people find moderately painful. The stimulation takes one minute in total, but it is split up into 5 trains. Each train lasts one second, and then you get a 9-second break. So you'll have one second of stimulation, then 9 seconds' break, one second of stimulation, 9 seconds' break - and so on. After this, we take a 20-minute break for you to complete some questionnaires.

The screen will count you through the trains. I want you to concentrate on keeping your arm glued to the table when the first train starts because some people pull their arm back as a reflex and then you could pull out cables, which can be dangerous. As a safety precaution, I will keep my finger on the safety switch so that if you decide you want to pull out of the study you can say 'STOP' and I will immediately flick the switch down to deactivate the stimulator. I'll be ready in case you need me to stop it. If you pull out, we won't be able to continue with the study. As I say, there are only 5 trains, and each one lasts one second before you get 9 seconds' break - so just count yourself through. Please give me a rating on the scale for each train.

Now, put the headphones on, listen carefully and let me know when it says the stimulation will start. The first site is the RIGHT arm. Your risk rating for the right arm will be shown on the computer screen in front of you and, assuming the arm's risk score hasn't caused a safety rejection, you will receive the intense electrical stimulation on your right arm. After that, you get a 30-second break and the process is repeated for the left arm. Make sense? [tell participant to click mouse. and HFS will start in about 12sec. Keep hand on safety switch.]

---

10. HFS

Start stopwatch at first train. Note clock time of HFS onto participation record. Record SPARS rating for each train.

***BE SURE TO SWITCH ELECTRODE SITE TO LEFT ARM AFTER 5TH TRAIN!**

[there is a 30-second break between arms, after which the screen will cue you to opt to continue with a mouse click]

11. Questionnaires

Now, can you please answer some questionnaires on the screen in front of you. I am going to go outside the room and turn off the screen on my side because I don't want to see what answers you are giving. All your responses will be labelled with your participant ID code so that they are not linked to your name and remain confidential. When you're finished with the questionnaires, this screen will show me a message saying that you're done. [start qu, take ppt next door.]

12. Follow-up testing

Do not try to rate the different tests relative to one another. Each time we test you with a new modality, don't try to compare it to the previous modality. Just consider each test in isolation, and start afresh with the scale. We are interested in your experience of each stimulation modality separately. You may also use decimals. Remember that -50 means no sensation at all; 0 is the exact point at which what you feel transitions to pain; +50 is the most intense pain you can imagine. Please stay with these reference points during the whole experiment! I will ask you to close your eyes when we test your skin, but in between the test runs you can open your eyes and look at the scale so that you have a visual image of it – most people find that a helpful approach. [Always start with right arm].

13. **Surface area testing**

Now, I will also use the pinprick (show) to test for an area of higher sensitivity.

I'm going to apply the pinprick in two spots and I want you to tell me if there is a very obvious difference in the sensation.

[apply to right arm first]: Does it feel different if I touch you here [distal]… and here [adjacent to electrode]?
[if no, repeat from proximal to electrode]
[repeat on left arm.]
Ok, now I want to map out the area of higher sensitivity. So I will apply the pinprick repeatedly along each of these radial lines, moving towards the electrode. If you feel a distinct change in sensation please say "now". Please close your eyes. [test. Repeat this instruction at each mapping time point.]
[Always start with right arm].

14. **Post-procedural questionnaires**

This is your last task. Please can you read these questions very carefully and answer them as honestly as possible.

Ask why they did/didn't feel anxious when receiving the intense electrical stimulation on the right/left arm (ask for each arm). Ask why the were/weren't concerned that the intense electrical stimulation would injury the skin on their right/left arm (ask for each arm). [write answers on post-procedural questionnaire sheet].

15. **Debriefing**

The skin examination was a sham examination procedure – it was not a true reflection of the robustness of your skin. It was part of the procedure for us to tell you that the skin on your one arm is more fragile than the other. We did not actually test the fragility of your arm skin; there is no indication that your arm skin is fragile, or we would actually not have let you undergo the intense electrical stimulation.

Appendix 21: Procedure checklist

Check equipment:

- DS7A input connected at the back, turned on, settings: voltage max, X1, current 0.01, pulse width 2000μs.
- Output from DS7A to remote electrode selector (D188); output from selector to electrodes.
- Set out sensory testing equipment, remove covers.
- Open Affect5 file
- Research assistant to designate the site allocation in the Affect5 programme
- Prepare marker pens, mark-up template, electrode stickers, alcohol balls and gauze swabs.
- Prepare forms:
    - Study information sheet
    - Informed consent
    - Exclusion criteria
    - Demographic information

Appendix 22: Study information sheet

UNIVERSITY OF CAPE TOWN

DIVISION OF PHYSIOTHERAPY

FACULTY OF HEALTH SCIENCES

Study information sheet

In this study we are interested in how people perceive different stimuli.

The entire procedure will be approximately 60 – 90 minutes. First, I will place two electrodes on your forearms, one on each arm. I will repeatedly assess your sensation and pain ratings to light touch, blunt-ended pinpricks and single electrical stimulations. You will listen to a short audio recording about the high-frequency stimulation. Then you will receive 5 high-frequency electrical stimulations, each only lasting 1 second with a 9 second break in between. This part of the procedure will only last 1 minute. **It may be moderately painful.** It may require some effort to tolerate, but it is short-lived and predictable, so most people find it tolerable. This is a well-established procedure and we do not anticipate any tissue damage to occur. However, you may develop a small area of sensitivity which could last for a few days. Full recovery is anticipated. Then you will fill out a few questionnaires looking at the following:

1. Basic demographic data (e.g age, sex, ethnicity, medication use and caffeine intake).
2. Pain awareness and thoughts about pain.
3. Stress, resilience, social support and previous trauma, including physical, emotional and sexual trauma.
4. Your experience of the procedure.

Importantly, you will complete these questionnaires on a computer and your responses will be linked to a participation code, not to your name, so that your responses are confidential. The data from these questionnaires will be given a new identification code when we analyse it, so that the data can in no way be linked back to you.

After the questionnaires, I will repeatedly reassess your sensation and pain ratings to light touch, slight pinpricks, and single electrical stimulations.

You may withdraw from the study at any stage during the procedure. If you choose to withdraw, it is your choice whether I keep the data collected up to that point or if you would like me to destroy the information.

All participants will be provided with an identification code so that the public will not know who took part in this study, nor will participants be able to distinguish their own data from those of other participants. At the time of publication, the de-identified data will be made publicly available in accordance with university policy and the principles of Open Science.

In order to partake in this study, you will need to sign the letter of informed consent as well as not have any of the exclusion criteria.

Appendix 23: Exclusion criteria

UNIVERSITY OF CAPE TOWN

DIVISION OF PHYSIOTHERAPY

FACULTY OF HEALTH SCIENCES

Exclusion criteria

Below is the list of exclusion criteria. If you agree with any of the below statements, you may **not** participate in this study.

1. I have chronic pain (pain on most days for the past three months)
2. I have pain today, the day of testing
3. I have used analgesics (painkillers) in the last 24 hours
4. I have sensory impairments
5. I have a mental illness (depression and anxiety count as a mental illness for this study)
6. I am pregnant
7. I have electrical or metal implants
8. I have a tattoo on my arm
9. I have a heart/cardiovascular condition
10. I have diabetes mellitus
11. I have neurological problems (e.g. epilepsy)
12. I have peripheral vascular disease
13. I have problems with skin healing
14. I use medication that could alter my skin sensitivity or healing (e.g. analgesic medication, topical medical creams or immune modulators)

I, _____, have read the exclusion criteria and declare that none of them applies to me.

Sign _____ Date _____

Appendix 24: Informed consent form

UNIVERSITY OF CAPE TOWN

DIVISION OF PHYSIOTHERAPY

FACULTY OF HEALTH SCIENCES

Informed Consent Form

Who am I?

I am a Master's student at the University of Cape Town and I am conducting an experimental study. The procedure will be run by either myself, or Caron Louw who is my research assistant.

Are there any risks?

This procedure involves some pain. However, it is a well-established procedure and is known not to cause any skin damage. You could be at risk of harm if you do not disclose any information listed in the exclusion criteria.

Are there any benefits?

There are no personal benefits to participating in this study. However, the findings of study could benefit society by helping us to understand how chronic pain works and thus to improve treatment for people who have chronic pain.

What else should I know?

You will be required to listen to a short audio recording about the high-frequency stimulation procedure and could also undergo a skin examination prior to the procedure taking place. You will be asked to answer a few questionnaires about your basic demographics, pain awareness and thoughts about pain, stress, resilience, social support, and previous trauma (including physical, emotional and sexual trauma) and lastly about your experience of the procedure. All information will be kept confidential. We will give you a participant code so that your data are not linked to your name when we analyse the data. Only the study research team will be able to link the data to you. Furthermore, all data will be kept on a password-protected computer. When the study results have

been published, all the study data will be made available to the public online, but no one will be able to link the data to you. The de-identified data are made publicly available to adhere with the University's policies and the principles of Open Science.

What happens if I get hurt taking part in this study?

This research study is covered by an insurance policy taken out by the University of Cape Town if you suffer a bodily injury because you are taking part in the study.

The insurer will pay for all reasonable medical costs required to treat your bodily injury, according to the SA Good Clinical Practice Guidelines 2006. The insurer will pay without your having to prove that the research was responsible for your bodily injury. You may ask the study doctor for a copy of these guidelines.

The insurer will *not* pay for harm if, during the study, you:

- Use medicines or other substances that are not allowed
- Do not follow the study investigator's instructions
- Do not tell the study investigator that you have a bad side effect from the procedure
- Do not take reasonable care of yourself and your study medicine
- Do not disclose any of the items listed in the exclusion criteria

If you are harmed and the insurer pays for the necessary medical costs, usually you will be asked to accept that insurance payment as full settlement of the claim for medical costs. However, accepting this offer of insurance cover does not mean you give up your right to make a separate claim for other losses based on negligence, in a South African court.

It is important to follow the study investigator's instructions and to report straight away if you have a side effect from the study procedure.

What if I have any questions?
If you have any questions or concerns about the study, you may contact Gillian Bedwell at bdwgil001@myuct.ac.za or 083 366 1784. Alternatively, you can contact Dr Tory Madden, the study supervisor, at tory.madden@uct.ac.za.

The UCT's Faculty of Health Sciences Human Research Ethics Committee can be contacted on 021 406 6338 in case you have any ethical concerns or questions about your rights or welfare as a participant on this research study.

I, _____, have read and understand the study information sheet.

I, _____, have read and understand the consent form and am willing to take part in this study.

Sign _____ Date _____

I, _____, have explained the study information sheet to the participant.

I, _____, have explained the consent form to the participant.

Sign _____ Date _____

Appendix 25: Information pamphlet

MENTAL HEALTH INFORMATION CENTRE
Southern Africa

For more information on mental health visit the Mental Health Information Centre website. There are many information sheets in both English and isiXhosa

FAMSA
Families South Africa
Western Cape

"FAMSA Western Cape is a non-profit organisation (NPO) specialising in relationship counselling "

- Trauma debriefing and support
- Domestic violence counselling
- Relationship counselling

Contact

All appointments can be made online by visiting their website:
http://www.famsawc.org.za/contact

Easy ways to access help!

This pamphlet contains information of some of the non-profit organisations available that can assist with a variety of mental health conditions.

THE SOUTH AFRICAN DEPRESSION AND ANXIETY GROUP

"SADAG is South Africa's largest health support and advocacy group"

Contact

For counselling queries e-mail:
zane@sadag.org

To contact a counsellor between 8am-8pm Monday to Sunday,
Call: **011 234 4837** / Fax number: **011 234 8182**

For a suicidal Emergency contact us on **0800 567 567**

24hr Helpline **0800 12 13 14**

Website below:

http://www.sadag.org/

LifeLine

"Anyone experiencing a personal crisis can contact LifeLine. Our trained counsellors are available to listen, provide support and referals. Confidentiality is one of the cornerstones of our counselling service. We respect your right to privacy and no information you give to us will be divulged without your permission to do so. Please note that all calls to LifeLine are charged at the cost of a local call, if you live within the Cape Town calling area (021). "

Contact

Landline: 021 461 1111 (09h30—22h00)

Whatsapp Call: 063 709 2620 (10h00—14h00)

365 days of the year

Face-to-face counselling

Book an appointment: 021 461 1113

(Monday—Friday, during office hours)

*Appointment is free of charge

Website below:

http://www.lifelinewc.org.za/

UCT Student Wellness Services

"We are a student counselling service, offering predominantly short-term counselling and psychotherapy, with the aim of ensuring that whatever personal, emotional or psychological problems you experience, the impact of these on your academic studies are kept to a minimum and your capacity for achievement is optimised."

Problems that we can assist with
Some of the problems for which the Counselling service can provide assistance with include:

- adjusting to UCT
- homesickness
- loneliness
- cultural transition
- social skills
- self-esteem issues
- confidence building
- individuation from parents
- stress and anxiety
- depression and suicidal feelings
- loss and bereavement
- trauma and crisis intervention
- rape and sexual assault
- HIV/AIDS counselling
- sexual identity issues
- addictions
- academic problems including motivation and concentration
- relationship issues
- family problems
- any other personal, emotional, social or psychological problem

Telephone: 021 650 1017 Email: lerushda.cheddie@uct.ac.za

Appendix 26: Community healthcare centres providing psychological counselling

Vanguard CHC 021 695 0232 Sr Mhlonyane and Mr Holo 071 7167419	**Greenpoint CHC** Mr Landolt-Tessa 021 4210289 072 4269750	**Rocklands CHC** 021 392 5121 Sr Jacobs 0781159195
Hanover Park Sr Cader and Sr Berg 021 692 4250 072 4310922 072 9397942	**Guguletu** Sr Banzi 021 637 1281	**Lentegeur CHC** Sr Adonis and Sr Martin 021 371 2126 0720385235
Dr Abduraghman Sr Lottering 021 637 9071 076 2321489	**Dunoon CHC** Mr Lewellyn Jakoet 021 2004551 073 5451057	**Strand CHC/Gustrow** Sr Stephens 021 397 8906/ 8195 0842732180
Lotus River Sr Shah 021 703 3131 / 2 084 4856601	**Heideveld** Mr Briekwa 021 6378036 083 7650532 Sr Williams	**Tafelsig** 021 444 2025 Sr Jacobs 0781159195
Lady Michaelis Sr Sias 021 797-8171 082 4210212	**Woodstock/Maitland** Sr George/Sr Thembi 0214609269/0215106473 08303171177	**Macassar Clinic** 021 857 2330/3502 Sr Hendriks 0837790086
Retreat **Sr Hendricks-** 021-712-5105 071 3559016	**Mitchells Plain Day Hospital** Mr Williams 021 392 5161 0607777398	

Appendix 27: Private practice psychologists within the University of Cape Town's neighbouring communities

Both psychologists were contacted via email to request permission to include their information for potential psychological referral. This was done before the protocol was submitted for ethical approval.

Name: Cheryl Baker

Phone number: 021 6711576 and 0826464250

Email address: cherylbaker@iafrica.com

Private Practice: Claremont

Name: Mireille Landman

Phone number: 021 6854250 (w) and 082 3391605

Email address: mlandman@cybersmart.co.za

Private Practice: Rondebosch

Appendix 28: Data analysis using pilot study data

Pilot data analysis

The effect of stimulus threat on experimentally induced secondary hyperalgesia

Tory Madden and Gillian Bedwell

07 Jan 2020

Table of Contents

Background

The purpose of this analysis script is to specify how the formal analysis of the full, final data set will be done. The current script uses the pilot data. Note that this script was planned and run on a very small sample, so some of the model assumptions may have been violated. We have not tested for this in the current version of the script, but we will test for violations and make the necessary adjustments to our statistical analysis with the final data set.

Analysis plan

Manipulation checks

- Describe and compare HFS ratings between arms.
- Describe and compare participant anxiety self-ratings between arms.
- Describe and compare participant threat self-ratings between arms.

Primary aim

To determine the intensity of secondary hyperalgesia (SH) on each arm, and compare between arms.

Secondary aim

To determine the surface area (SA) of secondary hyperalgesia (SH) on each arm, and compare between arms.

Exploratory aims

Sensory measurements

- To determine the intensity of static light touch on each arm, and compare between arms.
- To determine the intensity of dynamic light touch on each arm, and compare between arms.
- To determine the intensity of e-stim on each arm, and compare between arms.

Questionnaires

- Describe the Childhood Trauma Questionnaire (CTQ) and recent trauma scores (CIDI).
- Explore for relationship with area of SH (with results from both arms pooled).

NB: we also collected data using a few other questionnaires, but those data were intended to address other research questions, so we do not handle, report or analyse them here.

Import data

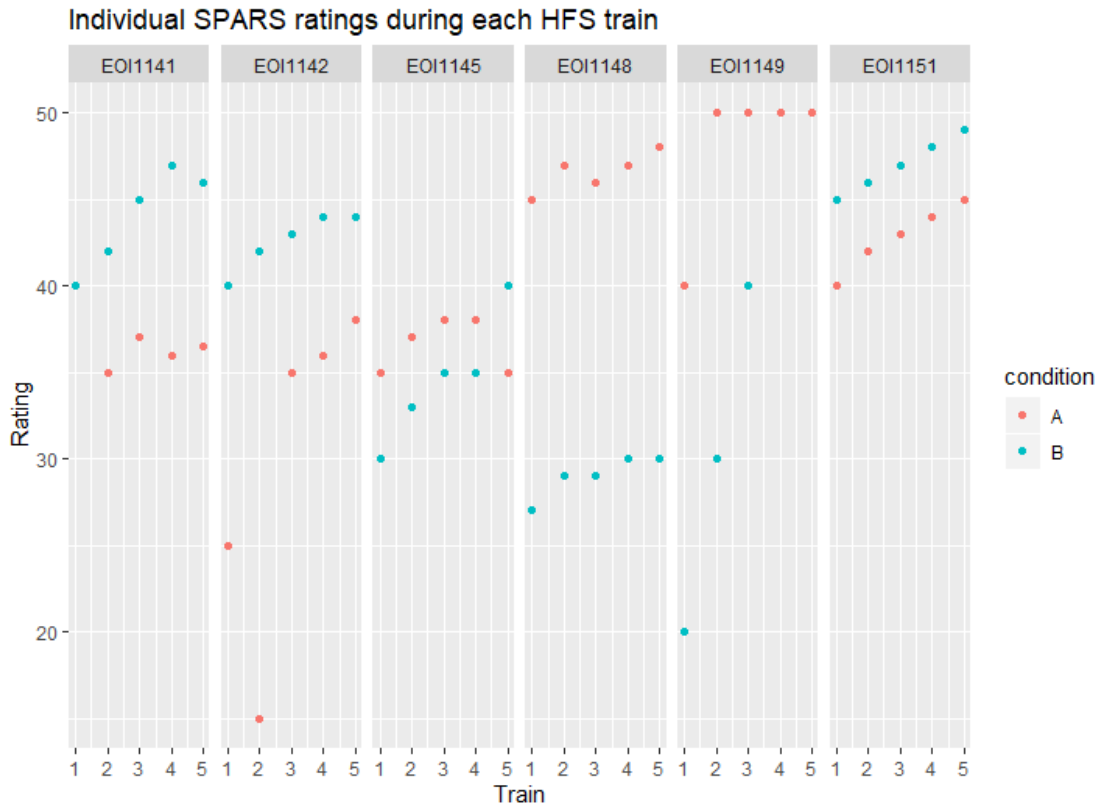
Manipulation checks

HFS ratings

We plan to use a mixed model analysis to compare ratings of the HFS trains between conditions. The order in which the two arms were stimulated was kept consistent across participants (right forearm first), but the allocation of forearm to condition was counterbalanced (random allocation to one of two equally sized groups). Therefore, the 'group' and 'site' information have been used to code for a 'condition' variable that is specified as A or B to maintain blinding of the analyst to actual experimental conditions. Here, we test for a main effect of condition on ratings, whilst allowing for a random intercept for each participant.

```
hfs <- master_data %>%
  filter(phase == 'hfs') %>%
  select(id, condition, rating)
```

```r
hfs$rating <- as.numeric(hfs$rating)

hfs %<>% group_by(id, condition) %>%
  mutate(train = row_number()) %>%
  ungroup()

hfs_wide <- hfs %>%
  group_by(id) %>%
  spread(key = condition,
         value = rating)

ggplot(data = hfs) +
  aes(x = train,
      y = rating,
      colour = condition) +
  geom_point() +
  facet_grid(~ id) +
  labs( y = 'Rating',
        x = 'Train',
        title = 'Individual SPARS ratings during each HFS train')
```




```r
# Null model
hfs_null <- lmer(rating ~ 1 + (1|id),
                 data = hfs)
hfs_condition <- lmer(rating ~ condition + (1|id),
                 data = hfs)
anova(hfs_condition, hfs_null) # hfs_condition is no better than null
```

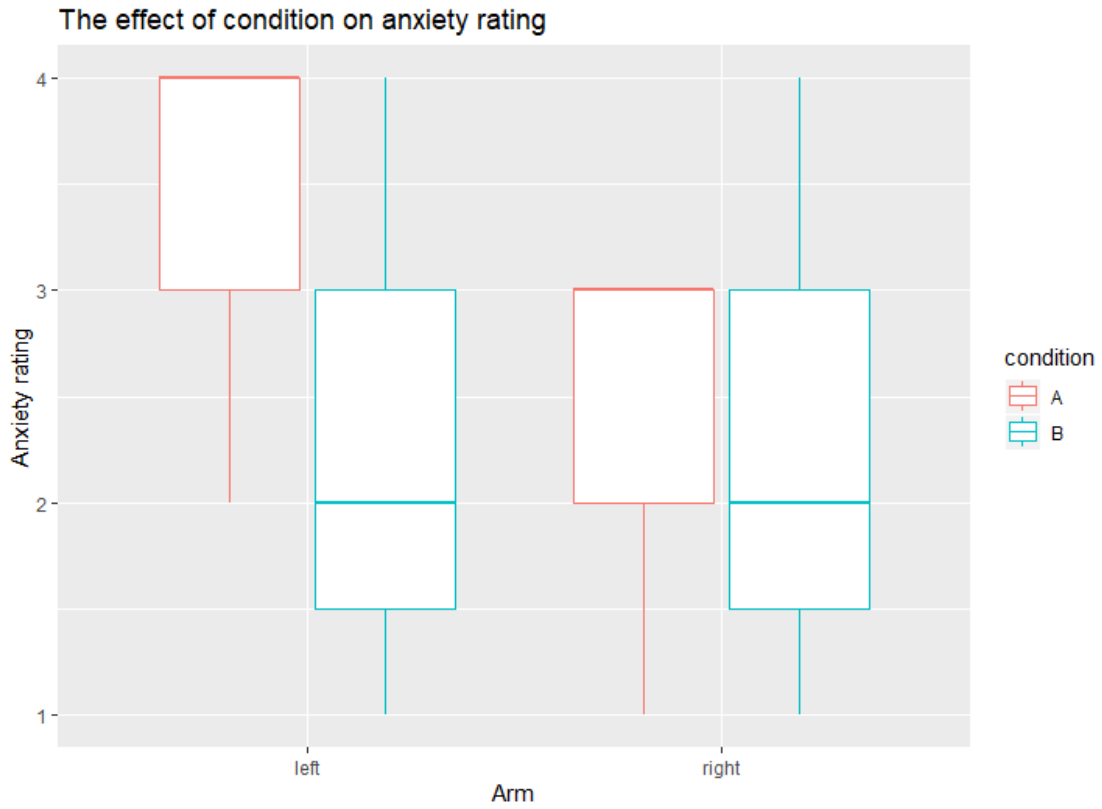
```
## Data: hfs
## Models:
## hfs_null: rating ~ 1 + (1 | id)
## hfs_condition: rating ~ condition + (1 | id)
##               Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## hfs_null       3 420.16 426.44 -207.08   414.16
## hfs_condition  4 421.92 430.30 -206.96   413.92 0.2347      1     0.6281
```

```r
# Conclude: HFS ratings were not predicted by condition.

summary(hfs_condition)

## Linear mixed model fit by REML ['lmerMod']
## Formula: rating ~ condition + (1 | id)
##    Data: hfs
##
## REML criterion at convergence: 408.2
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -3.1105 -0.4616  0.0471  0.8254  1.2217
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  id       (Intercept)  8.765   2.961
##  Residual             54.608   7.390
## Number of obs: 60, groups:  id, 6
##
## Fixed effects:
##             Estimate Std. Error t value
## (Intercept)  40.1167     1.8114   22.15
## conditionB   -0.9167     1.9080   -0.48
##
## Correlation of Fixed Effects:
##            (Intr)
## conditionB -0.527
```

Anxiety ratings

We are interested in a main effect of condition on anxiety ratings.

```r
anxiety <- master_data %>% select(group,
                                  id,
                                  anxious_right,
                                  anxious_left)

anxiety <- unique(anxiety)

anxiety %<>%
  gather(key = arm,
         value = anxiety,
         3:4) %>%
  mutate(arm = case_when(
    arm == 'anxious_left' ~ 'left',
    arm == 'anxious_right' ~ 'right'
  ))

anxiety %<>% mutate(condition = case_when(
  group == '1' & arm == 'right' ~ 'A',
  group == '1' & arm == 'left' ~ 'B',
  group == '2' & arm == 'right' ~ 'B',
  group == '2' & arm == 'left' ~ 'A')) %>%
  select(-group)

ggplot(data = anxiety) +
  aes(x = arm,
      y = anxiety,
      group = interaction(arm,condition),
      colour = condition) +
  geom_boxplot() +
  labs(title = 'The effect of condition on anxiety rating',
       y = 'Anxiety rating',
       x = 'Arm')
```



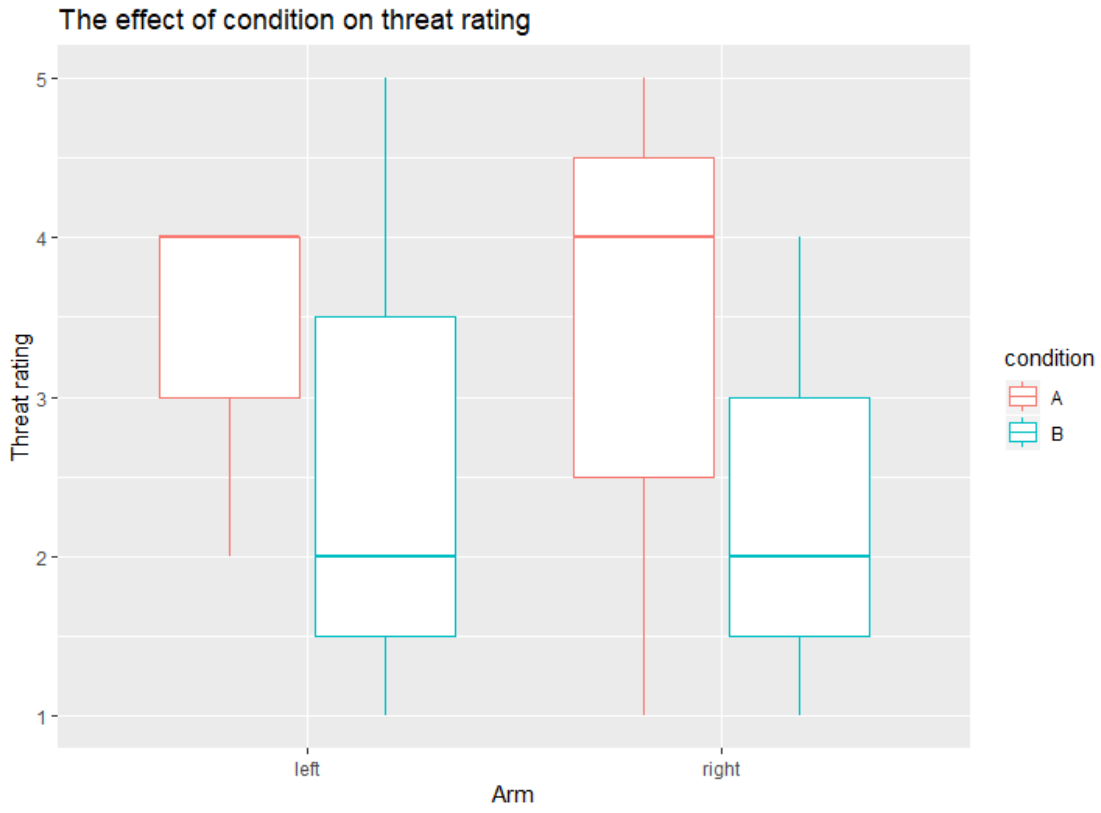

```
a1 <- ggplot(data = anxiety) +
  aes(x = arm,
      y = anxiety,
      group = condition,
      colour = condition) +
  geom_point() +
  facet_grid(~ id)+
  labs(title = 'Individual effect of condition on anxiety rating',
       y = 'Anxiety rating',
       x = 'Arm')

anxiety_null <- lmer(anxiety ~ 1 + (1|id),
                     data = anxiety)
anxiety_condition <- lmer(anxiety ~ condition + (1|id),
                     data = anxiety)
anova(anxiety_condition, anxiety_null) # No effect of condition.

## Data: anxiety
## Models:
## anxiety_null: anxiety ~ 1 + (1 | id)
## anxiety_condition: anxiety ~ condition + (1 | id)
##                    Df    AIC    BIC  logLik deviance  Chisq Chi Df
## anxiety_null        3 43.392 44.847 -18.696   37.392
## anxiety_condition   4 44.513 46.452 -18.256   36.513 0.8796      1
##                    Pr(>Chisq)
## anxiety_null
## anxiety_condition     0.3483
```

Threat ratings

We are interested in a main effect of condition on threat ratings.

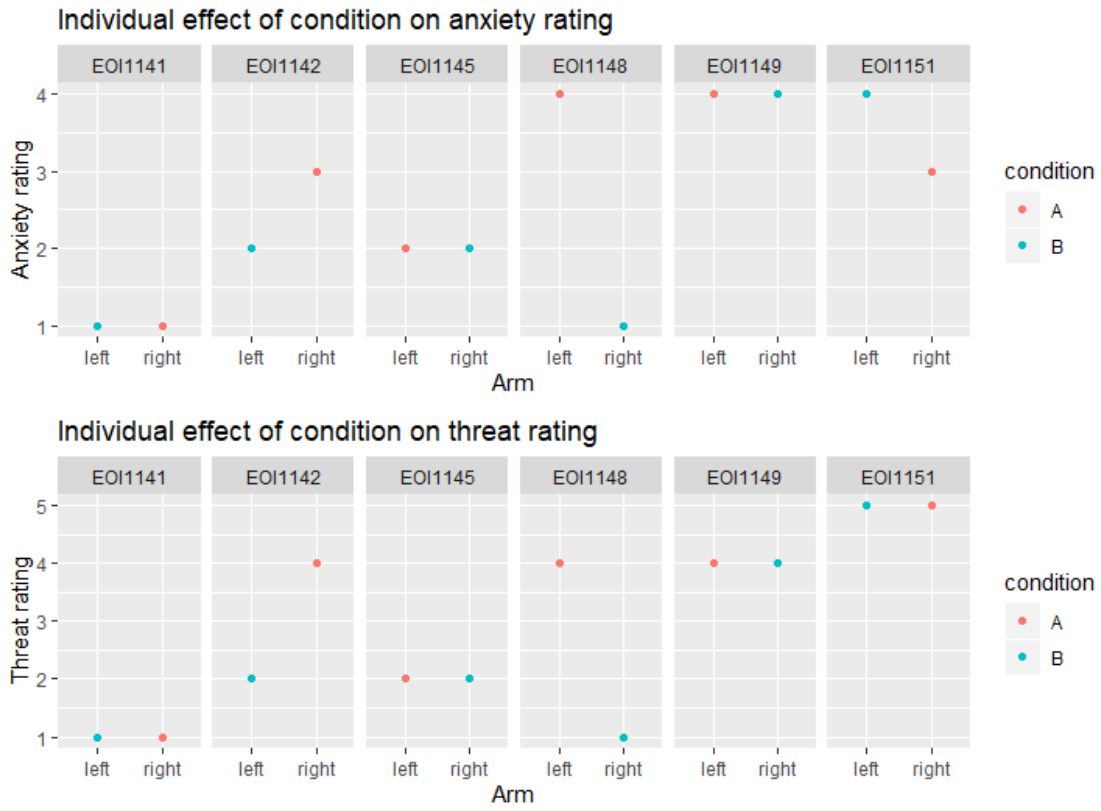
```
threat <- master_data %>% select(group,
                                 id,
                                 damage_right,
```

```
                                    damage_left)
threat <- unique(threat)

threat %<>%
  gather(key = arm,
         value = threat,
         3:4) %>%
  mutate(arm = case_when(
    arm == 'damage_left' ~ 'left',
    arm == 'damage_right' ~ 'right'
  ))

threat %<>% mutate(condition = case_when(
  group == '1' & arm == 'right' ~ 'A',
  group == '1' & arm == 'left' ~ 'B',
  group == '2' & arm == 'right' ~ 'B',
  group == '2' & arm == 'left' ~ 'A')) %>%
  select(-group)

ggplot(data = threat) +
  aes(x = arm,
      y = threat,
      group = interaction(arm,condition),
      colour = condition) +
  geom_boxplot() +
  labs(title = 'The effect of condition on threat rating',
       y = 'Threat rating',
       x = 'Arm')
```




```
t1 <- ggplot(data = threat) +
  aes(x = arm,
      y = threat,
      group = condition,
      colour = condition) +
  geom_point() +
  facet_grid(~ id)+
```

```
  labs(title = 'Individual effect of condition on threat rating',
       y = 'Threat rating',
       x = 'Arm')

threat_null <- lmer(threat ~ 1 + (1|id),
                    data = threat)
threat_condition <- lmer(threat ~ condition + (1|id),
                    data = threat)
anova(threat_condition, threat_null) # No effect of condition.

## Data: threat
## Models:
## threat_null: threat ~ 1 + (1 | id)
## threat_condition: threat ~ condition + (1 | id)
##                  Df    AIC    BIC  logLik deviance  Chisq Chi Df
## threat_null       3 47.882 49.337 -20.941   41.882
## threat_condition  4 47.564 49.503 -19.782   39.564 2.3185      1
##                  Pr(>Chisq)
## threat_null
## threat_condition     0.1278

grid.arrange(a1, t1)
```



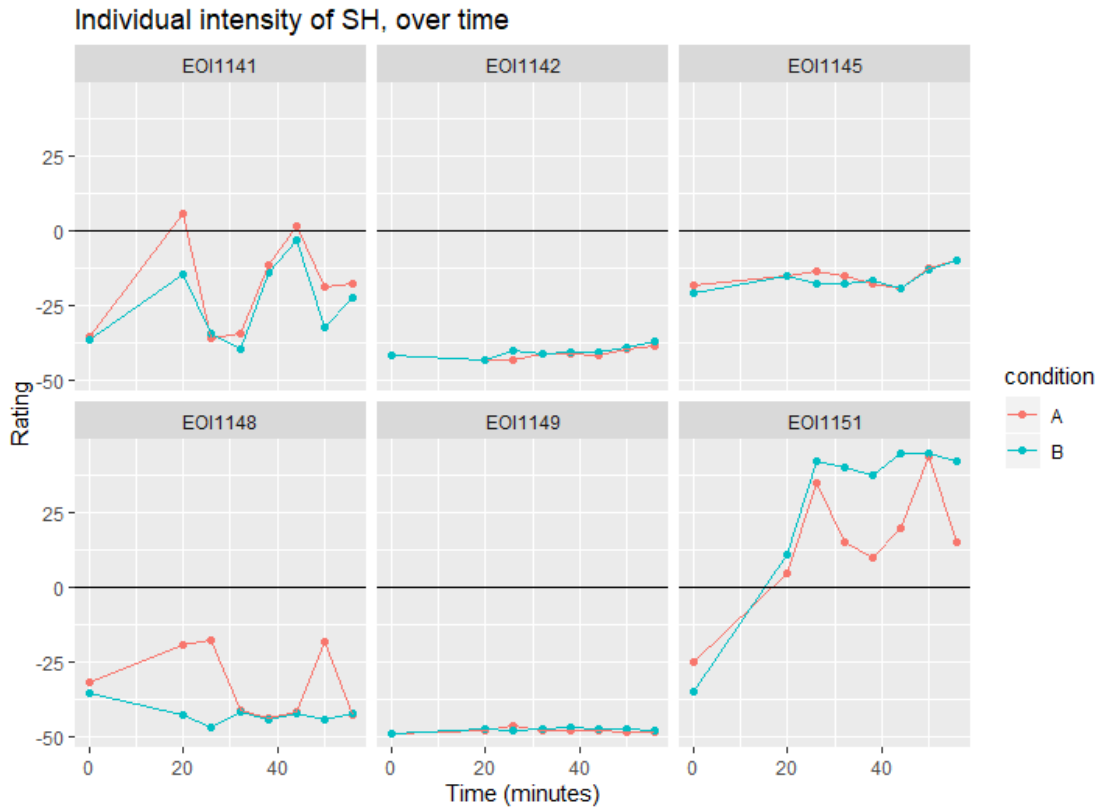
Primary outcome: intensity of SH

We are interested in determining the intensity of secondary hyperalgesia (SH) on each arm, and the comparison between arms.

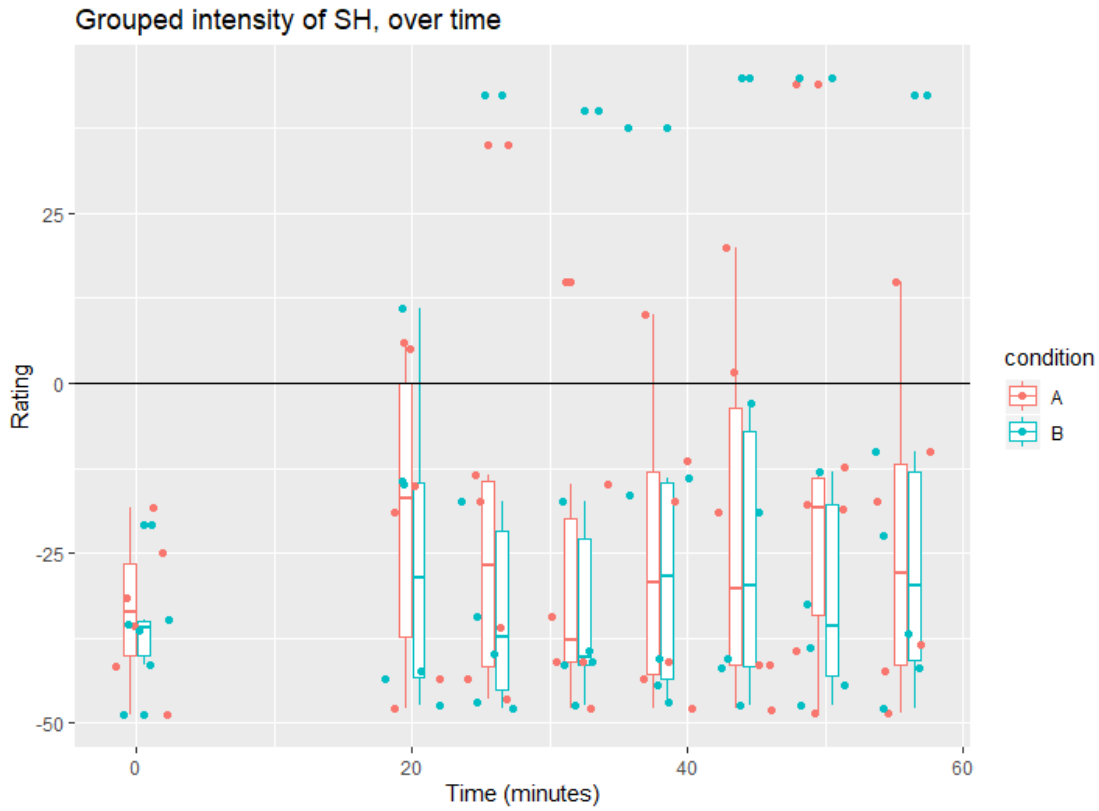
We have data from the baseline time points as well as follow-up time points, and for both arms (control and experimental sites). We are interested in the change in ratings (from baseline to follow-up time points), and how these differed between the arms/conditions. Therefore, for the formal analysis, we: 1) calculated the mean rating for all baseline time

points; 2) calculated the mean rating for all follow-up time points; 3) subtracted the mean baseline rating from the mean follow-up rating (within participant and condition), to provide the indication of change in rating (i.e. hyperalgesia) for each condition, within each participant.

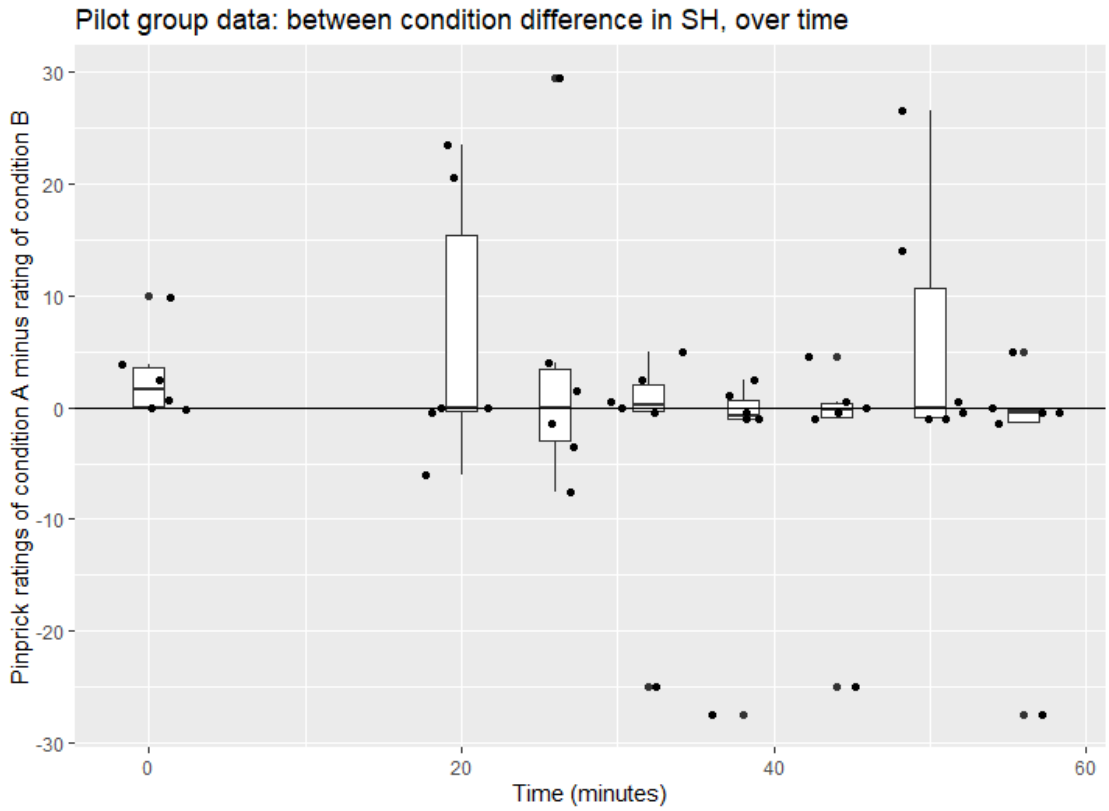
```
intensity <- master_data %>%
  select(group,
         id,
         time,
         phase,
         site,
         modality,
         rating) %>%
  filter(modality == '128' | modality == '256') %>%
  filter(phase != 'orientation') %>%
  mutate(time = case_when(
    phase == 'baseline' ~ time*-1,
    phase != 'baseline' ~ time
  ))

intensity %<>% mutate(condition = case_when(
  group == '1' & site == 'right' ~ 'A',
  group == '1' & site == 'left' ~ 'B',
  group == '2' & site == 'right' ~ 'B',
  group == '2' & site == 'left' ~ 'A')) %>%
  select(-group,
         -site)
intensity$rating <- as.numeric(intensity$rating)
intensity %<>% group_by(id,
           time,
           phase,
           condition) %>%
  summarise(pp_rating = mean(rating))

# Management of rating data: we controlled for baseline ratings. We compared individual rat
ings over time and group ratings over time.

int_bl <- intensity %>% filter(phase == 'baseline') %>%
  group_by(id, condition) %>%
  summarise(rating = mean(pp_rating)) %>%
  ungroup() %>%
  mutate(time = 0) %>% ungroup()

int_fu <- intensity %>% filter(phase != 'baseline') %>% ungroup () %>%
  select(-phase) %>%
  rename(rating = pp_rating)

intensity <- rbind(int_bl, int_fu)

# For interest, plot the actual pilot data

ggplot(data = intensity) +
  aes(x =time,
      y = rating,
      group = condition,
      colour = condition) +
  facet_wrap(~ id) +
  geom_point() +
  geom_line() +
  geom_hline(yintercept = 0) +
  labs(title = 'Individual intensity of SH, over time',
       y = 'Rating',
       x = 'Time (minutes)')
```




```r
# All participants, group data represented by boxplots
ggplot(data = intensity) +
  aes(x = time,
      y = rating,
      group = interaction(condition, time),
      colour = condition) +
  geom_boxplot(width = 2) +
  geom_point(position = 'jitter') +
  geom_hline(yintercept = 0) +
  labs(title = 'Grouped intensity of SH, over time',
       y = 'Rating',
       x = 'Time (minutes)')
```




```r
 # Calculate diff between conditions for group boxplots

intensity_wide <- intensity %>%
  group_by(id,
           time) %>%
  spread(key = condition,
         value = rating) %>%
  mutate(site_diff = A-B)

# All participants, group data represented by boxplots
ggplot(data = intensity_wide) +
  aes(x = time,
      y = site_diff,
      group = time) +
  geom_boxplot(width = 2) +
  geom_point(position = 'jitter') +
  geom_hline(yintercept = 0) +
  labs(title = 'Pilot group data: between condition difference in SH, over time',
       y = 'Pinprick ratings of condition A minus rating of condition B',
       x = 'Time (minutes)')
```




```
# Collapse all post-baseline ratings for each condition and then compare them using a RM AN
OVA type model. Time is nested within (and fully crossed with) ID.

int_bl %<>% select(-time)
names(int_bl)[names(int_bl) == 'rating'] <- 'baseline_rating'
collapse <- int_fu %>% # resolve each rating relative to baseline
  right_join(int_bl) %>%
  mutate(rating_controlled = rating-baseline_rating) %>%
  select(id,
         time,
         condition,
         rating_controlled) # spread to calculate difference between conditions

# We want to find out whether rating_cotrolled (for baseline) is predicted by condition.

model_null <- lmer(rating_controlled ~ (1|id),
                   data = collapse)
model_condition <- lmer(rating_controlled ~ condition + (1|id),
                        data = collapse)
anova(model_condition, model_null) # No improvement

## Data: collapse
## Models:
## model_null: rating_controlled ~ (1 | id)
## model_condition: rating_controlled ~ condition + (1 | id)
##                  Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## model_null        3 665.14 672.43 -329.57   659.14
## model_condition   4 665.88 675.60 -328.94   657.88 1.2618      1     0.2613

model_pre_condition_crossed <- lmer(rating_controlled ~ condition + (1|id/time),
                                    data = collapse)

anova(model_pre_condition_crossed, model_null)

## Data: collapse
## Models:
```

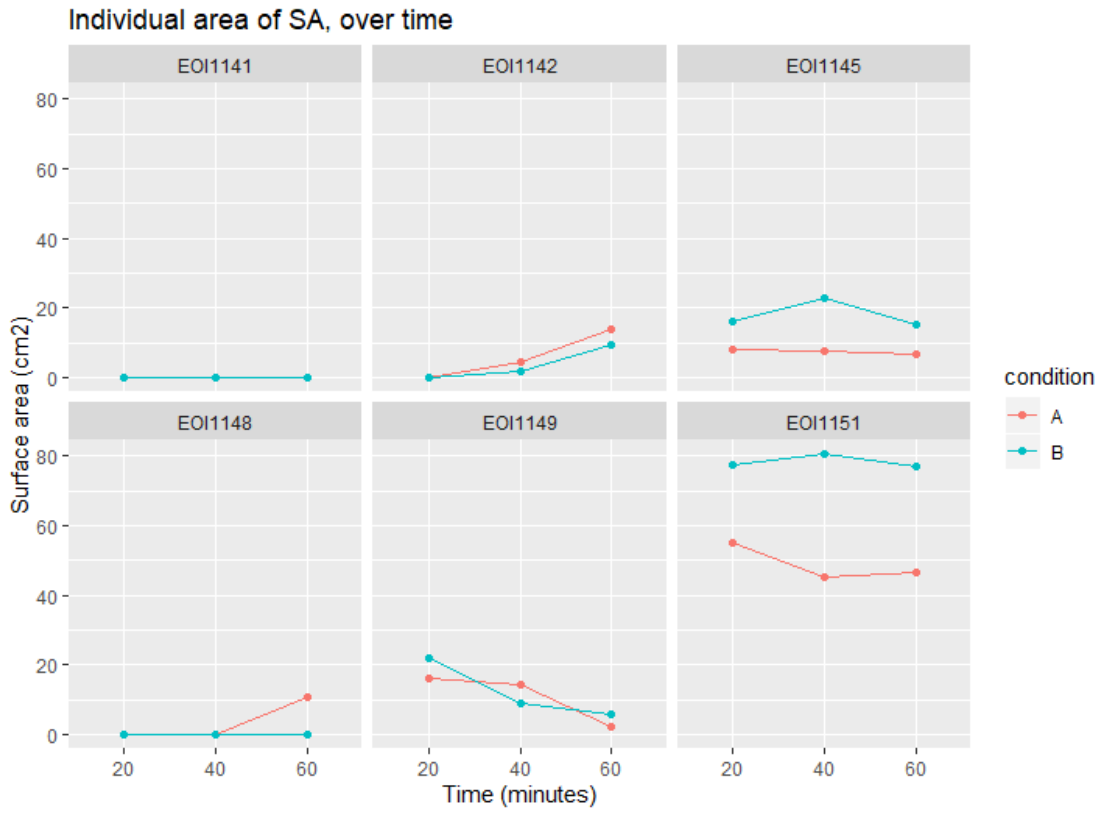
```
## model_null: rating_controlled ~ (1 | id)
## model_pre_condition_crossed: rating_controlled ~ condition + (1 | id/time)
##                             Df    AIC    BIC  logLik deviance Chisq Chi Df
## model_null                   3 665.14 672.43 -329.57   659.14
## model_pre_condition_crossed  5 666.93 679.08 -328.46   656.93  2.21      2
##                             Pr(>Chisq)
## model_null
## model_pre_condition_crossed     0.3312

# not a significant improvement

# Secondary hyperalgesia intensity is not predicted by condition.
```

Secondary outcome: area of SH

We are interested in determining the surface area (SA) of secondary hyperalgesia (SH) on each arm, and the comparison between arms.

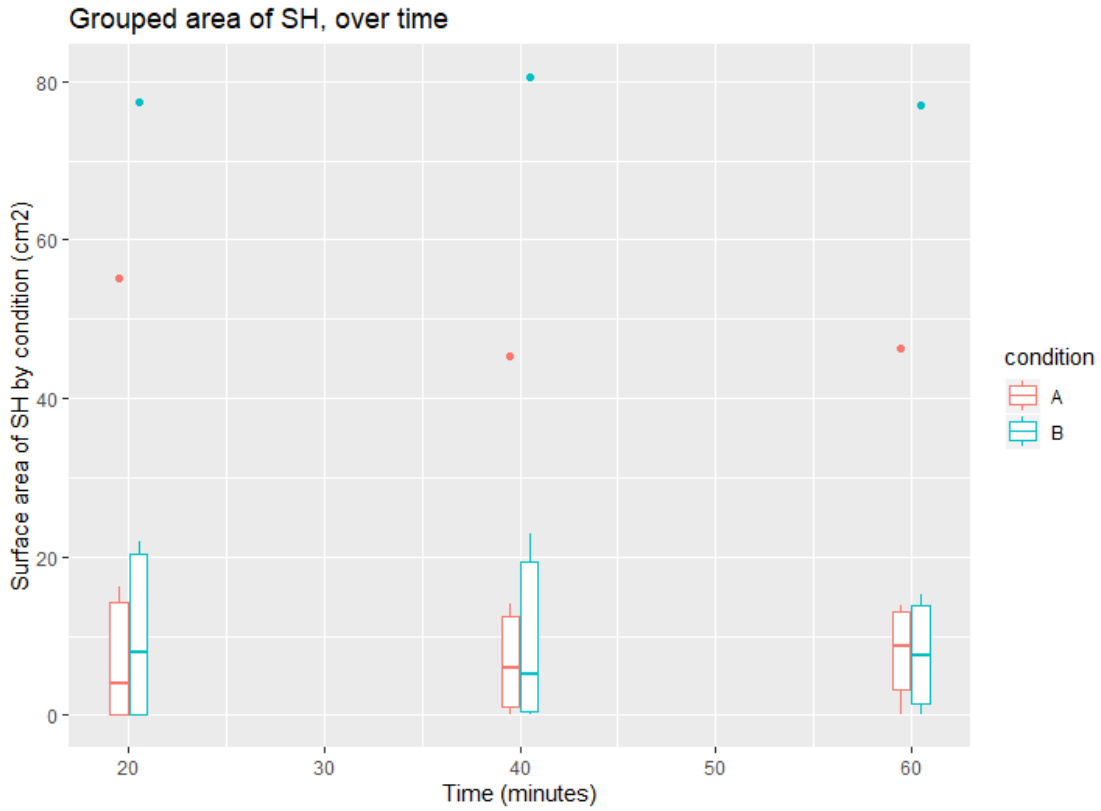
```
SA <- master_data %>%
  select(group,
         id,
         time,
         phase,
         site,
         modality,
         rating) %>%
  filter(phase == 'test_sa')

SA %<>% mutate(condition = case_when(
  group == '1' & site == 'right' ~ 'A',
  group == '1' & site == 'left' ~ 'B',
  group == '2' & site == 'right' ~ 'B',
  group == '2' & site == 'left' ~ 'A')) %>%
  select(-group,
         -site,
         -phase)

# replace ND with 0

SA$rating[SA$rating == 'ND'] <- 0

names(SA)[names(SA) == 'rating'] <- 'point1'
names(SA)[names(SA) == 'modality'] <- 'radial_line'

SA %<>% group_by(id, time) %>%
  mutate(point2 = case_when(radial_line == 'A' ~ lead(point1, 5),
                            radial_line == 'B' ~ lag(point1, 2),
                            radial_line == 'C' ~ lag(point1, 2),
                            radial_line == 'D' ~ lag(point1, 2),
                            radial_line == 'E' ~ lead(point1, 7),
                            radial_line == 'F' ~ lag(point1, 2),
                            radial_line == 'G' ~ lag(point1, 2),
                            radial_line == 'H' ~ lag(point1, 2))) %>%
  ungroup()

SA$point1 <- as.numeric(SA$point1)
SA$point2 <- as.numeric(SA$point2)
# Now calculate surface area for each triangle
library(REdaS)
sinangle <- sin((deg2rad(45)))
sinangle

## [1] 0.7071068
```

```r
SA %<>% mutate(triangle = (point1*point2*sinangle)/2)
# Now calculate SA for each ID, site and time

SA %<>% select(-radial_line,
                      -point1,
                      -point2)

SA %<>% group_by(id,
                 time,
                 condition) %>%
  summarise(SA = sum(triangle)) %>%
  ungroup()

SA_plot <- ggplot(data = SA) +
  aes(x = as.factor(time),
      y = SA,
      colour = condition,
      group = condition) +
  geom_point() +
  geom_line(aes(colour = condition)) +
  facet_wrap(~ id,
             nrow = 2) +
  labs(x = 'Time (minutes)',
       y = 'Surface area (cm2)',
       title = 'Individual area of SA, over time')
SA_plot
```




```r
# All participants, group data represented by boxplots
ggplot(data = SA) +
  aes(x = time,
      y = SA,
      group = interaction(time,condition),
      colour = condition) +
  geom_boxplot(width = 2) +
  labs(title = 'Grouped area of SH, over time',
```

```
       y = 'Surface area of SH by condition (cm2)',
       x ='Time (minutes)')
```




```
# We are interested in a main effect of condition on SA

model_null_sa <- lmer(SA ~ (1|id),
                      data = SA)
model_condition_sa <- lmer(SA ~ condition + (1|id),
                           data = SA)
anova(model_condition_sa, model_null_sa) # Condition predicts SA! (p = 0.02703)

## Data: SA
## Models:
## model_null_sa: SA ~ (1 | id)
## model_condition_sa: SA ~ condition + (1 | id)
##                    Df    AIC    BIC  logLik deviance  Chisq Chi Df
## model_null_sa       3 283.30 288.05 -138.65   277.30
## model_condition_sa  4 280.41 286.74 -136.21   272.41 4.8891      1
##                    Pr(>Chisq)
## model_null_sa
## model_condition_sa    0.02703 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

model_pre_condition_crossed_sa <- lmer(SA ~ condition + (1|id/time),
                                       data = SA)

anova(model_pre_condition_crossed_sa, model_condition_sa)

## Data: SA
## Models:
## model_condition_sa: SA ~ condition + (1 | id)
## model_pre_condition_crossed_sa: SA ~ condition + (1 | id/time)
##                                Df    AIC    BIC  logLik deviance Chisq
## model_condition_sa              4 280.41 286.74 -136.21   272.41
## model_pre_condition_crossed_sa  5 282.41 290.33 -136.21   272.41     0
```

```
##                                Chi Df Pr(>Chisq)
## model_condition_sa
## model_pre_condition_crossed_sa      1          1

# not a significant improvement

# Secondary hyperalgesia surface area is predicted by condition, and the model that does no
t allow for full crossing of time and ID is a better model.
```

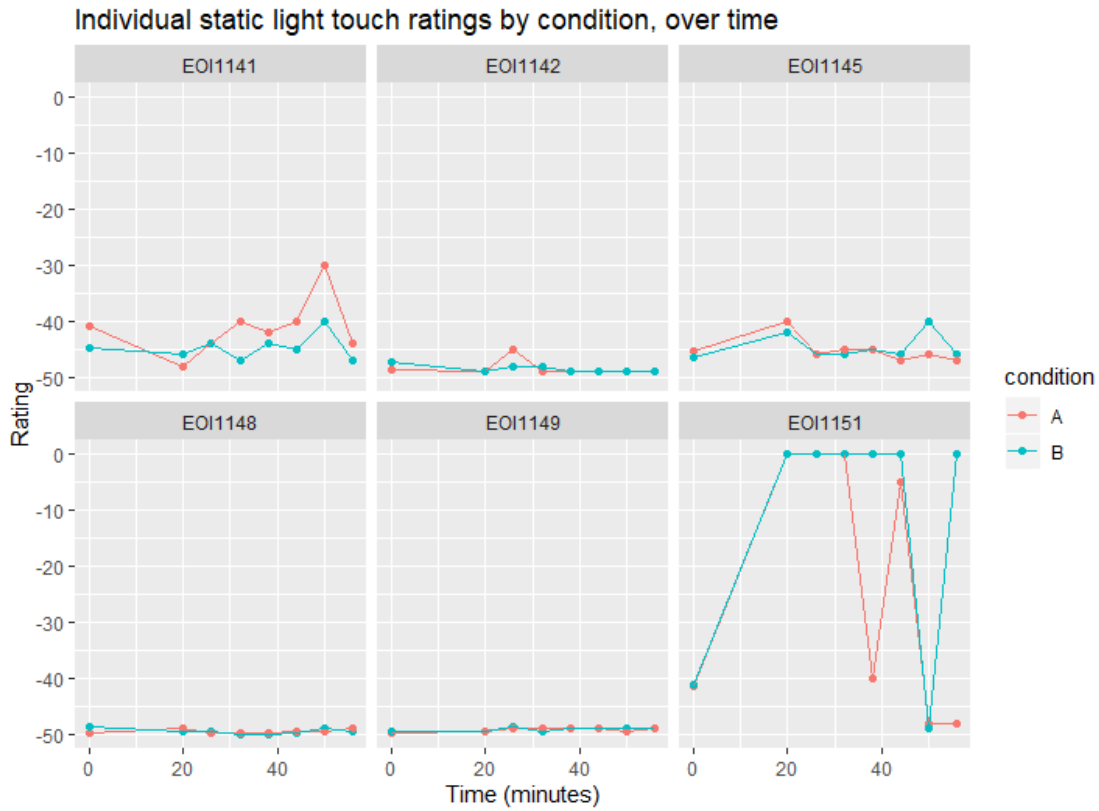
Exploratory outcomes

Sensory outcomes

- To determine the intensity of static light touch on each arm, and the comparison between arms.
- To determine the intensity of dynamic light touch on each arm, and the comparison between arms.
- To determine the intensity of e-stim on each arm, and the comparison between arms.

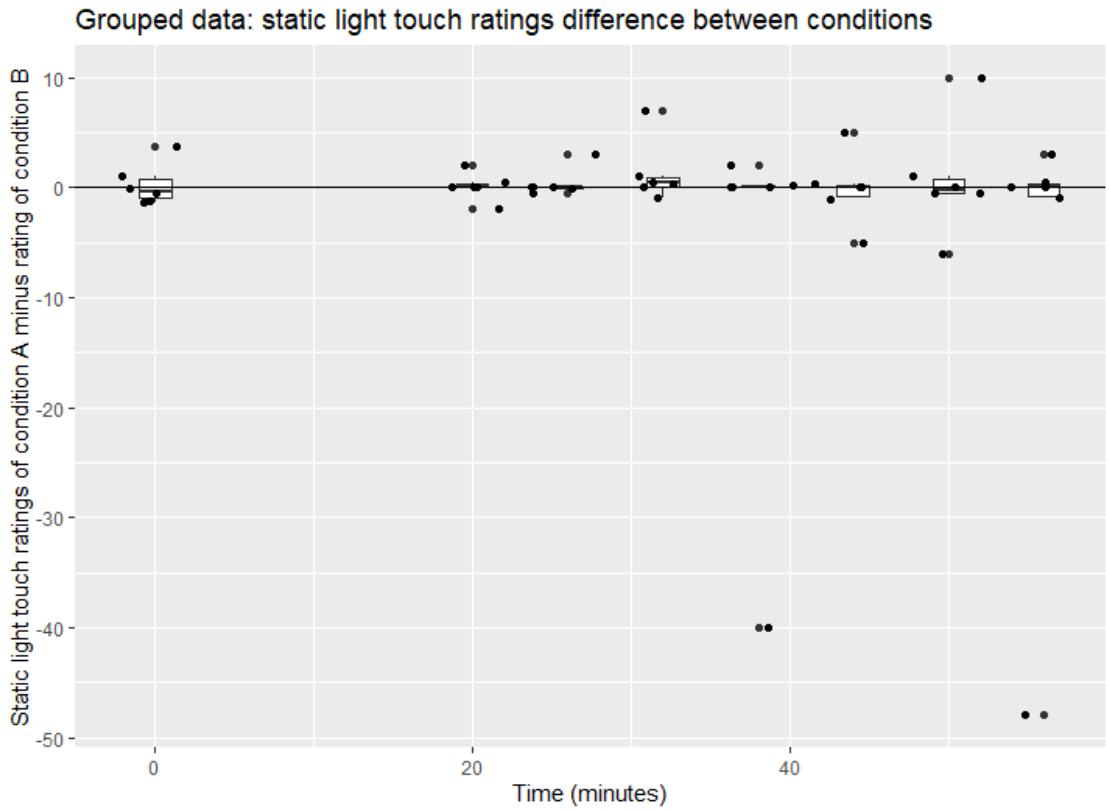
```
## Static light touch

slt <- master_data %>%
  select(group,
         id,
         time,
         phase,
         site,
         modality,
         rating) %>%
  filter(modality == 'VFF') %>%
  filter(phase != 'orientation') %>%
  mutate(time = case_when(
    phase == 'baseline' ~ time*-1,
    phase != 'baseline' ~ time
  ))

slt %<>% mutate(condition = case_when(
  group == '1' & site == 'right' ~ 'A',
  group == '1' & site == 'left' ~ 'B',
  group == '2' & site == 'right' ~ 'B',
  group == '2' & site == 'left' ~ 'A')) %>%
  select(-group,
         -site)
slt$rating <- as.numeric(slt$rating)

slt_bl <- slt %>% filter(phase == 'baseline') %>%
  group_by(id, condition) %>%
  summarise(rating = mean(rating)) %>%
  ungroup() %>%
  mutate(time = 0) %>% ungroup()

slt_fu <- slt %>% filter(phase != 'baseline') %>% ungroup () %>%
  select(-phase,
         -modality)

slt <- rbind(slt_bl, slt_fu)

# For interest, plot the pilot data

ggplot(data = slt) +
  aes(x = time,
      y = rating,
      group = condition,
      colour = condition) +
```

```
  facet_wrap(~ id) +
  geom_point() +
  geom_line() +
  labs(title = 'Individual static light touch ratings by condition, over time',
       y = 'Rating',
       x = 'Time (minutes)')
```




```
# All participants, group data represented by boxplots
ggplot(data = slt) +
  aes(x = time,
      y = rating,
      group = interaction(condition, time),
      colour = condition) +
  geom_boxplot(width = 2) +
  geom_point(position = 'jitter') +
  labs(title = 'Group data: static light touch ratings by condition, over time',
       y = 'Rating',
       x = 'Time (minutes)')
```

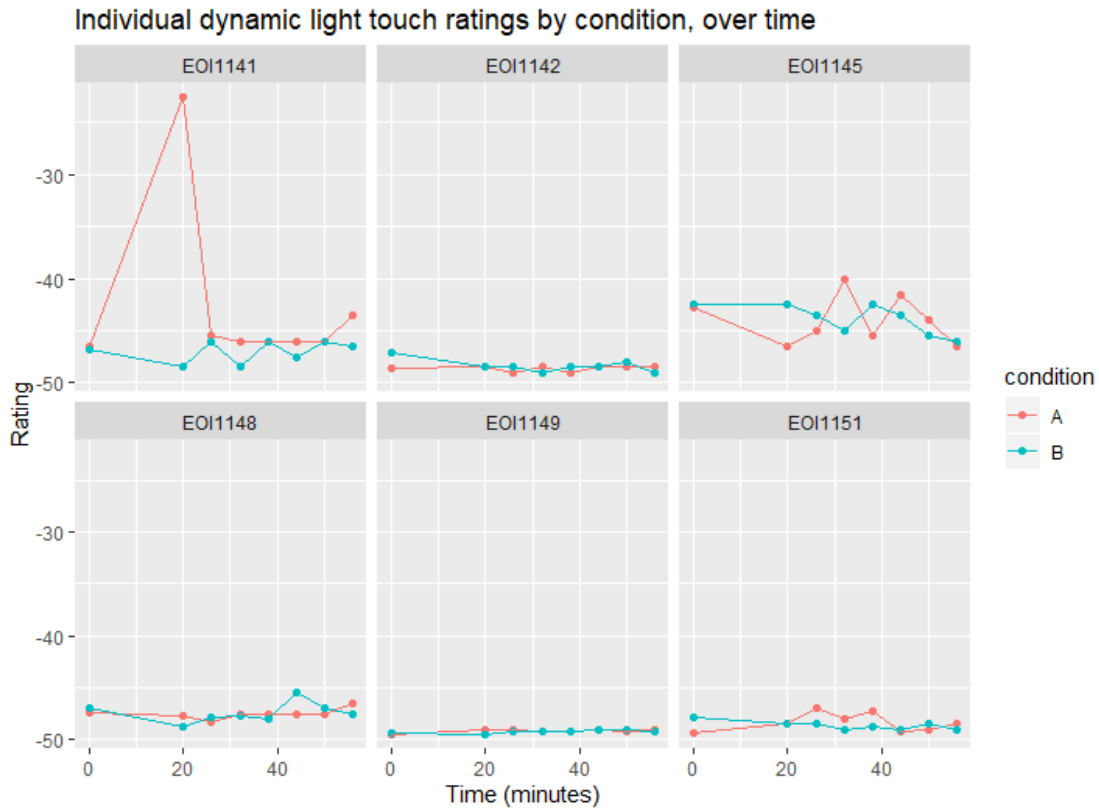

```
 # Calculate diff between conditions for group boxplots

slt_wide <- slt %>%
  group_by(id,
           time) %>%
  spread(key = condition,
         value = rating) %>%
  mutate(site_diff = A-B)

# All participants, group data represented by boxplots
ggplot(data = slt_wide) +
  aes(x = time,
      y = site_diff,
      group = time) +
  geom_boxplot(width = 2) +
  geom_point(position = 'jitter') +
  geom_hline(yintercept = 0) +
  labs(title = 'Grouped data: static light touch ratings difference between conditions',
       y = 'Static light touch ratings of condition A minus rating of condition B',
       x = 'Time (minutes)')
```



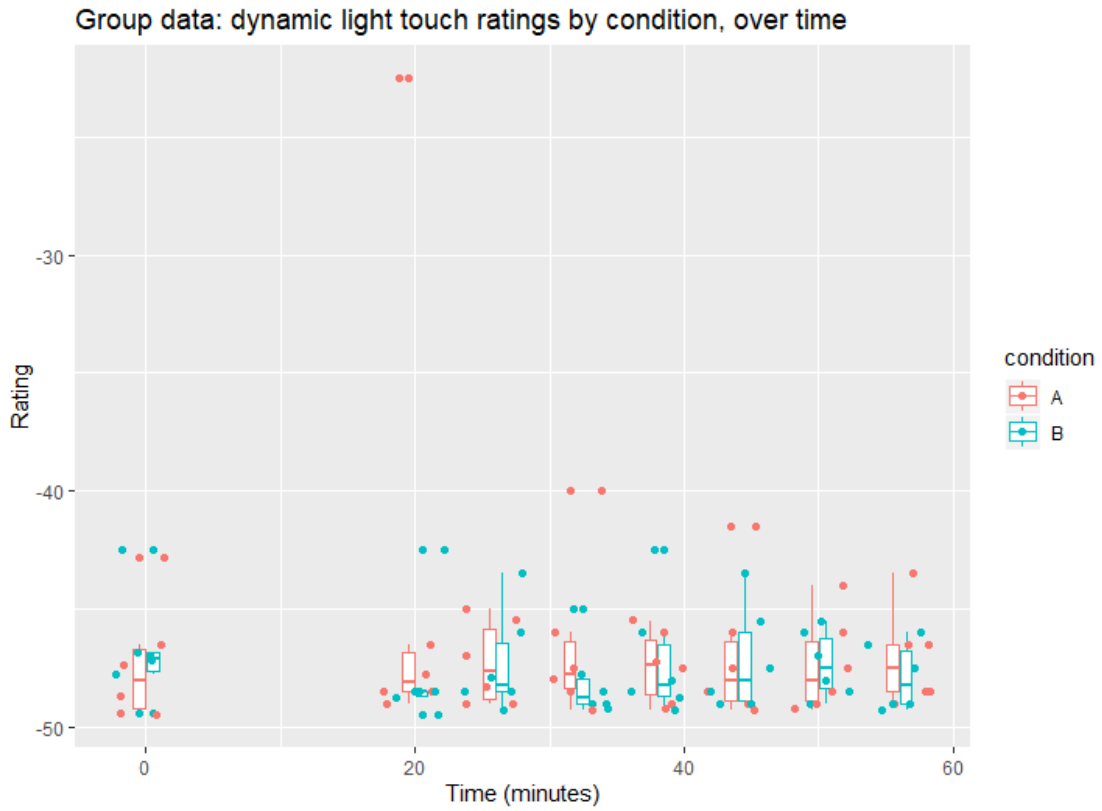

```
# Collapse all post-baseline ratings for each condition and then compare them using a RM AN
OVA type model. Time is nested within (and fully crossed with) ID.

slt_bl %<>% select(-time)
names(slt_bl)[names(slt_bl) == 'rating'] <- 'baseline_rating'
collapse_slt <- slt_fu %>% # resolve each rating relative to baseline
  right_join(slt_bl) %>%
  mutate(rating_controlled = rating-baseline_rating) %>%
  select(id,
         time,
         condition,
         rating_controlled) # spread to calculate difference between conditions

# We want to find out whether rating_cotrolled (for baseline) is predicted by condition.

model_null_slt <- lmer(rating_controlled ~ (1|id),
                   data = collapse_slt)
model_condition_slt <- lmer(rating_controlled ~ condition + (1|id),
                       data = collapse_slt)
anova(model_condition_slt, model_null_slt) # No improvement

## Data: collapse_slt
## Models:
## model_null_slt: rating_controlled ~ (1 | id)
## model_condition_slt: rating_controlled ~ condition + (1 | id)
##                      Df    AIC    BIC  logLik deviance  Chisq Chi Df
## model_null_slt        3 631.37 638.67 -312.69   625.37
## model_condition_slt   4 632.44 642.16 -312.22   624.44 0.9367      1
##                      Pr(>Chisq)
## model_null_slt
## model_condition_slt     0.3331

model_pre_condition_crossed_slt <- lmer(rating_controlled ~ condition + (1|id/time),
                                   data = collapse_slt)

anova(model_pre_condition_crossed_slt, model_null_slt)
```

```
## Data: collapse_slt
## Models:
## model_null_slt: rating_controlled ~ (1 | id)
## model_pre_condition_crossed_slt: rating_controlled ~ condition + (1 | id/time)
##                                 Df    AIC    BIC  logLik deviance  Chisq
## model_null_slt                   3 631.37 638.67 -312.69   625.37
## model_pre_condition_crossed_slt  5 625.73 637.89 -307.87   615.73 9.6406
##                                 Chi Df Pr(>Chisq)
## model_null_slt
## model_pre_condition_crossed_slt      2   0.008064 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# Significant improvement... condition predicts static light touch ratings but only in full
y crossed model.

# Dynamic light touch

dlt <- master_data %>%
  select(group,
         id,
         time,
         phase,
         site,
         modality,
         rating) %>%
  filter(modality == 'CW' | modality == 'BR') %>%
  filter(phase != 'orientation') %>%
  mutate(time = case_when(
    phase == 'baseline' ~ time*-1,
    phase != 'baseline' ~ time
  ))

dlt %<>% mutate(condition = case_when(
  group == '1' & site == 'right' ~ 'A',
  group == '1' & site == 'left' ~ 'B',
  group == '2' & site == 'right' ~ 'B',
  group == '2' & site == 'left' ~ 'A')) %>%
  select(-group,
         -site)
dlt$rating <- as.numeric(dlt$rating)

dlt_bl <- dlt %>% filter(phase == 'baseline') %>%
  group_by(id, condition) %>%
  summarise(dlt_rating = mean(rating)) %>%
  ungroup() %>%
  mutate(time = 0) %>% ungroup()

dlt_fu <- dlt %>% filter(phase != 'baseline') %>% ungroup() %>%
  group_by(id,
           time,
           condition) %>%
  summarise(dlt_rating = mean(rating)) %>%
  ungroup()

dlt <- rbind(dlt_bl, dlt_fu)

# For interest, plot the pilot data

ggplot(data = dlt) +
  aes(x = time,
      y = dlt_rating,
      group = condition,
      colour = condition) +
  facet_wrap(~ id) +
  geom_point() +
```

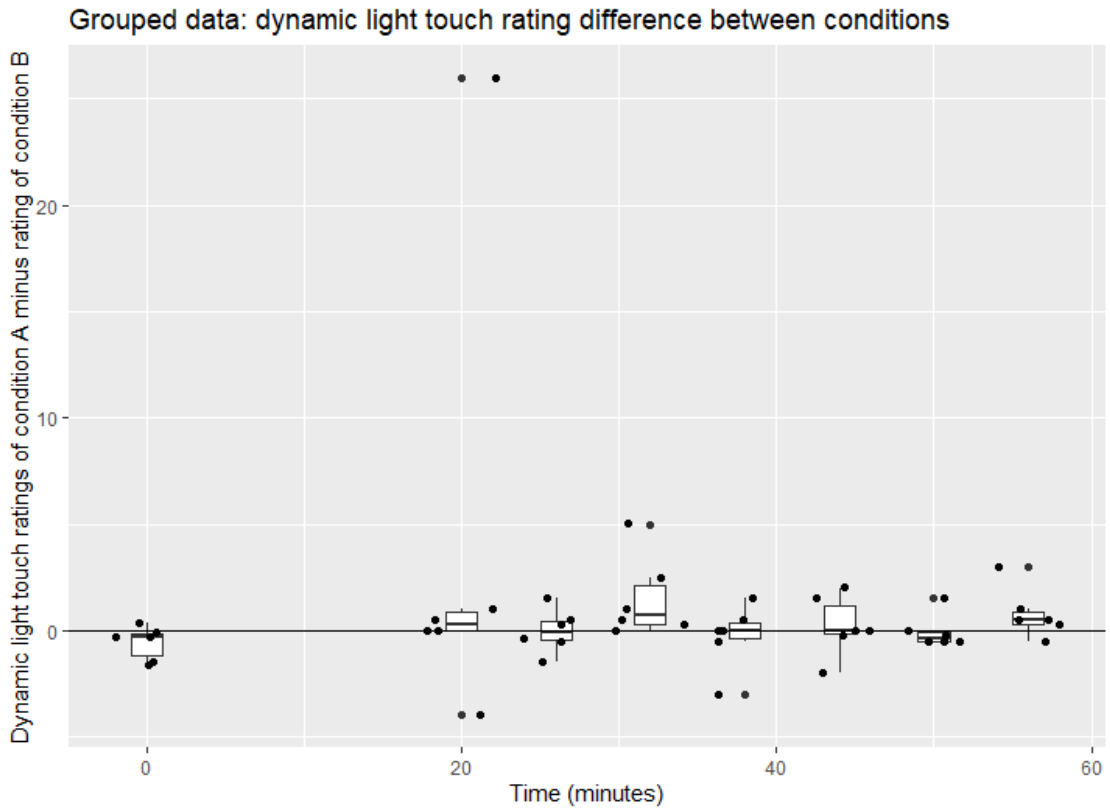
```
  geom_line() +
  labs(title = 'Individual dynamic light touch ratings by condition, over time',
       y = 'Rating',
       x = 'Time (minutes)')
```




```
# All participants, group data represented by boxplots
ggplot(data = dlt) +
  aes(x = time,
      y = dlt_rating,
      group = interaction(condition, time),
      colour = condition) +
  geom_boxplot(width = 2) +
  geom_point(position = 'jitter') +
  labs(title = 'Group data: dynamic light touch ratings by condition, over time',
       y = 'Rating',
       x = 'Time (minutes)')
```



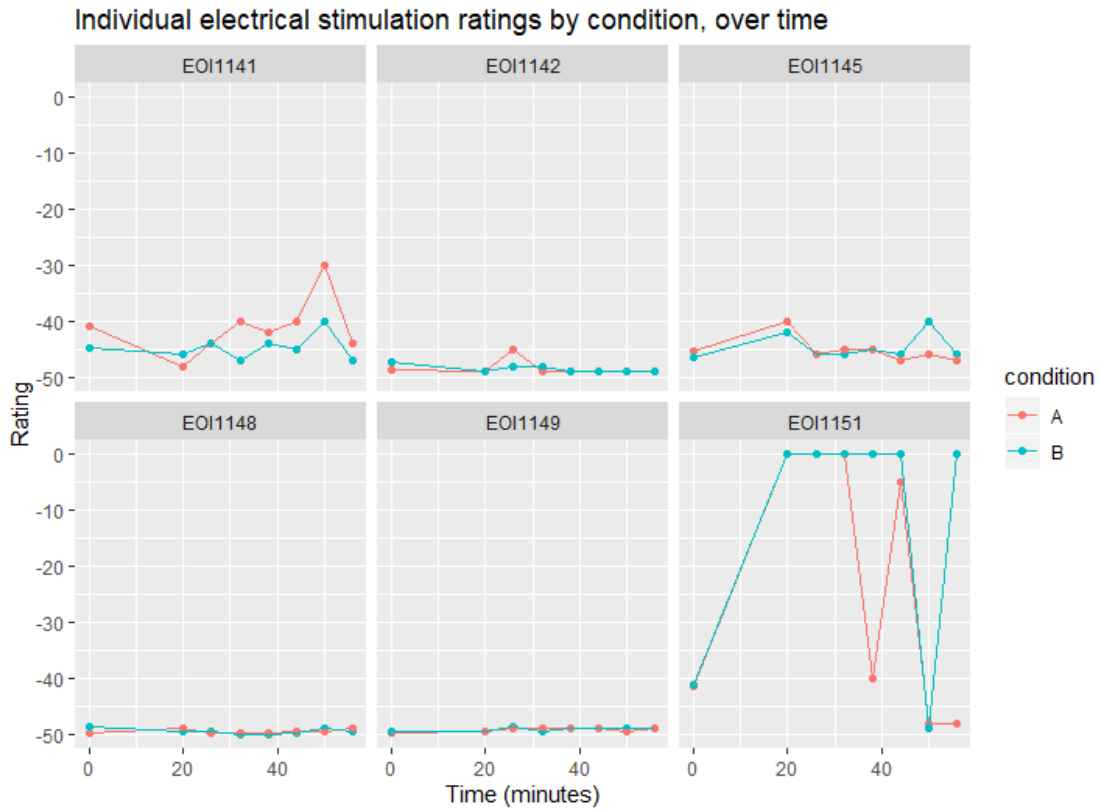

```
 # Calculate diff between conditions for group boxplots

dlt_wide <- dlt %>%
  group_by(id,
           time) %>%
  spread(key = condition,
         value = dlt_rating) %>%
  mutate(site_diff = A-B)

# All participants, group data represented by boxplots
ggplot(data = dlt_wide) +
  aes(x = time,
      y = site_diff,
      group = time) +
  geom_boxplot(width = 2) +
  geom_point(position = 'jitter') +
  geom_hline(yintercept = 0) +
  labs(title = 'Grouped data: dynamic light touch rating difference between conditions',
       y = 'Dynamic light touch ratings of condition A minus rating of condition B',
       x = 'Time (minutes)')
```



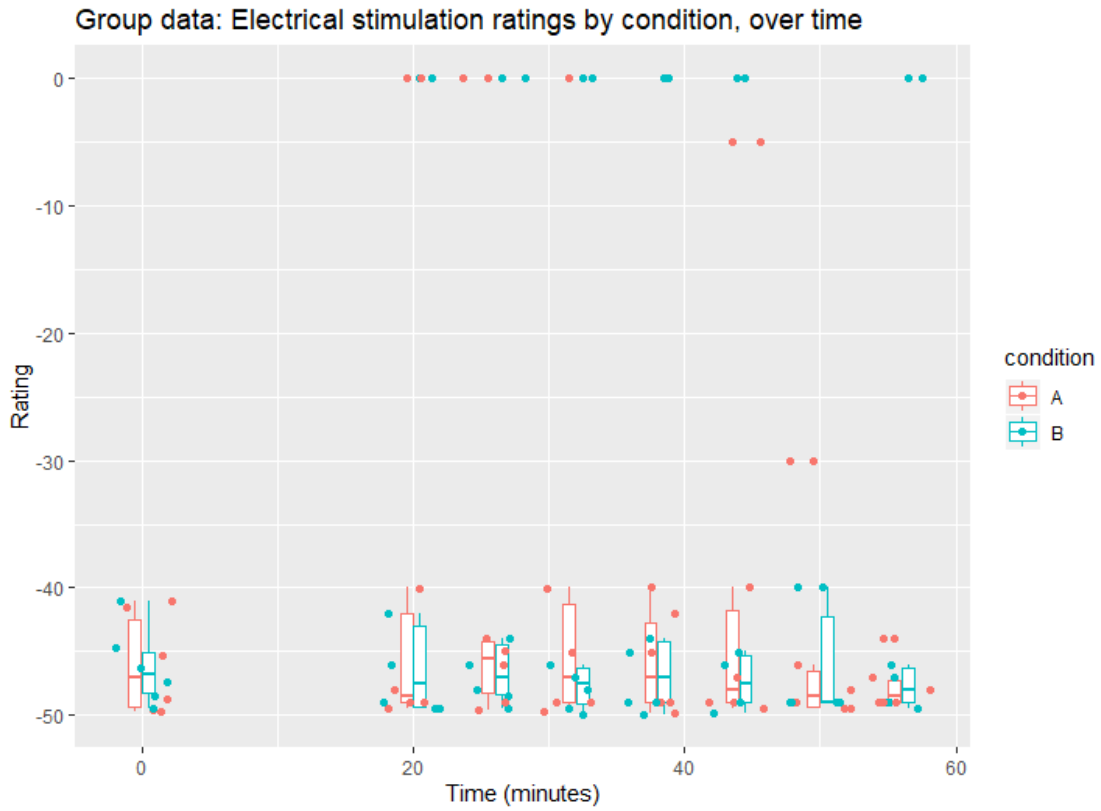

```
# Collapse all post-baseline ratings for each condition and then compare them using a RM AN
OVA type model. Time is nested within (and fully crossed with) ID.

dlt_bl %<>% select(-time)
names(dlt_bl)[names(dlt_bl) == 'dlt_rating'] <- 'baseline_rating'
collapse_dlt <- dlt_fu %>% # resolve each rating relative to baseline
  right_join(dlt_bl) %>%
  mutate(rating_controlled = dlt_rating-baseline_rating) %>%
  select(id,
         time,
         condition,
         rating_controlled) # spread to calculate difference between conditions

# We want to find out whether rating_cotrolled (for baseline) is predicted by condition.

model_null_dlt <- lmer(rating_controlled ~ (1|id),
                   data = collapse_dlt)
model_condition_dlt <- lmer(rating_controlled ~ condition + (1|id),
                       data = collapse_dlt)
anova(model_condition_dlt, model_null_dlt) # Condition predicts DLT rating

## Data: collapse_dlt
## Models:
## model_null_dlt: rating_controlled ~ (1 | id)
## model_condition_dlt: rating_controlled ~ condition + (1 | id)
##                      Df    AIC    BIC  logLik deviance  Chisq Chi Df
## model_null_dlt        3 423.36 430.65 -208.68   417.36
## model_condition_dlt   4 419.47 429.19 -205.74   411.47 5.8896      1
##                      Pr(>Chisq)
## model_null_dlt
## model_condition_dlt    0.01523 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

model_pre_condition_crossed_dlt <- lmer(rating_controlled ~ condition + (1|id/time),
                                    data = collapse_dlt)
```

```r
anova(model_pre_condition_crossed_dlt, model_null_dlt) # Not better

## Data: collapse_dlt
## Models:
## model_null_dlt: rating_controlled ~ (1 | id)
## model_pre_condition_crossed_dlt: rating_controlled ~ condition + (1 | id/time)
##                                 Df    AIC    BIC  logLik deviance  Chisq
## model_null_dlt                   3 423.36 430.65 -208.68   417.36
## model_pre_condition_crossed_dlt  5 421.47 433.62 -205.74   411.47 5.8896
##                                 Chi Df Pr(>Chisq)
## model_null_dlt
## model_pre_condition_crossed_dlt      2    0.05261 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# Condition predicts dynamic light touch ratings but only the un-crossed model is tolerated
.

# Electrical stimulation

estim <- master_data %>%
  select(group,
         id,
         time,
         phase,
         site,
         modality,
         rating) %>%
  filter(modality == 'VFF') %>%
  filter(phase != 'orientation') %>%
  mutate(time = case_when(
    phase == 'baseline' ~ time*-1,
    phase != 'baseline' ~ time
  ))

estim %<>% mutate(condition = case_when(
  group == '1' & site == 'right' ~ 'A',
  group == '1' & site == 'left' ~ 'B',
  group == '2' & site == 'right' ~ 'B',
  group == '2' & site == 'left' ~ 'A')) %>%
  select(-group,
         -site)
estim$rating <- as.numeric(estim$rating)

estim_bl <- estim %>% filter(phase == 'baseline') %>%
  group_by(id, condition) %>%
  summarise(rating = mean(rating)) %>%
  ungroup() %>%
  mutate(time = 0) %>% ungroup()

estim_fu <- estim %>% filter(phase != 'baseline') %>% ungroup () %>%
  select(-phase,
         -modality)

estim <- rbind(estim_bl, estim_fu)

# For interest, plot the pilot data

ggplot(data = estim) +
  aes(x = time,
      y = rating,
      group = condition,
      colour = condition) +
  facet_wrap(~ id) +
  geom_point() +
```

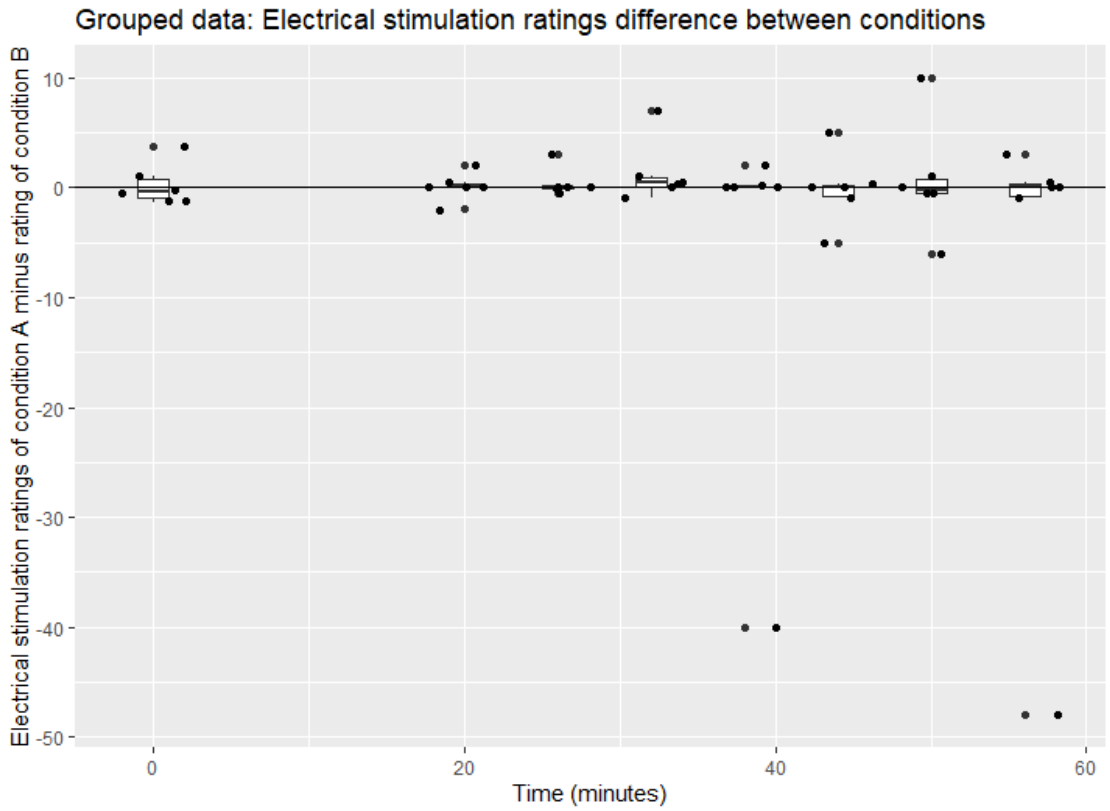
```
  geom_line() +
  labs(title = 'Individual electrical stimulation ratings by condition, over time',
       y = 'Rating',
       x = 'Time (minutes)')
```




```
# All participants, group data represented by boxplots
ggplot(data = estim) +
  aes(x = time,
      y = rating,
      group = interaction(condition, time),
      colour = condition) +
  geom_boxplot(width = 2) +
  geom_point(position = 'jitter') +
  labs(title = 'Group data: Electrical stimulation ratings by condition, over time',
       y = 'Rating',
       x = 'Time (minutes)')
```




```
 # Calculate diff between conditions for group boxplots

estim_wide <- estim %>%
  group_by(id,
           time) %>%
  spread(key = condition,
         value = rating) %>%
  mutate(site_diff = A-B)

# All participants, group data represented by boxplots
ggplot(data = estim_wide) +
  aes(x = time,
      y = site_diff,
      group = time) +
  geom_boxplot(width = 2) +
  geom_point(position = 'jitter') +
  geom_hline(yintercept = 0) +
  labs(title = 'Grouped data: Electrical stimulation ratings difference between conditions'
,
       y = 'Electrical stimulation ratings of condition A minus rating of condition B',
       x = 'Time (minutes)')
```




```
# Collapse all post-baseline ratings for each condition and then compare them using a RM AN
OVA type model. Time is nested within (and fully crossed with) ID.

estim_bl %<>% select(-time)
names(estim_bl)[names(estim_bl) == 'rating'] <- 'baseline_rating'
collapse_estim <- estim_fu %>% # resolve each rating relative to baseline
  right_join(estim_bl) %>%
  mutate(rating_controlled = rating-baseline_rating) %>%
  select(id,
         time,
         condition,
         rating_controlled) # Spread to calculate difference between conditions.

# We want to find out whether rating_cotrolled (for baseline) is predicted by condition.

model_null_estim <- lmer(rating_controlled ~ (1|id),
                  data = collapse_estim)
model_condition_estim <- lmer(rating_controlled ~ condition + (1|id),
                      data = collapse_estim)
anova(model_condition_estim, model_null_estim) # No improvement

## Data: collapse_estim
## Models:
## model_null_estim: rating_controlled ~ (1 | id)
## model_condition_estim: rating_controlled ~ condition + (1 | id)
##                       Df    AIC    BIC  logLik deviance  Chisq Chi Df
## model_null_estim       3 631.37 638.67 -312.69   625.37
## model_condition_estim  4 632.44 642.16 -312.22   624.44 0.9367      1
##                       Pr(>Chisq)
## model_null_estim
## model_condition_estim     0.3331

model_pre_condition_crossed_estim <- lmer(rating_controlled ~ condition + (1|id/time),
                                  data = collapse_estim)

anova(model_pre_condition_crossed_estim, model_null_estim)
```

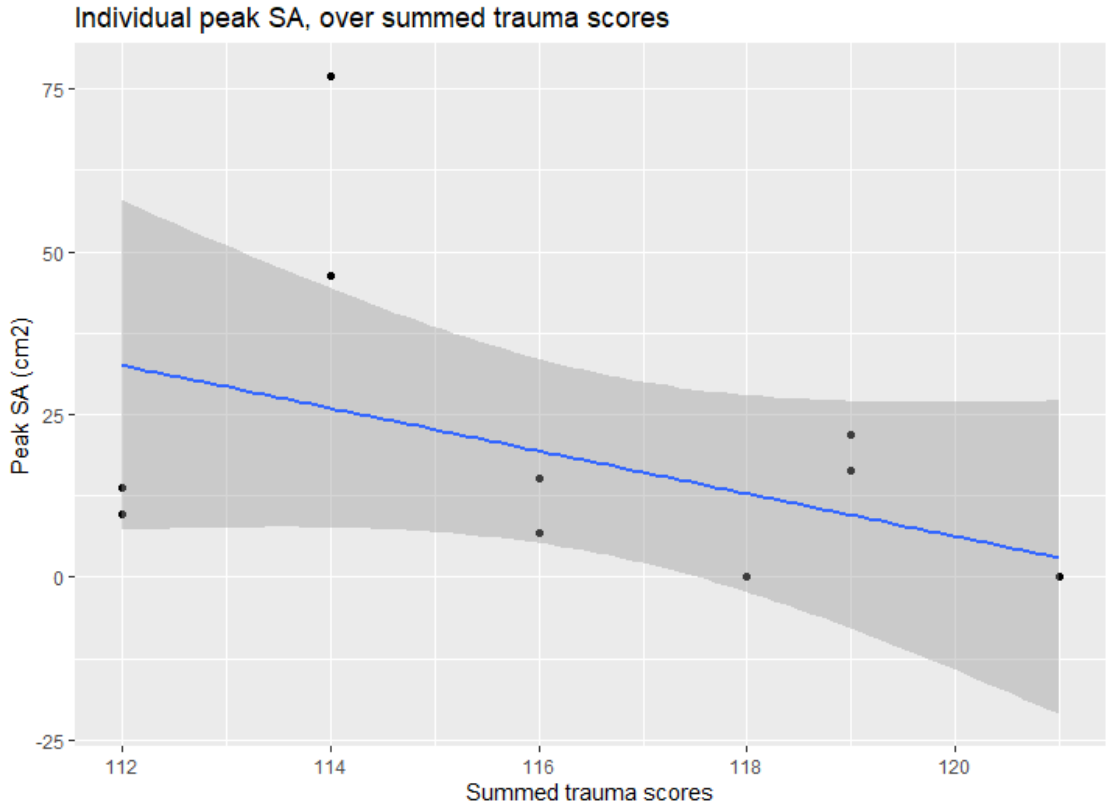
```
## Data: collapse_estim
## Models:
## model_null_estim: rating_controlled ~ (1 | id)
## model_pre_condition_crossed_estim: rating_controlled ~ condition + (1 | id/time)
##                                  Df    AIC    BIC  logLik deviance  Chisq
## model_null_estim                  3 631.37 638.67 -312.69   625.37
## model_pre_condition_crossed_estim  5 625.73 637.89 -307.87   615.73 9.6406
##                                  Chi Df Pr(>Chisq)
## model_null_estim
## model_pre_condition_crossed_estim      2   0.008064 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# Significant improvement... condition predicts Electrical stimulation ratings but only in
fully crossed model.
```

Childhood and recent trauma scores

We are interested in exploring the relationship between trauma history and area of SA.

```
cidi <- master_data[ , grep('cidi', colnames(master_data))]
cidi$cidi_total <- rowSums(cidi)
ctq <- master_data[ , grep('ctq', colnames(master_data))]
ctq$ctq_total <- rowSums(ctq)

trauma <- cbind(master_data$id, cidi, ctq)
names(trauma)[names(trauma) == 'master_data$id'] <- 'id'
trauma %<>% mutate(trauma = cidi_total + ctq_total) %>%
  select(id,
         trauma)
trauma <- unique(trauma)

SA_trauma <- SA %>% right_join(trauma)

# We need to identify the best time point from which to draw the SA data for each participa
nt. We will do this by identifying the time of peak SH intensity individually, and then use
the SA time point closest to that time.

# Considering that secondary hyperalgesia needs first to be verified before the surface are
a becomes a meaningful outcome, we first identified the time at which each participant show
ed the peak SH intensity (mean of both conditions). We then used that individualised time p
oint for each participant and identified the SA of SH at that time point, using that as the
peak (relevant) SA for that participant.

intensity_time <- intensity %>% group_by(id, time) %>%
  summarise(mean_int = mean(rating)) %>%
  filter(time != 0) %>%
  ungroup() %>%
  group_by(id) %>%
  filter(mean_int == max(mean_int))

# Now identify SA at those time point, using the df 'SA_trauma'. Adjust values to closest a
pplicable SA assessment times - i.e. 20 or 26 (int) to 20 mins, 32 or 38 or 44 to 40 mins,
50 or 56 to 60 mins.

SA_at_peak <- SA_trauma %>%
  filter(id == 'EOI1141' & time == 40 |
           id == 'EOI1142' & time == 60 |
           id == 'EOI1145' & time == 60 |
           id == 'EOI1148' & time == 20 |
           id == 'EOI1149' & time == 20 |
           id == 'EOI1151' & time == 60 )


names(SA_at_peak)[names(SA_at_peak) == 'SA'] <- 'peak_SA'
```

```r
ggplot(data = SA_at_peak) +
  aes(x = trauma,
      y = peak_SA) +
  geom_point() +
  geom_smooth(method = 'lm')+
  labs(title = 'Individual peak SA, over summed trauma scores',
       y = 'Peak SA (cm2)',
       x = 'Summed trauma scores')
```




```r
# First check distributional assumptions for correlation.
# Shapiro-Wilk normality test for peak surface area
with(SA_at_peak, shapiro.test(peak_SA)) # p = 0.03 therefore not normally distributed.

##
##  Shapiro-Wilk normality test
##
## data:  peak_SA
## W = 0.75336, p-value = 0.002898

# Spearman rank-order correlation

correlation <- cor.test(SA_at_peak$trauma, SA_at_peak$peak_SA, method= "spearman")
correlation

##
##  Spearman's rank correlation rho
##
## data:  SA_at_peak$trauma and SA_at_peak$peak_SA
## S = 409.46, p-value = 0.1611
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##       rho
## -0.4316658
```

Session information

```
sessionInfo()
```

```
## R version 3.6.1 (2019-07-05)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 17763)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_South Africa.1252
## [2] LC_CTYPE=English_South Africa.1252
## [3] LC_MONETARY=English_South Africa.1252
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_South Africa.1252
##
## attached base packages:
## [1] grid      stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] REdaS_0.9.3     gridExtra_2.3   lme4_1.1-21     Matrix_1.2-17
##  [5] readxl_1.3.1    magrittr_1.5    forcats_0.4.0   stringr_1.4.0
##  [9] dplyr_0.8.3     purrr_0.3.3     readr_1.3.1     tidyr_1.0.0
## [13] tibble_2.1.3    ggplot2_3.2.1   tidyverse_1.2.1
##
## loaded via a namespace (and not attached):
##  [1] tidyselect_0.2.5 xfun_0.10        reshape2_1.4.3   splines_3.6.1
##  [5] haven_2.1.1      lattice_0.20-38  colorspace_1.4-1 vctrs_0.2.0
##  [9] generics_0.0.2   htmltools_0.4.0  yaml_2.2.0       rlang_0.4.1
## [13] nloptr_1.2.1     pillar_1.4.2     glue_1.3.1       withr_2.1.2
## [17] modelr_0.1.5     plyr_1.8.4       lifecycle_0.1.0  munsell_0.5.0
## [21] gtable_0.3.0     cellranger_1.1.0 rvest_0.3.4      evaluate_0.14
## [25] labeling_0.3     knitr_1.25       broom_0.5.2      Rcpp_1.0.2
## [29] scales_1.0.0     backports_1.1.5  jsonlite_1.6     hms_0.5.2
## [33] digest_0.6.22    stringi_1.4.3    cli_1.1.0        tools_3.6.1
## [37] lazyeval_0.2.2   crayon_1.3.4     pkgconfig_2.0.3  zeallot_0.1.0
## [41] ellipsis_0.3.0   MASS_7.3-51.4    xml2_1.2.2       lubridate_1.7.4
## [45] minqa_1.2.4      assertthat_0.2.1 rmarkdown_1.16   httr_1.4.1
## [49] rstudioapi_0.10  boot_1.3-22      R6_2.4.0         nlme_3.1-140
## [53] compiler_3.6.1
```

Appendix 29: Study protocol preregistered with Open Science Framework

The effect of stimulus threat on experimentally induced secondary hyperalgesia: an experimental protocol

Authors and contributions

Gillian J Bedwell refined the study design, contributed to the pilot data analysis, led the writing of the protocol, and will lead the execution of the study.

Romy Parker contributed to study design and the writing of the protocol.

Emanuel van den Broeke contributed to the study design and approved the protocol.

Johan WS Vlaeyen conceived the study, contributed to the study design and approved the protocol.

G Lorimer Moseley conceived the study, contributed to the study design and approved the protocol.

Victoria J Madden conceived the study, refined the study design, conducted the sample size estimation, prepared the pilot data analysis, contributed to the writing of the protocol and will supervise the execution of the study.

Author affiliations

GJB: Department of Health and Rehabilitation Sciences, University of Cape Town, Cape Town, South Africa. Pain Unit, Department of Anaesthesia and Perioperative Medicine, University of Cape Town, Groote Schuur Hospital, Cape Town, South Africa.

RP: Pain Unit, Department of Anaesthesia and Perioperative Medicine, Neuroscience Institute, University of Cape Town, Groote Schuur Hospital, Observatory, Cape Town, South Africa.

EVDB: UC Louvain, Institute of Neuroscience, Division Cognitive and Systems, Avenue Mounier 53, B-1200, Brussels, Belgium.

JWSV: Research Group Health Psychology, KU Leuven – University of Leuven, Tiensestraat 102, 3000, Leuven, Belgium; Experimental Health Psychology, Maastricht University, Universiteitssingel 40, 6229 ER, Maastricht, the Netherlands

GLM: University of South Australia, Adelaide, SA, Australia; Neuroscience Research Australia, Sydney, NSW, Australia

VJM: Pain Unit, Department of Anaesthesia and Perioperative Medicine and Department of Psychiatry and Mental Health, Neuroscience Institute, University of Cape Town, Groote Schuur Hospital, Observatory, Cape Town 7925, South Africa.

Correspondence

torymadden@gmail.com

Funding

Funding for this study was provided by PainSA, the South African Physiotherapy Society and the University of Cape Town. GJB is supported by Postgraduate Research Grants from PainSA and the South African Physiotherapy Society and Merit Scholarships from the University of Cape Town. GLM was supported by a Principal Research Fellowship from the National Health and Medical Research Council of Australia. VJM was supported by an Innovation postdoctoral fellowship from the National Research Foundation of South Africa. Research collaboration supported by the IASP Developing Countries Collaborative Research Grant. JWSV is supported by the Asthenes research program "From acute aversive sensations to chronic bodily symptoms", a long term structural Methusalem funding (METH/15/011) by the Flemish government, Belgium.

Conflicts of interest

In the last 5 years, GLM has received support from: Seqirus, Kaiser Permanente, Workers' Compensation Boards in Australia, Europe and North America, AIA Australia, the International Olympic Committee, Port Adelaide Football Club and Arsenal Football Club. Professional and scientific bodies have reimbursed him for travel costs related to the presentation of research on pain at scientific conferences/symposia. He has received speaker fees for lectures on pain and rehabilitation. He receives book royalties from NOI group publications (Adelaide, Australia), Dancing Giraffe Press (Canberra, Australia) & OPTP (Minneapolis, Minnesota, USA). VJM receives speakers' fees for lectures on pain and rehabilitation. The authors declare that there are no other conflicts of interest.

Protocol 1.0, posted 08 January 2020

Background

Chronic pain is poorly understood, poorly treated and burdensome worldwide. In the American 2012 National Health Interview Survey, just over half (55,7%) of the adult population reported having had pain within the three months before the survey. Of these, 25,5 million (11,2%) participants reported having had "pain every day for the past three months" (Nahin, 2015). A large-scale survey investigating the prevalence and severity of chronic pain in 15 European countries and Israel found that of the 46,394 participants, 19% reported having had moderate or severe pain for at least the preceding six months (Breivik et al., 2006). The socio-economic burden of chronic pain in Sweden costs the healthcare sector 32 billion Euros per year (Gustavsson et al., 2012). Back and neck pain is the second greatest cause of years lived with disability in Southern Sub-Saharan Africa, second only to HIV (Institute for Health Metrics and Evaluation, 2017). The magnitude of pain problems may be due to a limited understanding of pain such that optimising clinical treatment is difficult (Breivik et al., 2013, Briggs et al., 2015, Madden and Moseley, 2016). Treatment strategies ought to be targeted to specific mechanisms of chronic pain (Woolf and Mannion, 1999). A thorough understanding of physiological mechanisms of chronic pain would allow development of improved treatment modalities.

Introduction

Chronic pain can be partially attributed to structural and functional changes in the central and peripheral nervous systems, referred to as central and peripheral sensitisation, respectively (Sandkuhler and Gruber-Schoffnegger, 2012, van Wilgen and Keizer, 2012). Some of these changes cause the nervous system to have an increased responsiveness to input - specifically, within the neuronal synapses of the dorsal horn of the spinal cord (Sandkuhler and Gruber-Schoffnegger, 2012). This enhanced synaptic activity is referred to as spinal long-term potentiation (LTP). Spinal LTP underlies the amplification and prolongation of noxious afferent stimuli (Nickel et al., 2012). As such, it is an important physiological mechanism underlying central sensitisation and increased pain sensitivity.

Spinal LTP can underlie primary hyperalgesia in the absence of peripheral nociceptor sensitisation, and some forms of secondary hyperalgesia - common clinical findings in patients with neuropathic pain (Jensen and Finnerup, 2014). Spinal LTP can be assessed using multiple outcome measures. This study will focus on three specific indicators of spinal LTP: allodynia, primary hyperalgesia, and secondary hyperalgesia. Allodynia is increased sensitivity to a stimulus that is normally non-painful (IASP, 2017). Hyperalgesia is formally defined as "increased pain from a stimulus that normally provokes pain" (IASP, 2017) within the area of tissue damage (primary hyperalgesia) and in the area surrounding tissue damage (secondary hyperalgesia).

There are many methods of experimentally inducing secondary hyperalgesia, including repeated thermal stimulation (Salomons et al., 2014), high-frequency electrical stimulation (Henrich et al., 2015), low-frequency electrical stimulation (Klein et al., 2004), and intradermal or topical application of capsaicin (Serra et al., 2004). Recent research has shown that secondary hyperalgesia can be manipulated with pharmacological and non-pharmacological modalities (Andersen et al., 1996, van den Broeke et al., 2014, Ditre et al., 2018). Cognitive behavioural therapy, aimed at reducing negative emotions and thoughts related to pain, has been shown to reduce thermally induced secondary hyperalgesia (Salomons et al., 2014), but the mechanisms of this effect are unclear. The effect could be attributed to an alteration of the threat value of the stimulation, or to other features of the cognitive behavioural therapy intervention.

Pain is known to be influenced by the threat value of a stimulus (Arntz and Claassens, 2004, Wiech et al., 2010). Instructions signifying that a stimulus will be of high intensity appear to increase reported pain intensities of the stimulus, despite the intensity of the stimulus being unchanged (Moseley and Arntz, 2007). The meaning behind a stimulus - for example, the degree to which a person feels threatened that the stimulus can cause tissue damage - is subjective (Arntz and Claassens, 2004). Personal and cultural beliefs, previous experiences, and expectations all play a role in determining the threat value of a stimulus. However, the physiological mechanisms by which threat influences pain are unclear. Specifically, it is not known if threat influences the extent to which synaptic activity is enhanced after sustained or intense neural signalling (spinal LTP). Therefore, the aim of this study is to investigate the effect of the threat value of a stimulus on experimentally induced secondary hyperalgesia, using high-frequency electrical stimulation (HFS) in healthy human volunteers.

The objectives of the study are:

a) to differentially manipulate the threat associated with high-frequency electrical stimulation at two different sites within each participant;
b) to assess and compare (1) intensity ratings (primary outcome) and (2) surface area (secondary outcome) of experimentally induced secondary hyperalgesia (as assessed using mechanical punctate stimulation) between the sites, and
c) to assess and compare intensity ratings given for (exploratory outcomes) static light touch, dynamic light touch, and a single electrical stimulation, before and after the experimental induction of secondary hyperalgesia, between the sites.

Ratings will be provided using the Sensation and Pain Rating Scale (SPARS) (Madden et al., 2016, Madden et al., 2019b, Madden et al., 2019a).

We hypothesise that greater threat will be associated with (hypothesis 1) greater intensity and (hypothesis 2) greater surface area of induced secondary hyperalgesia. Data obtained from assessing static light touch, dynamic light touch and single electrical stimulation will be used for exploratory purposes only.

Methods

Study design

The study is designed as a within-subject, double-blinded, experimental study. It will be conducted at the University of Cape Town, South Africa. The protocol has been approved by Faculty of Health Sciences Human Research Ethics Committee (REF 498/2019), University of Cape Town.

Recruitment

Volunteers will be recruited from the general public using flyers, advertisements, social media channels, such as Facebook and Twitter, and word of mouth. They will be provided with a study information sheet outlining the details of the procedure and will be screened for exclusion criteria by completing an online eligibility quiz using the Responster platform. After completing the screening quiz, eligible volunteers will be contacted via email to organise a booking slot. Volunteers not eligible for this study will be thanked for their interest and given the opportunity to have their details added to our database for notifications about future studies. Participants may withdraw from the study at any stage during the procedure or up to 48 hours after the procedure. They will be compensated ZAR100, in cash, for their time and inconvenience, even if they withdraw from the study.

Inclusion and exclusion criteria

Volunteers must be healthy, pain-free adults between the ages of 18 – 65 (to account for the possible presence of age-related peripheral loss of sensation), to be able to consent autonomously, and be fluent in speaking, understanding and reading English (all as per volunteers' self-reports).

Volunteers will be excluded from the study if they report one or more of the following: chronic pain – pain for most days for the past three months (Blyth et al., 2001), pain on the day of testing, self-reported pregnancy, electronic implant (e.g. pacemaker), any kind of heart/cardiovascular problem, diabetes mellitus, neurological problems (e.g. epilepsy), peripheral vascular disease, problems with skin healing, use of analgesics within 24 hours before testing, use of medication that could alter skin sensitivity or healing (analgesic medication, topical medical creams or immune modulators, for example), history of psychiatric problems (fear or anxiety disorder, or clinical depression, for example), and previous participation in this study or a closely related study. Additionally, volunteers with upper limb tattoos distal to the anode will be ineligible to participate as some tattoo inks contain metals. Tattoo ink containing iron oxide (usually within black pigmented tattoos) theoretically can conduct an electrical current (Ross and Matava, 2011). Therefore, applying HFS

near these tattoos could cause minor skin burns. Due to difficulty identifying the composition of tattoo ink, all volunteers with tattoos at or distal to the intended site of the anode will be excluded from participating in this study.

Randomisation and blinding

Participants will receive the HFS on both forearms separately. One arm will receive the HFS under a condition of threat (experimental) and the other arm will not (control), thus providing a within-subject comparison. Site allocation for the threat manipulation will be counterbalanced across participants.

Participants will be blinded to the study goals: they will be informed that the researchers are investigating how people experience painful and non-painful stimulations. The researcher undertaking the measurements will be blinded to site allocation, i.e. which arm (right or left) received the HFS under a condition of threat, thus mitigating verification bias. The researcher will perform a blinding assessment after participants receive the HFS and before the sensory testing battery. The blinding assessment will consist of two questions: first, they will be asked which arm they think received the HFS under a condition of threat. If they are uncertain, they will be instructed to guess. Second, they will rate their confidence in their answer to the first question on five-point Likert scale consisting of “not at all confident”, “not confident”, “neutral”, “confident”, “extremely confident”.

The effect of the threat manipulation on the outcomes will be deduced on the basis of a between-site comparison of the change in sensation or pain (before to after HFS) to punctate mechanical stimulation around the site of tissue stimulation (intensity of secondary hyperalgesia; primary outcome) and the surface area affected by secondary hyperalgesia (secondary outcome).

Equipment

High-frequency electrical stimulation will be provided using a constant current stimulator (DS7A, Digitimer Limited, Hertfordshire, UK). The electrical impulses will be controlled by the software programme Affect5 (Spruyt, 2010). Current will be directed from the DS7A to a pair of electrodes. The electrodes consist of two cathodes and two anodes. The cathodes have 10 blunt steel pins arranged in a circle and will be secured to both anterior forearms. The anodes are large surface electrodes, which will be secured to both upper arms.

Manipulated variables

High-frequency electrical stimulation

The cathodes will be secured on the anterior aspect of both the participant's forearms, with a double-sided sticker, approximately eight centimetres distal to the cubital fossa, and avoiding any visibly prominent vasculature. Large surface electrodes will be placed around both upper arms and will serve as the anodes.

The appropriate intensity of the HFS depends on the electrode used and individual's electrical detection threshold. Our electrodes most closely resemble those used by Klein et al. (2004), Klein et al. (2008) and Henrich et al. (2015). Their work and pilot work done by this current study's authors has shown robust secondary hyperalgesia with HFS delivered at 100Hz, at a current of ten times the individual detection threshold.

Participants will be orientated to the electrical stimulus and the stimulus will be calibrated to the participant's individual electrical detection threshold. This calibration will consist of single electrical stimuli, with a pulse width of 2ms. We will use an adaptive staircase approach to determine the individual electrical detection threshold. The electrical detection threshold will be used to determine the intensity of the HFS: ten times the electrical detection threshold. Klein et al. (2004) reported participants' electrical detection threshold to be 0.11 ± 0.06mA (mean ± SD). Therefore, it is anticipated that the range of currents will be similar in this study.

Threat manipulation

The threat manipulation procedure is similar to that used by Wiech et al. (2010) and will consist of a sham skin examination. The sham skin examination will be conducted after the baseline sensory assessment and before participants receive the HFS. The researcher will inform the participants that they are examining the robustness of the skin around the electrodes, while using an otoscope to shine light onto the skin and magnify the view of the area. The purpose of the sham skin examination will be framed as to determine the risk of skin damage associated with that site undergoing HFS. The (sham) results will be shown on a computer screen not visible to the researcher. For each participant, the screen will report a judgement of "approved with reservations", with participants instructed to closely monitor their "fragile" skin during the HFS as there is "moderate risk of injury" for the experimental site, and "fully approved", with participants informed that the skin is "robust" and there is "low risk of injury" for the control site.

Measured variables

Participants will verbally report sensation or pain ratings using the SPARS (Madden et al., 2016, Madden et al., 2019b, Madden et al., 2019a). This scale is useful as it provides for a range of non-painful and painful sensory experiences. The non-painful range, on the left-hand side of the scale, ranges from -50 – "no sensation" – to 0 – "the exact point at which what you feel transitions to pain". The SPARS is sensitive to change in both painful and non-painful sensory experiences (Madden et al., 2019a).

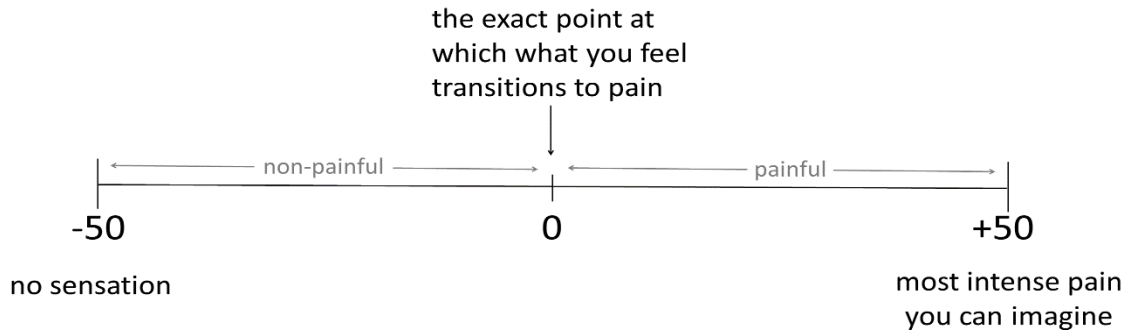


Figure 21: Sensation and Pain Rating Scale (SPARS)

Primary outcome

Mechanical punctate stimulation

Mechanical punctate stimulation will be provided with two pinprick stimulators (MRS Systems, Heidelberg, Germany), exerting forces of 128mN and 256mN. Increased ratings to these modalities in the region surrounding the distal electrode, after the HFS, will indicate the presence of secondary hyperalgesia. The mean ratings of the two different pinprick weights will be used to determine the overall mechanical punctate stimulation rating.

Secondary outcome

Mapping surface area of secondary hyperalgesia

Using the eight-radial-lines approach, the area of secondary hyperalgesia will be mapped with the use of a pinprick stimulator exerting a force of 128mN (You et al., 2014).

Exploratory outcomes

Static light touch

Static light touch sensation will be assessed with application of a von Frey filament (MARSTOCK, Schriesheim, Germany) that exerts a force of 32mN upon bending (Rolke et al., 2006).

Dynamic light touch

Dynamic light touch will be measured using a cotton wisp and soft brush stroke. Henrich et al. (2015) reported increased non-painful dynamic light touch sensation using cotton wool and a soft brush stroke one to two centimetres over the skin surrounding the area where the stimulus was provided.

Participants were asked to report pain (with the instruction that any sensation felt as pricking or burning should be reported as painful) on a numerical rating scale, where "0 = no pain and 100 = most intense pain imaginable". We will adapt this approach slightly by asking participants to report their sensation or pain ratings using the SPARS. Dynamic light touch stimulations are typically perceived as non-painful in unsensitised areas as they only activate low-threshold mechanoreceptors (Leem et al., 1993). Therefore, allodynia will be deemed present if participants indicate pain to dynamic light touch after HFS.

Single electrical stimulation

A single electrical stimulation will be used to assess primary hyperalgesia (homotopic spinal LTP). The electrical stimulus will be two milliseconds long with an intensity of ten times the individual's electrical detection threshold (Henrich et al., 2015).

Questionnaires

Trauma has been associated with increased area of secondary hyperalgesia (You et al., 2016). Women reporting childhood trauma and/or recent trauma displayed a larger surface area of secondary hyperalgesia after application of topical capsaicin than women without a history of trauma (You et al., 2016). Therefore, data from the Childhood Trauma Questionnaire short form (CTQ-SF) and a modified version of the World Mental Health Survey Initiative version of the World Health Organisation Composite International Diagnostic Interview for post-traumatic stress disorder (WMH-CIDI) will be used in a secondary analysis, to investigate the relationship between trauma history and experimentally induced secondary hyperalgesia, in an attempt to replicate the work by You et al. (2016). Participants will also complete several other questionnaires: 10-item Connor-Davidson Resilience Scale, Cohen's Perceived Stress Scale, Pain Catastrophising Scale, Multidimensional Scale of Perceived Social Support, and 16-item Pain Vigilance and Awareness Questionnaire, which will be used for exploratory analyses to inform the development of future research questions. The full list of questionnaires includes:

9. Basic demographic and participant information questionnaire, which will include the following items: participant ID code, group allocation, age, sex, ethnicity, medication use, caffeine intake, hand dominance, detection threshold on each arm, and time and day of procedure.
10. Childhood trauma and adult trauma will be screened for using the Childhood Trauma Questionnaire (CTQ-SF) (Bernstein et al., 2003) and a modified version of the World Mental Health Survey Initiative version of the World Health Organisation Composite International Diagnostic Interview for post-traumatic stress disorder (WMH-CIDI)

(Kessler et al., 2004), respectively. The CTQ-SF focuses on 5 criteria: emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect.

11. The modified version of the WMH-CIDI has questions drawn directly from the WMH-CIDI (Kessler et al., 2004). The modified version aims to determine whether participants have been through specific traumatic events. It will be used as an inventory and the researchers will not investigate the details of the traumatic event.
12. A customised questionnaire will be used to check the threat manipulation and provide qualitative data on participants' self-report of their experience of the HFS procedure. After the procedure, participants will be asked to indicate on a five-point Likert scale the extent to which they agree or disagree with the following statements: "At the time of receiving the intense electrical stimulation, I was concerned that it would cause damage to my skin on my right/left arm" and "At the time of receiving the intense electrical stimulation on my right/left arm, I felt anxious". Participants will complete these questions with reference to each arm separately.
13. Resilience will be assessed using the 10-item Connor-Davidson Resilience Scale (CD-RISC-10) (Campbell-Sills and Stein, 2007), which is a validated, shortened version of the 25-item Connor-Davidson Resilience Scale (CD-RISC).
14. Stress will be assessed using Cohen's Perceived Stress Scale (PSS) (Lee, 2012, Cohen, 1988).
15. Pain catastrophising is associated with increased pain sensitivity (Sullivan et al., 2005). Therefore, we plan to use the Pain Catastrophising Scale (PCS) – a valid and reliable outcome measure – to assess participants' catastrophic thoughts about pain (Sullivan et al., 1995). The instructions and text of this scale will be modified to prompt participants to respond with reference to their thoughts during the HFS.
16. Pain can be influenced by perceived social support. Social support has been shown to decrease pain intensity (López-Martínez et al., 2008). Therefore, we plan to assess participants' perceived social support using the Multidimensional Scale of Perceived Social Support (MSPSS) (Zimet et al., 1988). The MSPSS is a 12-item scale on which participants respond to certain statements about the support they receive from family, friends and a significant other using a 7-point Likert-type scale ranging from "very strongly disagree" to "very strongly agree". It has been shown to be valid with a South African cohort (Bruwer et al., 2008).
17. Attention to pain and changes in pain will be assessed using the 16-item Pain Vigilance and Awareness Questionnaire (PVAQ) (McCracken, 1997). The PVAQ is a valid

questionnaire for assessing awareness, intrusion and monitoring of pain in non-clinical samples (McWilliams and Asmundson, 2001).

These questionnaires do not require expert training to administer. They will all be administered via a computer. To ensure confidentiality, the researcher will not have viewing access to the relevant computer screen while the participant completes the questionnaires.

Overview of procedure

Participants will be orientated to the sensory testing battery and baseline measurements will be taken. The researcher will perform the sham skin examination and participants will receive the results of their skin rating on each arm separately. Thereafter, participants will receive the HFS on each arm separately. Participants will then have 20 minutes to complete the questionnaires before the researcher conducts the sensory testing battery again at 20, 26, 32, 38, 40, 44, 50, 56, and 60 minutes after participants receive the HFS.

Preparation

Before the experimental procedure, participants will be asked to re-read the study information sheet, confirm that none of the exclusion criteria applies to them, and sign the document of informed consent. Thereafter, participants will be asked to remove any jewellery (rings, watch or bracelets) from their arms and to turn off mobile phones or smart watches so that they will not be distracted by any alerts. They will be clearly briefed about the HFS procedure.

The researcher will use a stencil to mark locations for the electrodes and eight surrounding radial lines, arranged at 45-degree angles to one another, on the participant's skin. The cathodes will be secured, with a double-sided sticker, approximately eight centimetres distal to the cubical fossa on the anterior aspect of both the participants' forearms, avoiding any visibly prominent vasculature. Large surface electrodes will be placed on both upper arms and will serve as the anodes.

Participants will be orientated to the SPARS and the sensory testing battery. They will have an opportunity to practice using the SPARS while the researcher runs through a practice round of the sensory testing battery.

Baseline testing

Once the participants are comfortable with using the SPARS, the sensory testing battery will be conducted six times (three times on each arm) to obtain baseline sensory ratings of static light touch, dynamic light touch and punctate mechanical stimuli. Primary hyperalgesia will not be assessed at this time point as the electrical stimulation will not yet be calibrated to the participant.

High-frequency electrical stimulation

Participants will be thoroughly briefed on what to expect from the HFS. Participants will be informed that most people find the HFS "moderately painful" and they may withdraw with immediate effect at any point during the procedure. They will be instructed to say "STOP" if they wish to withdraw, in which case the researcher will flick the safety switch on the stimulator to deactivate the stimulator immediately. A comparison of the HFS SPARS ratings will serve as a manipulation check. Additionally, participants will complete a post-experiment comparative sensory rating question for each arm separately.

Waiting period

There will be a waiting period of approximately 20 minutes to allow time for the secondary hyperalgesia to develop. To optimise time, this period will be used to administer the questionnaires.

Follow-up testing

The battery of sensory testing will be conducted at the following time points after HFS: 20 minutes, 26 minutes, 32 minutes, 38 minutes, 40 minutes, 44 minutes, 50 minutes and 56 minutes. Importantly, the order of the sensory testing modalities will be randomised within each time point, for each participant, to decrease predictability and ensure accurate ratings (with the same order used for both arms, within each time point). The surface area of secondary hyperalgesia will be mapped at 20-minute intervals after receiving the HFS, namely: at 20 minutes, 40 minutes and 60 minutes after the HFS.

After the procedure, the electrodes will be removed, and participants will be asked to complete the post-experiment questionnaire. Finally, participants will be debriefed on the threat manipulation and reassured about the safety of the procedure.

Participants will complete the questionnaires in privacy, and on a computer. Details of any traumatic events will not be requested. For these reasons, it is unlikely the questionnaires will evoke strong emotional responses at the time of testing. Nevertheless, after the procedure, participants will be provided with an information pamphlet outlining the local non-profit organisations where they can access psychological assistance, if they wish to do so. Additionally, all participants will receive a list of the community health care centres in Cape Town that provide psychological counselling as well as a list of a few private practice psychologists within the University of Cape Town's neighbouring communities.

Sample size calculation

Pilot data and the GLIMMPSE online calculator (Kreidler et al., 2013) were used to calculate the estimated sample size required to achieve 80% power to detect a minimum 5-point difference in secondary hyperalgesia with alpha set at 0.05. We planned for a mixed linear regression in which the dependent variable was the mean rating to both pinprick stimulators (128 and 256mN) at each time point after HFS, minus the equivalent mean rating at the baseline time point (before HFS). The model structure allowed each participant to have their own intercept (i.e. individual participant (ID) was a random factor). The independent variable 'condition' (i.e. experimental or control site) was a fixed factor, and the repeated measures variable 'time' was nested within ID, because each participant was assessed at each time point. In the lme4 (Loy and Hofmann, 2014, Bates et al., 2015) of R (version 3.5.3 (2019-03-11)), the model structure would be: lmer(rating ~ condition + (1|ID/time).

We were interested in detecting a main effect of condition. GLIMMPSE estimated that we would require a sample size of 25 participants. Therefore, we opted for a sample size of 26, to allow for counterbalancing for the manipulation site.

Statistical analysis

Primary analysis

Data will be analysed using a linear mixed modelling approach, so as to account for individual variability in responses whilst still testing for a between-site effect at the group level. The study is designed to have within-subject controls of both pre- and post-induction measurements and control site measurements. Therefore, the change in sensitivity (pre-induction measurements subtracted from post-induction measurements) will be compared between arms (within subjects). The exact parameters for the analysis will be chosen on the basis of visual inspection of the data (including an assessment of distribution), and the appropriate tests to confirm or refute any assumptions of the analytical strategy. As specified above, the primary outcome will be the magnitude of secondary hyperalgesia.

It is plausible that the individual calibration approach could confound the results, because HFS delivered at a higher current could result in greater secondary hyperalgesia. We will test for this in our analysis although a previous, well powered (n = 170, unpublished) investigation of this relationship found none.

The planned data analysis using the full pilot study data (Appendix 1) has been finalised. A mixed linear modelling approach, likely using the 'lmer' option within the lme4 package (Loy and Hofmann, 2014, Bates et al., 2015) that allows for both random effects (including participant) and fixed effects (including threat condition) as used in our sample size calculation, will be used for the formal data

analysis. Initial processing of the formal data has commenced at the time of this protocol being locked online. However, the analysis of the formal data has not been completed. The pilot analysis was not substantively changed after initial processing of the formal data commenced.

Secondary analysis

A secondary analysis will be conducted to investigate the relationship between trauma history and experimentally induced secondary hyperalgesia, replicating the work by You et al. (2016). In their study, they summed the results of participants' individual scores from the Childhood Trauma Events Scale and the Recent Traumatic Events Scale to obtain an individual stressful life events score. Similarly, the plan for this current study is to sum the results of the CTQ-SF and WMH-CIDI. You et al. (2016) reported a larger surface area of capsaicin-induced secondary hyperalgesia in participants with a history of trauma than participants without a history of trauma. However, there were no differences in the intensity of secondary hyperalgesia between those with and those without a history of trauma. In this current study, a univariate regression analysis will be conducted to examine whether stressful life events correlate with the area of secondary hyperalgesia in this sample.

Distribution of findings

The plan for this current study is to write the results of the study into a manuscript, post the manuscript as a pre-print for initial feedback, and then submit it for publication in a peer-reviewed journal. The manuscript will include a link to this protocol.

References

ANDERSEN, O. K., FELSBY, S., NICOLAISEN, L., BJERRING, P., JENSEN, T. S. & ARENDT-NIELSEN, L. 1996. The effect of Ketamine on stimulation of primary and secondary hyperalgesic areas induced by capsaicin--a double-blind, placebo-controlled, human experimental study. *Pain,* 66**,** 51-62.

ARNTZ, A. & CLAASSENS, L. 2004. The meaning of pain influences its experienced intensity. *Pain,* 109**,** 20-5.

BATES, D., MAECHLER, M., BOLKER, B. & WALKER, S. 2015. Ime4: Linear mixedeffects models using Eigen and S4. R package version 1.1–9. Available a thttps. *CRAN. R-project. org/package= lme4*.

BERNSTEIN, D. P., STEIN, J. A., NEWCOMB, M. D., WALKER, E., POGGE, D., AHLUVALIA, T., STOKES, J., HANDELSMAN, L., MEDRANO, M., DESMOND, D. & ZULE, W. 2003. Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse & Neglect,* 27**,** 169-190.

BLYTH, F. M., MARCH, L. M., BRNABIC, A. J., JORM, L. R., WILLIAMSON, M. & COUSINS, M. J. 2001. Chronic pain in Australia: a prevalence study. *Pain,* 89**,** 127-134.

BREIVIK, H., COLLETT, B., VENTAFRIDDA, V., COHEN, R. & GALLACHER, D. 2006. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain,* 10**,** 287-333.

BREIVIK, H., EISENBERG, E. & O'BRIEN, T. 2013. The individual and societal burden of chronic pain in Europe: the case for strategic prioritisation and action to improve knowledge and availability of appropriate care. *BMC Public Health,* 13**,** 1229.

BRIGGS, E. V., BATTELLI, D., GORDON, D., KOPF, A., RIBEIRO, S., PUIG, M. M. & KRESS, H. G. 2015. Current pain education within undergraduate medical studies across Europe: Advancing the Provision of Pain Education and Learning (APPEAL) study. *BMJ open,* 5**,** e006984.

CAMPBELL-SILLS, L. & STEIN, M. B. 2007. Psychometric analysis and refinement of the Connor-davidson Resilience Scale (CD-RISC): Validation of a 10-item measure of resilience. *Journal Of Traumatic Stress,* 20**,** 1019-1028.

COHEN, S. 1988. Perceived stress in a probability sample of the United States.

DITRE, J. W., ZALE, E. L., LAROWE, L. R., KOSIBA, J. D. & DE VITA, M. J. 2018. Nicotine deprivation increases pain intensity, neurogenic inflammation, and mechanical hyperalgesia among daily tobacco smokers. *Journal of abnormal psychology,* 127**,** 578.

GUSTAVSSON, A., BJORKMAN, J., LJUNGCRANTZ, C., RHODIN, A., RIVANO-FISCHER, M., SJOLUND, K. F. & MANNHEIMER, C. 2012. Socio-economic burden of patients with a diagnosis related to chronic pain--register data of 840,000 Swedish patients. *Eur J Pain,* 16**,** 289-99.

HENRICH, F., MAGERL, W., KLEIN, T., GREFFRATH, W. & TREEDE, R. D. 2015. Capsaicin-sensitive C- and A-fibre nociceptors control long-term potentiation-like pain amplification in humans. *Brain,* 138**,** 2505-20.

IASP 2017. Classification of Chronic Pain. *In:* BOGDUK, H. M. A. N. (ed.) *Part III: Pain Terms, A Current List with Definitions and Notes on Usage.* Second Edition ed. Seattle: IASP Press.

INSTITUTE FOR HEALTH METRICS AND EVALUATION. 2017. *GBD Compare Data Visualization* [Online]. Available: http://vizhub.healthdata.org/gbd-compare [Accessed 01 June 2018].

JENSEN, T. S. & FINNERUP, N. B. 2014. Allodynia and hyperalgesia in neuropathic pain: clinical manifestations and mechanisms. *The Lancet Neurology,* 13**,** 924-935.

KESSLER, R. C., ÜSTUUML & N, T. B. 2004. The World Mental Health (WMH) Survey Initiative Version of the World Health Organization (WHO) Composite. International Diagnostic Interview (CIDI). *International Journal of Methods in Psychiatric Research,* 13**,** 93-121.

KLEIN, T., MAGERL, W., HOPF, H. C., SANDKUHLER, J. & TREEDE, R. D. 2004. Perceptual correlates of nociceptive long-term potentiation and long-term depression in humans. *J Neurosci,* 24**,** 964-71.

KLEIN, T., STAHN, S., MAGERL, W. & TREEDE, R. D. 2008. The role of heterosynaptic facilitation in long-term potentiation (LTP) of human pain sensation. *Pain,* 139**,** 507-519.

KREIDLER, S. M., MULLER, K. E., GRUNWALD, G. K., RINGHAM, B. M., COKER-DUKOWITZ, Z. T., SAKHADEO, U. R., BARÓN, A. E. & GLUECK, D. H. 2013. GLIMMPSE: online power computation for linear models with and without a baseline covariate. *Journal of statistical software,* 54.

LEE, E.-H. 2012. Review of the Psychometric Evidence of the Perceived Stress Scale. *Asian Nursing Research,* 6**,** 121-127.

LEEM, J. W., WILLIS, W. D., WELLER, S. C. & CHUNG, J. M. 1993. Differential activation and classification of cutaneous afferents in the rat. *Journal of Neurophysiology,* 70**,** 2411 - 2424.

LOY, A. & HOFMANN, H. 2014. HLMdiag: A suite of diagnostics for hierarchical linear models in R. *Journal of Statistical Software,* 56**,** 1-28.

MADDEN, V., CATLEY, M. J., BELLAN, V., RUSSEK, L. N., CAMFFERMANN, D. & MOSELEY, G. L. 2016. The FESTNRS- a new numerical rating scale to assess non-painful and painful experiences in humans [Poster]. Unpublished.

MADDEN, V., KAMERMAN PR, BELLAN V, CATLEY MJ, RUSSEK LN, CAMFFERMAN D & GL., M. 2019a. Was That Painful or Nonpainful? The Sensation and Pain Rating Scale Performs Well in the Experimental Context. *The Journal of Pain (in press)*.

MADDEN, V., KAMERMAN PR, CATLEY MJ, BELLAN V, RUSSEK LN, CAMFFERMAN D & L., M. 2019b. Rethinking pain threshold as a zone of uncertainty. *bioRxiv,* 521302.

MADDEN, V. J. & MOSELEY, G. L. 2016. Do clinicians think that pain can be a classically conditioned response to a non-noxious stimulus? *Manual therapy,* 22**,** 165-173.

NICKEL, F. T., SEIFERT, F., LANZ, S. & MAIHOFNER, C. 2012. Mechanisms of neuropathic pain. *Eur Neuropsychopharmacol,* 22**,** 81-91.

ROLKE, R., BARON, R., MAIER, C., TÖLLE, T. R., TREEDE, R. D., BEYER, A., BINDER, A., BIRBAUMER, N., BIRKLEIN, F., BÖTEFÜR, I. C., BRAUNE, S., FLOR, H., HUGE, V., KLUG, R., LANDWEHRMEYER, G. B., MAGERL, W., MAIHÖFNER, C., ROLKO, C., SCHAUB, C., SCHERENS, A., SPRENGER, T., VALET, M. & WASSERKA, B. 2006. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): Standardized protocol and reference values. *PAIN,* 123**,** 231-243.

ROSS, J. R. & MATAVA, M. J. 2011. Tattoo-Induced Skin "Burn" During Magnetic Resonance Imaging in a Professional Football Player:A Case Report. *Sports Health,* 3**,** 431-434.

SANDKUHLER, J. & GRUBER-SCHOFFNEGGER, D. 2012. Hyperalgesia by synaptic long-term potentiation (LTP): an update. *Curr Opin Pharmacol,* 12**,** 18-27.

SPRUYT, A., CLARYSSE, J., VANSTEENWEGEN, D., BAEYENS, F., & HERMANS, D. 2010. Affect 4.0: A free software package for implementing psychological and psychophysiological experiments. *Experimental Psychology* 57**,** 36 - 45.

SULLIVAN, M. J. L., LYNCH, M. E. & CLARK, A. J. 2005. Dimensions of catastrophic thinking associated with pain experience and disability in patients with neuropathic pain conditions. *Pain,* 113**,** 310-315.

VAN DEN BROEKE, E., GEENE, N., VAN RIJN, C., WILDER-SMITH, O. & OOSTERMAN, J. 2014. Negative expectations facilitate mechanical hyperalgesia after high-frequency electrical stimulation of human skin. *European journal of pain,* 18**,** 86-91.

VAN WILGEN, C. P. & KEIZER, D. 2012. The sensitization model to explain how chronic pain exists without tissue damage. *Pain Management Nursing,* 13**,** 60-65.

WIECH, K., LIN, C. S., BRODERSEN, K. H., BINGEL, U., PLONER, M. & TRACEY, I. 2010. Anterior insula integrates information about salience into perceptual decisions about pain. *J Neurosci,* 30**,** 16324-31.

WOOLF, C. J. & MANNION, R. J. 1999. Neuropathic pain: aetiology, symptoms, mechanisms, and management. *The Lancet,* 353**,** 1959-1964.

YOU, D. S., CREECH, S. K. & MEAGHER, M. W. 2016. Enhanced Area of Secondary Hyperalgesia in Women with Multiple Stressful Life Events: A Pilot Study. *Pain Medicine,* 17**,** 1859-1864.

YOU, D. S., CREECH, S. K., VICHAYA, E. G., YOUNG, E. E., SMITH, J. S. & MEAGHER, M. W. 2014. Effect of written emotional disclosure on secondary hyperalgesia in women with trauma history. *Psychosom Med,* 76**,** 337-46.

Appendix 30: Formal data analysis

Formal data analysis

The effect of stimulus threat on experimentally induced secondary hyperalgesia

Tory Madden and Gillian Bedwell

29 Mar 2020

Table of Contents

Demographic data

- To determine the median (range) age in years
- To determine the number of male and female participants
- To determine the mean (SD) intensity used from the HFS induction

Manipulation check

- Describe and compare HFS ratings between arms.
- Describe and compare participant anxiety self-ratings between arms.
- Describe and compare participant threat self-ratings between arms.
- Assess whether blinding of Researcher 2 (who conducted the experiment and sensory testing) was upheld

Primary outcome

To determine the intensity of secondary hyperalgesia (SH) on each arm, and compare between arms.

Secondary outcome

To determine the surface area (SA) of secondary hyperalgesia (SH) on each arm, and compare between arms.

Exploratory outcomes

Sensory measurements

- To determine the intensity of static light touch on each arm, and compare between arms.
- To determine the intensity of dynamic light touch on each arm, and compare between arms.
- To determine the intensity of e-stim on each arm, and compare between arms.

Questionnaires

- Describe the Childhood Trauma Questionnaire (CTQ) and recent trauma scores (CIDI).
- Explore for relationship with area of SH (with results from both arms pooled).

Post-hoc analysis

- To determine the mean (range) of the Pain Catastrophising Scale
- To determine the mean (range) of the 10-item Connor-Davidson Resilience Scale.

Note: These analyses were not initially planned nor described in this study's protocol. These analyses were performed for exploratory purposes to inform the development of future research questions.

```
master_data <- read_csv("C:/Users/Gill/Desktop/Formal data analysis Jan 2020/Gill_formal_da
ta_HFS_ST_080120/formal_data.csv")
```

Demographic information

```
library(readr)
demo_info <- read_delim("C:/Users/Gill/Desktop/Formal data analysis Jan 2020/Gill_formal_da
ta_HFS_ST_080120/Demographic_info.csv",
    ";", escape_double = FALSE, trim_ws = TRUE)

# Median age and range.
demo_info$Age

##  [1] 51 19 19 26 21 22 21 21 20 36 21 21 23 55 26 23 20 24 30 23 21 19 19
## [24] 21 18 29

median(demo_info$Age, na.rm = FALSE)

## [1] 21

range(demo_info$Age, na.rm = FALSE)

## [1] 18 55

# Sex
demo_info$Sex <- (as.factor(demo_info$Sex))# First change sex to be a character

freq_sex <- table(demo_info$Sex)
view(freq_sex)

# Average detection threshold on each arm across all participants.
mean(demo_info$Detection_threshold_R)# mean detection threshold on the right arm was 1.53

## [1] 1.534231

mean(demo_info$Detection_threshold_L)# mean detection threshold on the left was 1.56

## [1] 1.557692

# Average detection threshold across both arms across all participants.
mean(demo_info$Intensity_used, trim = 0, na.rm = TRUE)# Overall mean detection threshold wa
s 1.53

## [1] 1.596154

#Standard deviation of individual detection threshold
sd(demo_info$Intensity_used, na.rm = TRUE)

## [1] 0.6352831
```

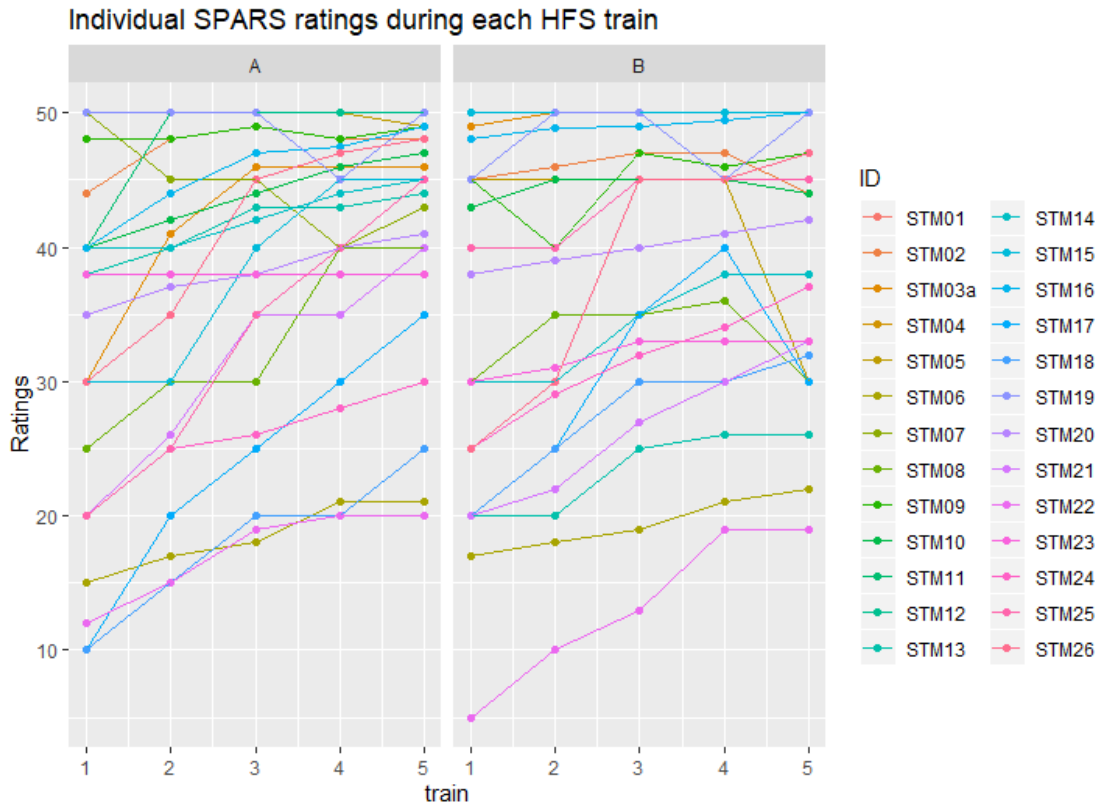
Manipulation checks

HFS ratings

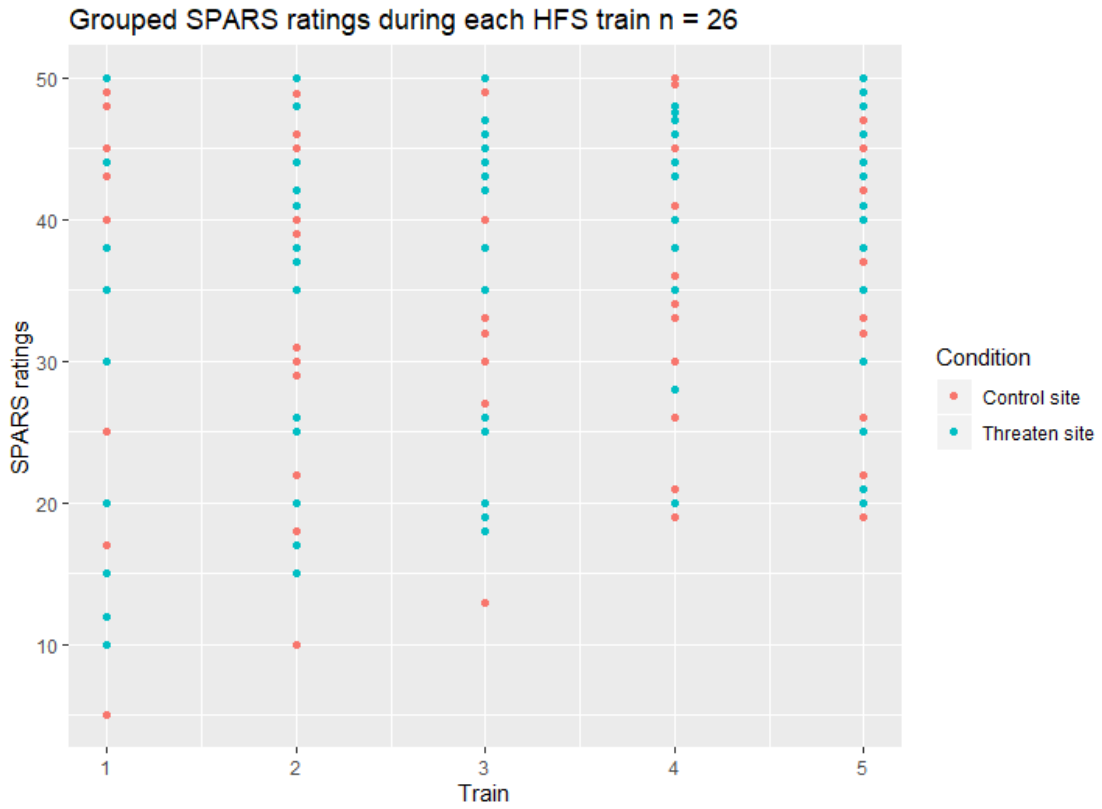
A mixed model analysis was used to compare ratings of the HFS trains between conditions. The order in which the two arms were stimulated was kept consistent across participants (right forearm first), but the allocation of forearm to condition was counterbalanced (random allocation to one of two equally sized groups). Therefore, the 'group' and 'site' information have been used to code for a 'condition' variable that is specified as A or B to maintain blinding of the analyst to actual experimental conditions. Here, we test for a main effect of condition on ratings, whilst allowing for a random intercept for each participant.

```
hfs <- master_data %>%
  filter(phase == 'hfs') %>%
  select(id, condition, rating)
```

```r
hfs$rating <- as.numeric(hfs$rating)

hfs %<>% group_by(id, condition) %>%
  mutate(train = row_number()) %>%
  ungroup()

hfs_wide <- hfs %>%
  group_by(id) %>%
  spread(key = condition,
         value = rating)

#Determining the mean (SD) per condition

mean(hfs_wide$A)

## [1] 38.76538

sd(hfs_wide$A)

## [1] 11.34453

mean(hfs_wide$B)

## [1] 39.07231

sd(hfs_wide$B)

## [1] 11.31391

ggplot(data = hfs) +
  aes(x = train,
      y = rating,
      group = id,
      colour = id) +
  geom_point() +
  facet_grid(~ condition) +
  geom_line() +
  guides(colour = guide_legend("ID"), size = guide_legend("ID")) +
  labs( y = 'Ratings',
        x = 'train',
        title = 'Individual SPARS ratings during each HFS train')
```




```r
#unblinding to make graph clear
hfs$condition[hfs$condition == 'A'] <- 'Threaten site'

hfs$condition[hfs$condition == 'B'] <- 'Control site'


ggplot(data = hfs) +
  aes(x = train,
      y = rating,
      group = condition,
      colour = condition) +
  geom_point() +
  guides(colour = guide_legend("Condition"), size = guide_legend("Condition")) +
  labs( y = 'SPARS ratings',
        x = 'Train',
        title = 'Grouped SPARS ratings during each HFS train n = 26')
```




```
range(hfs$rating)

## [1]  5 50

# Null model
hfs_null <- lmer(rating ~ 1 + (1|id),
                 data = hfs)
hfs_condition <- lmer(rating ~ condition + (1|id),
                 data = hfs)
anova(hfs_condition, hfs_null) # hfs_condition is no better than null

## Data: hfs
## Models:
## hfs_null: rating ~ 1 + (1 | id)
## hfs_condition: rating ~ condition + (1 | id)
##               Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## hfs_null       3 1710.2 1720.9 -852.11   1704.2
## hfs_condition  4 1712.0 1726.3 -852.01   1704.0 0.2126      1     0.6447

# Conclude: HFS ratings were not predicted by condition.

summary(hfs_condition)

## Linear mixed model fit by REML ['lmerMod']
## Formula: rating ~ condition + (1 | id)
##    Data: hfs
##
## REML criterion at convergence: 1699.8
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -3.3241 -0.4205  0.1031  0.5164  2.3287
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  id       (Intercept) 102.63   10.131
```

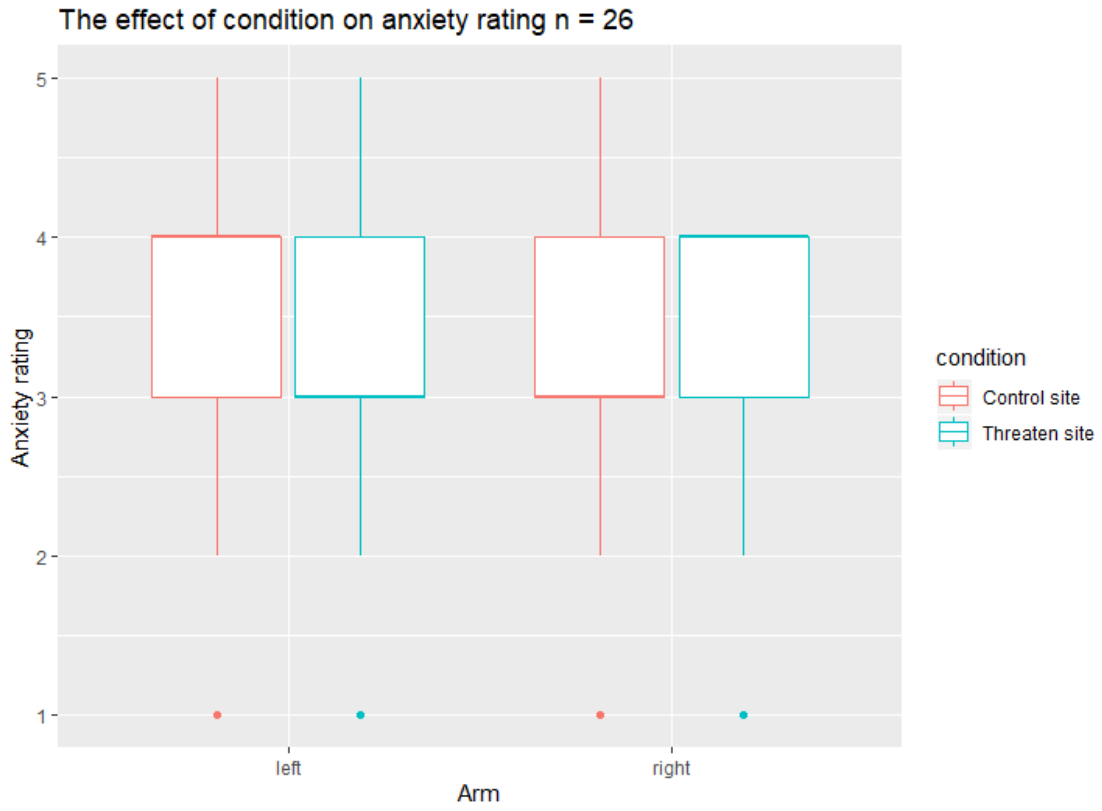
```
##  Residual              28.91    5.376
## Number of obs: 260, groups:  id, 26
##
## Fixed effects:
##                         Estimate Std. Error t value
## (Intercept)              39.0723     2.0420   19.14
## conditionThreaten site  -0.3069     0.6669   -0.46
##
## Correlation of Fixed Effects:
##             (Intr)
## cndtnThrtns -0.163
```

Anxiety ratings

We are interested in a main effect of condition on anxiety ratings.

```
anxiety <- master_data %>% select(group,
                                  id,
                                  anxious_right,
                                  anxious_left)
anxiety <- unique(anxiety)

anxiety %<>%
  gather(key = arm,
         value = anxiety,
         3:4) %>%
  mutate(arm = case_when(
    arm == 'anxious_left' ~ 'left',
    arm == 'anxious_right' ~ 'right'
  ))


anxiety %<>% mutate(condition = case_when(
  group == '1' & arm == 'right' ~ 'A',
  group == '1' & arm == 'left' ~ 'B',
  group == '2' & arm == 'right' ~ 'B',
  group == '2' & arm == 'left' ~ 'A')) %>%
  select(-group)


anxiety_wide <- anxiety %>%
  group_by(id) %>%
  spread(key = condition,
         value = anxiety) %>%
  select(-arm)

#Determining the mean (SD) anxiety rating per condition
mean(anxiety_wide$A, na.rm = TRUE)

## [1] 3.307692

sd(anxiety_wide$A, na.rm = TRUE)

## [1] 1.123182

mean(anxiety_wide$B, na.rm = TRUE)

## [1] 3.423077

sd(anxiety_wide$B, na.rm = TRUE)

## [1] 1.137474

var(anxiety_wide$B, na.rm = TRUE)
```

```
## [1] 1.293846

#unblinding
anxiety$condition[anxiety$condition == 'A'] <- 'Threaten site'

anxiety$condition[anxiety$condition == 'B'] <- 'Control site'


ggplot(data = anxiety) +
  aes(x = arm,
      y = anxiety,
      group = interaction(arm,condition),
      colour = condition) +
  geom_boxplot() +
  labs(title = 'The effect of condition on anxiety rating n = 26',
       y = 'Anxiety rating',
       x = 'Arm')
```




```
a1 <- ggplot(data = anxiety) +
  aes(x = arm,
      y = anxiety,
      group = condition,
      colour = condition) +
  geom_point() +
  facet_grid(~ id)+
  labs(title = 'Individual effect of condition on anxiety rating',
       y = 'Anxiety ratings',
       x = 'Arm')


anxiety_null <- lmer(anxiety ~ 1 + (1|id),
                     data = anxiety)
anxiety_condition <- lmer(anxiety ~ condition + (1|id),
                     data = anxiety)
anova(anxiety_condition, anxiety_null) # No effect of condition.
```

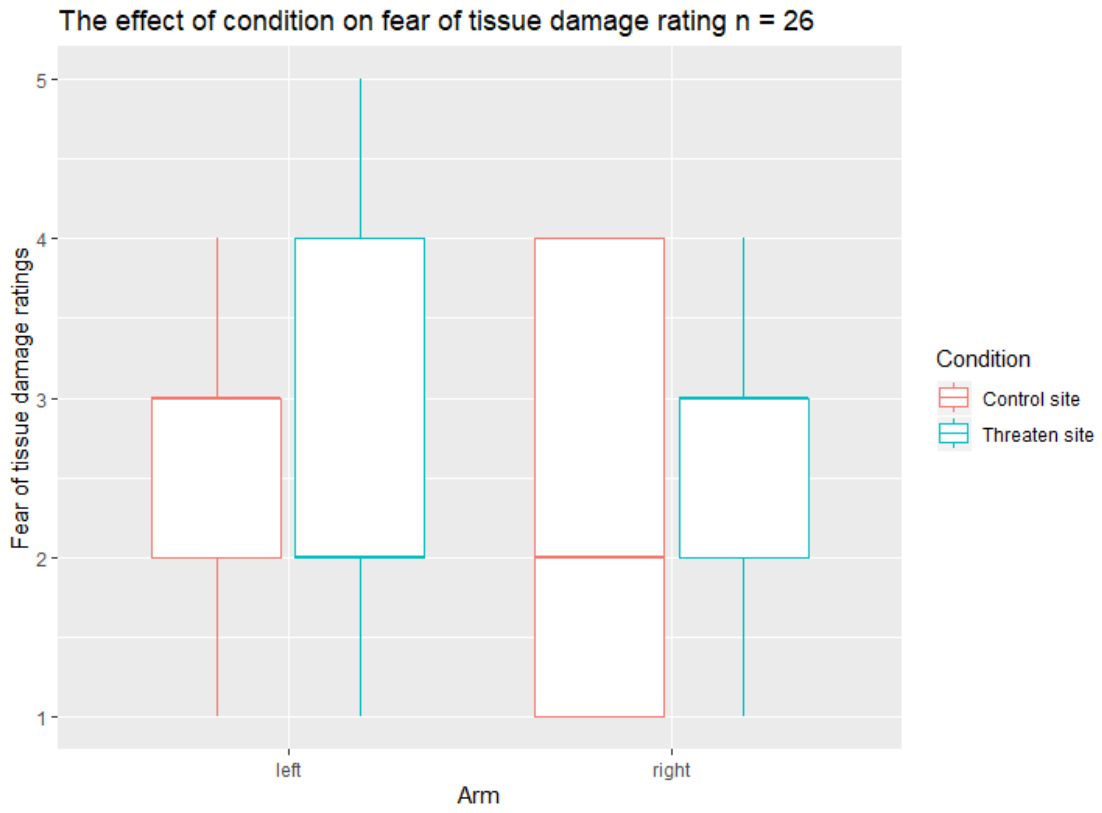
```
## Data: anxiety
## Models:
## anxiety_null: anxiety ~ 1 + (1 | id)
## anxiety_condition: anxiety ~ condition + (1 | id)
##                   Df    AIC    BIC  logLik deviance  Chisq Chi Df
## anxiety_null       3 129.51 135.37 -61.758   123.52
## anxiety_condition  4 130.50 138.30 -61.248   122.50 1.0197      1
##                   Pr(>Chisq)
## anxiety_null
## anxiety_condition     0.3126
```

Threat ratings

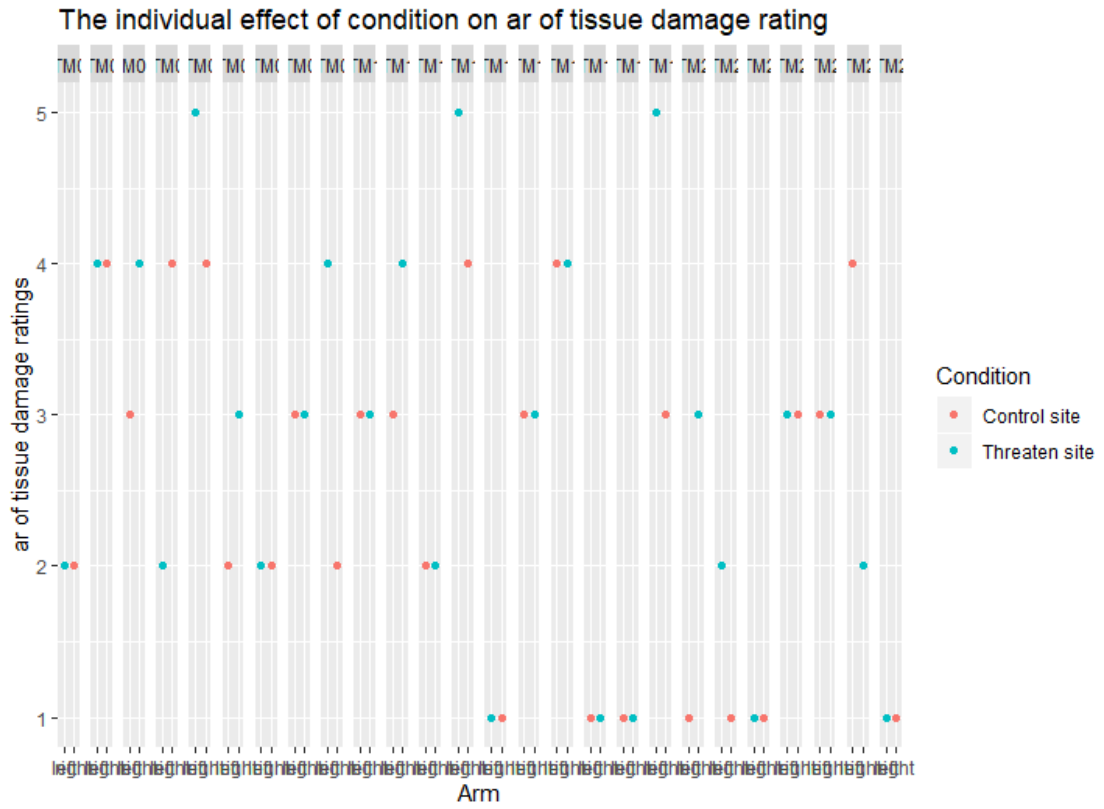
We are interested in a main effect of condition on threat ratings.

```
threat <- master_data %>% select(group,
                                 id,
                                 damage_right,
                                 damage_left)

threat <- unique(threat)

threat %<>%
  gather(key = arm,
         value = threat,
         3:4) %>%
  mutate(arm = case_when(
    arm == 'damage_left' ~ 'left',
    arm == 'damage_right' ~ 'right'
  ))

threat %<>% mutate(condition = case_when(
  group == '1' & arm == 'right' ~ 'A',
  group == '1' & arm == 'left' ~ 'B',
  group == '2' & arm == 'right' ~ 'B',
  group == '2' & arm == 'left' ~ 'A')) %>%
  select(-group)

threat_wide <- threat %>%
  group_by(id) %>%
  spread(key = condition,
         value = threat) %>%
  select(-arm)

#Determining the mean (SD) threat rating per condition
mean(threat_wide$A, na.rm = TRUE)

## [1] 2.807692

sd(threat_wide$A, na.rm = TRUE)

## [1] 1.296741

mean(threat_wide$B, na.rm = TRUE)

## [1] 2.5

sd(threat_wide$B, na.rm = TRUE)

## [1] 1.140175

#unbliunding
threat$condition[threat$condition == 'A'] <- 'Threaten site'

threat$condition[threat$condition == 'B'] <- 'Control site'
```

```r
ggplot(data = threat) +
  aes(x = arm,
      y = threat,
      group = interaction(arm,condition),
      colour = condition) +
  geom_boxplot() +
  guides(colour = guide_legend("Condition"), size = guide_legend("Condition")) +
  labs(title = 'The effect of condition on fear of tissue damage rating n = 26',
       y = 'Fear of tissue damage ratings',
       x = 'Arm')
```



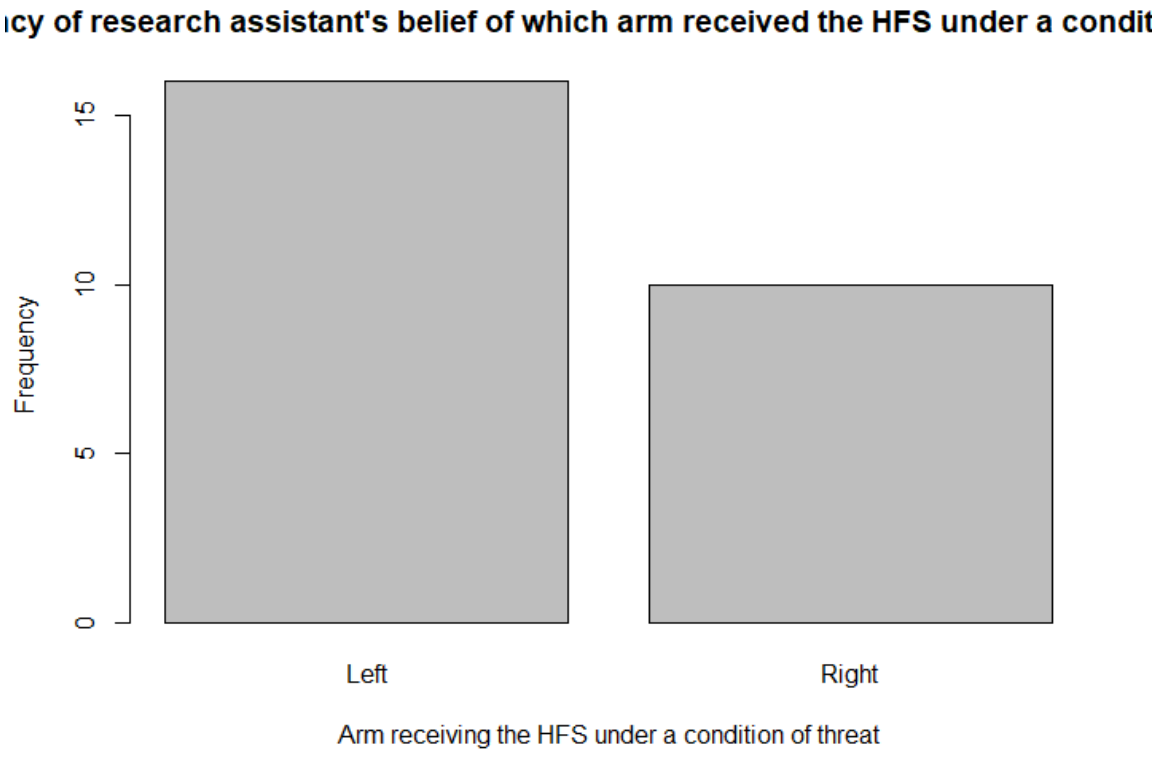

```r
ggplot(data = threat) +
  aes(x = arm,
      y = threat,
      group = condition,
      colour = condition) +
  geom_point() +
  facet_grid(~ id) +
  guides(colour = guide_legend("Condition"), size = guide_legend("Condition")) +
  labs(title = 'The individual effect of condition on ar of tissue damage rating',
       y = 'ar of tissue damage ratings',
       x = 'Arm')
```




```
threat_null <- lmer(threat ~ 1 + (1|id),
                    data = threat)
threat_condition <- lmer(threat ~ condition + (1|id),
                    data = threat)
anova(threat_condition, threat_null) # No effect of condition.

## Data: threat
## Models:
## threat_null: threat ~ 1 + (1 | id)
## threat_condition: threat ~ condition + (1 | id)
##                  Df    AIC    BIC  logLik deviance Chisq Chi Df Pr(>Chisq)
## threat_null       3 158.46 164.32 -76.232   152.46
## threat_condition  4 157.88 165.68 -74.939   149.88 2.586      1     0.1078
```

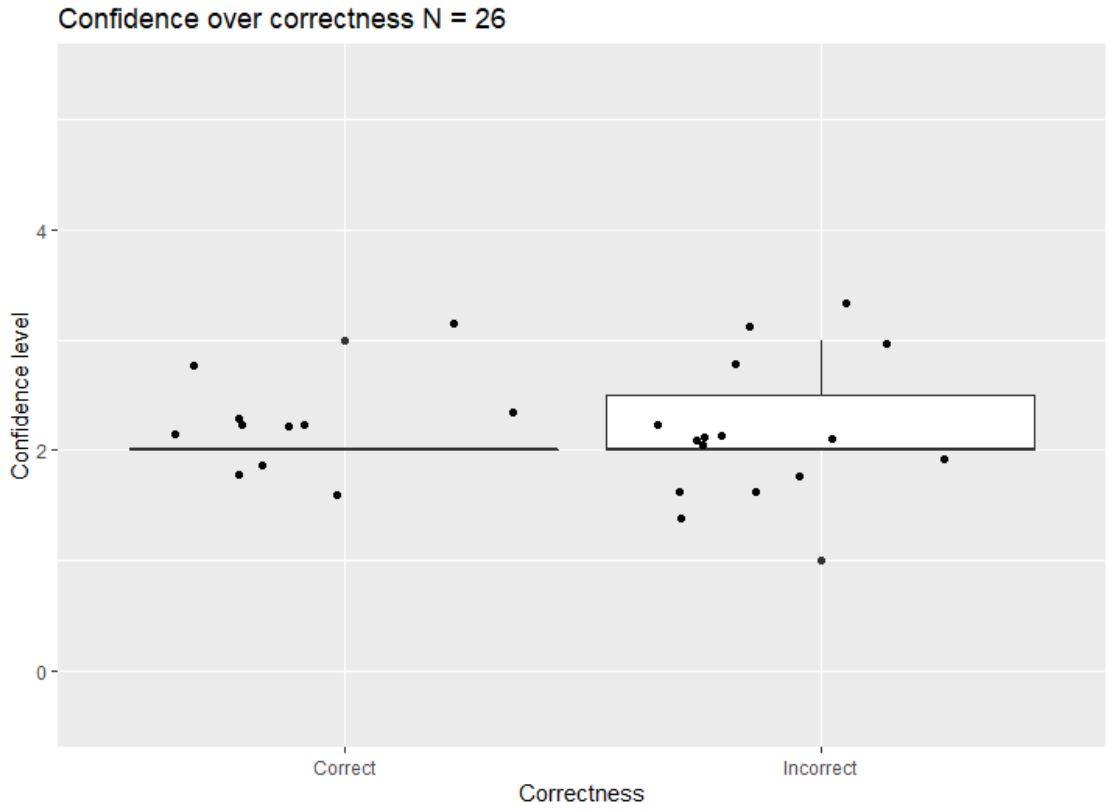
Blinding assessment

```
# Importing data

library(readr)
RA_blinding <- read_delim("C:/Users/Gill/Desktop/RA_blinding_ax.cvs.csv",
    ";", escape_double = FALSE, trim_ws = TRUE)

# Renaming column

names(RA_blinding)[names(RA_blinding) == 'Which are do you think received the HFS under a c
ondition of threat?'] <- 'threat_arm_RA'

freq <- table(RA_blinding$threat_arm_RA)


barplot(freq, main = "Frequency of research assistant's belief of which arm received the HF
S under a condition of threat", xlab = "Arm receiving the HFS under a condition of threat",
ylab = "Frequency")
```




```r
# compare RA's results to actual group allocation

actual_condition <- master_data %>%
  select(id,
         Group_allocation)

actual_condition <- unique(actual_condition)

names(actual_condition)[names(actual_condition) == 'Group_allocation'] <- 'threat_arm_actua
l'

# rename "group 1" to "right" and "group 2" to "left"

actual_condition %<>% mutate(threat_arm_actual = case_when(
  threat_arm_actual == '1' ~ 'Right',
  threat_arm_actual == '2' ~ 'Left'))

comparison <- merge(x = actual_condition, y = RA_blinding, by.x = 'id' , by.y = 'id' , all
= TRUE)

# Now add a new column to comparison to see whether RA was correct or not. When threat_arm_
actual and threat_arm_RA are the same then outcome is "correct". When different, outcome is
"incorrect"

comparison %<>% mutate(accuracy = if_else(threat_arm_actual == threat_arm_RA, 'Correct','In
correct'))

#Determine the percentage of correct responses and 95% CI

Correctness <- table(comparison$accuracy)

view(Correctness)

# Percentage correct: 42.31%
(11/26)*100
```

```
## [1] 42.30769

# Determine Cohen kappa's coefficient

blinding_ax_table <- table(comparison$threat_arm_actual,
                           comparison$threat_arm_RA)
blinding_ax_table

##
##         Left Right
##   Left     7     6
##   Right    9     4

p0 <- (7 + 9 )/26
pa <- (13/26)*(16/26)
pb <- (13/26)*(10/26)
pe <- pa + pb

  kappa <- (p0 - pe) / (1 - pe)  #Kappa is 0.23

# Plot degree of correctness

ggplot(data = comparison) +
  aes(x = accuracy,
      y = Confidence_level,
      confidence_level, ymax = 5, ymin = 0) +
geom_boxplot(width = 2) +
  geom_point(position = 'jitter') +
  labs(title = 'Confidence over correctness N = 26',
       y = 'Confidence level',
       x = 'Correctness')
```




```
# Could the RA reliably predict condition? No from this plot the RA made confident assertio
ns that were incorrect. Meaning they were effectively blinded.
```

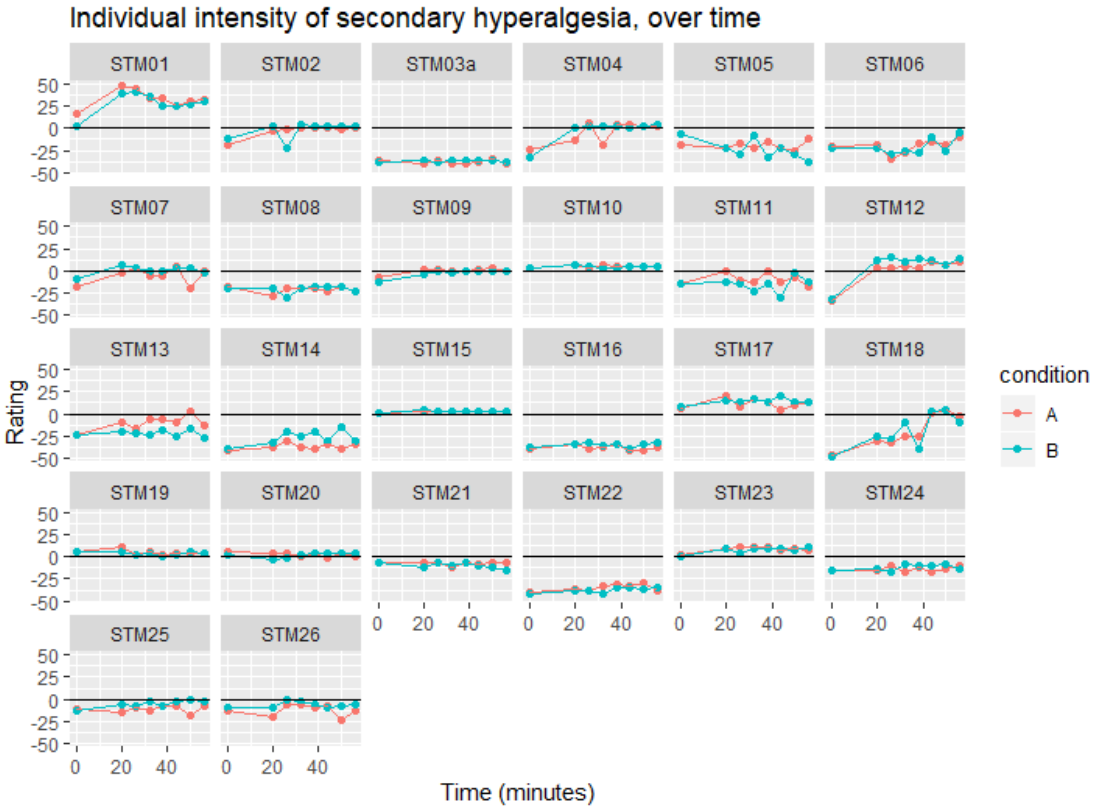
Primary outcome: intensity of secondary hyperalgesia

Aim: to determine the intensity of secondary hyperalgesia (SH) on each arm, and the comparison between arms.

Data were from the baseline time points as well as follow-up time points, and for both arms (control and experimental sites). The main interest was the change in ratings (from baseline to follow-up time points), and how these differed between the arms/conditions. Therefore, for the formal analysis, researchers: 1) calculated the mean rating for all baseline time points; 2) calculated the mean rating for all follow-up time points; 3) subtracted the mean baseline rating from the mean follow-up rating (within participant and condition), to provide the indication of change in rating (i.e. hyperalgesia) for each condition, within each participant.

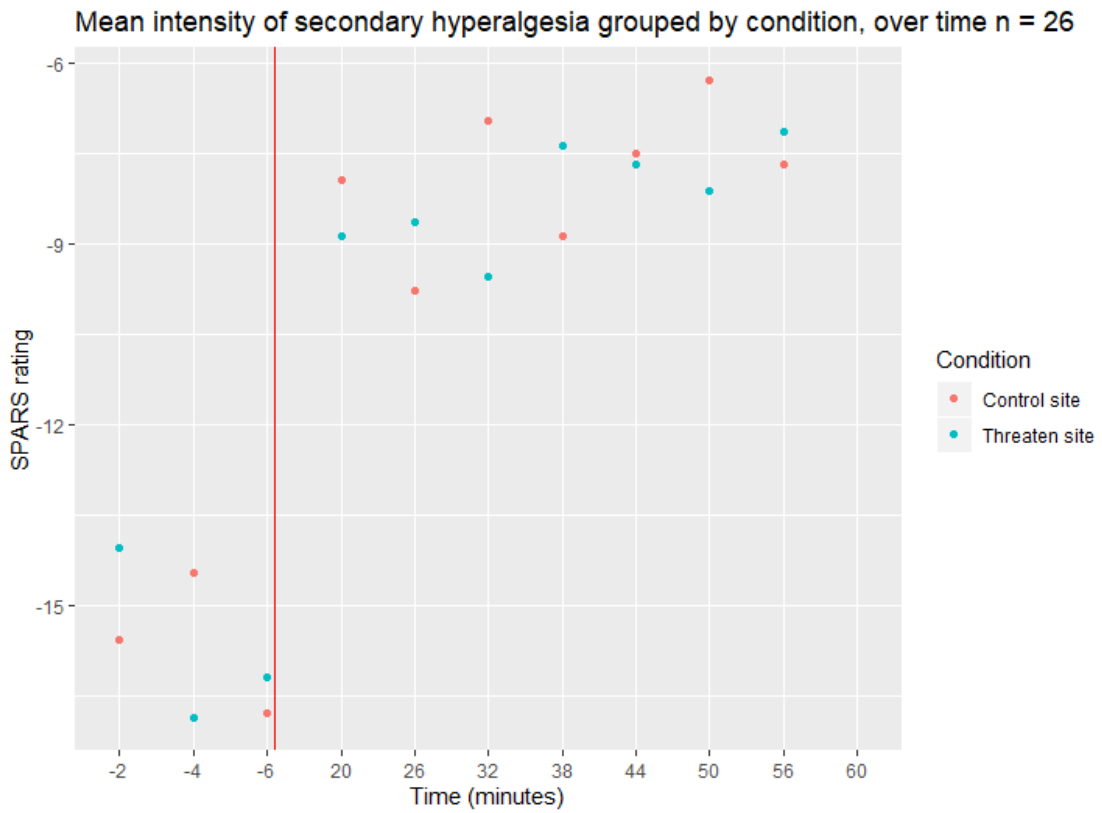
```
intensity <- master_data %>%
  select(group,
         id,
         time,
         phase,
         site,
         modality,
         rating) %>%
filter(modality == '128' | modality == '256') %>%
filter(phase != 'orientation') %>%
mutate(time = if_else(phase == 'baseline', time*-1L, time))

intensity %<>% mutate(condition = case_when(
  group == '1' & site == 'right' ~ 'A',
  group == '1' & site == 'left' ~ 'B',
  group == '2' & site == 'right' ~ 'B',
  group == '2' & site == 'left' ~ 'A')) %>%
  select(-group,
         -site)

intensity$rating <- as.numeric(intensity$rating)
intensity %<>% group_by(id,
           time,
           phase,
           condition) %>%
  summarise(pp_rating = mean(rating))


int_bl <- intensity %>% filter(phase == 'baseline') %>%
  group_by(id, condition) %>%
  summarise(rating = mean(pp_rating)) %>%
  ungroup() %>%
  mutate(time = 0) %>% ungroup()

int_fu <- intensity %>% filter(phase != 'baseline') %>% ungroup () %>%
  select(-phase) %>%
  rename(rating = pp_rating)

intensity1 <- rbind(int_bl, int_fu)

# For interest, plot the actual data

ggplot(data = intensity1) +
  aes(x =time,
      y = rating,
      group = condition,
      colour = condition) +
```

```
  facet_wrap(~ id) +
  geom_point() +
  geom_line() +
  geom_hline(yintercept = 0) +
  labs(title = 'Individual intensity of secondary hyperalgesia, over time',
       y = 'Rating',
       x = 'Time (minutes)')
```

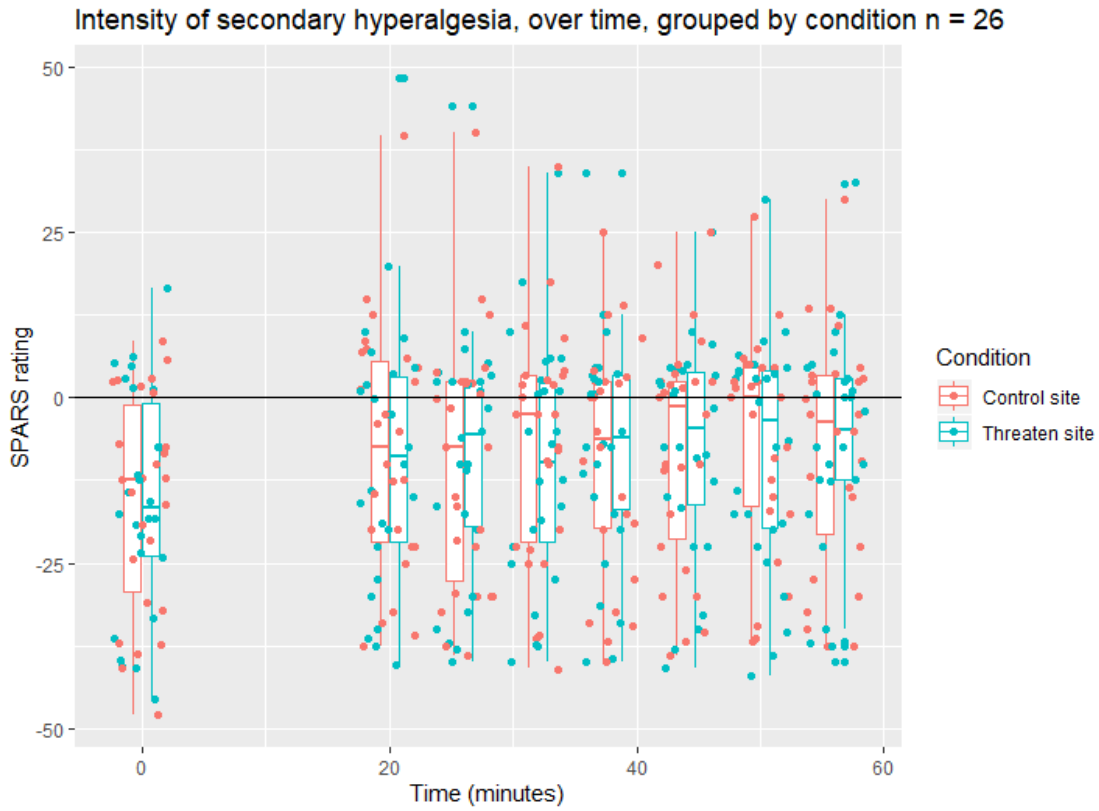



```
# Plot group mean ratings by condition over time

mean_intensity <- intensity1 %>%
  group_by(condition, time) %>%
  summarise(mean_rating = mean(rating, na.rm=TRUE))

# Plot group intensity of secondary hyperalgesia by condition, over time

intensity_groupmean <- intensity %>%
  ungroup() %>%
  select(-phase) %>%
  group_by(time, condition) %>%
  summarise(rating = mean(pp_rating)) %>%
  ungroup()

intensity_groupmean$time <- as.factor(intensity_groupmean$time)

#Unblinding condition for graphs
intensity_groupmean$condition[intensity_groupmean$condition == 'A'] <- 'Threaten site'

intensity_groupmean$condition[intensity_groupmean$condition == 'B'] <- 'Control site'

ggplot(data = intensity_groupmean) +
    aes(x = time,
      y = rating,
      group = condition,
      colour = condition,
      xmax = '60') +
```

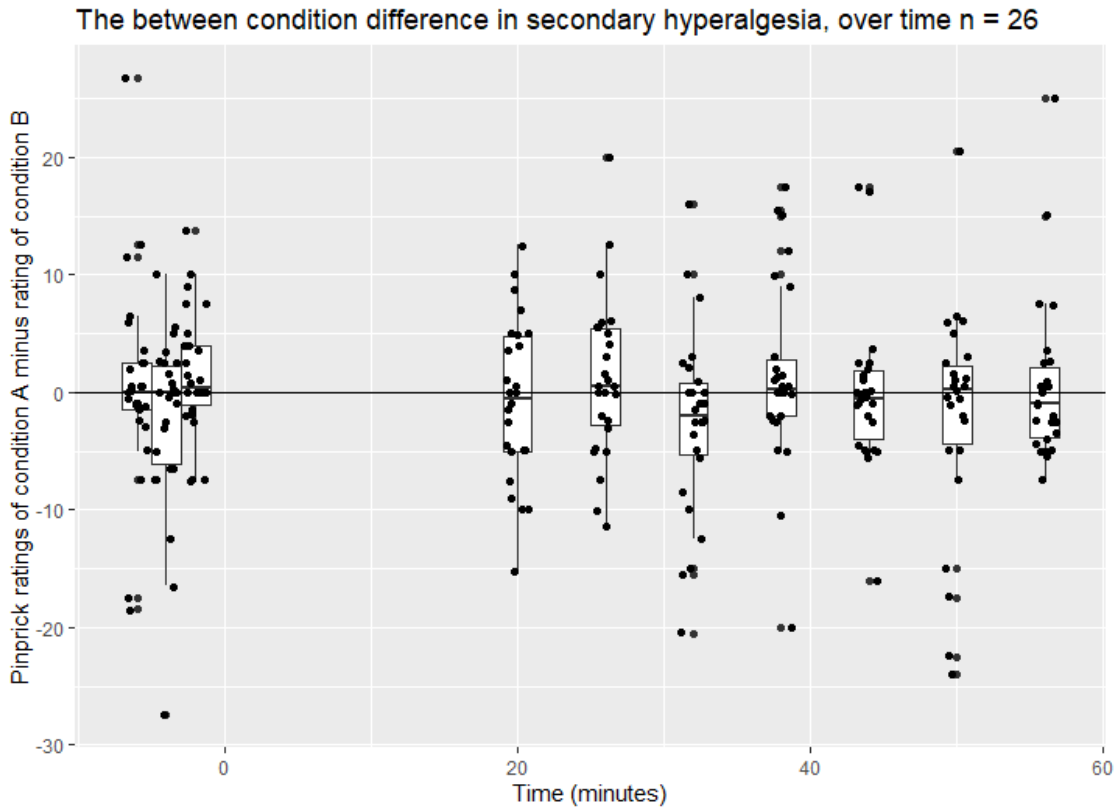
```
  geom_point() +
  geom_vline(xintercept = 3.1, colour = 'red') +
  guides(colour = guide_legend("Condition"), size = guide_legend("Condition"),
  shape = guide_legend("Condition")) +
  labs(title = 'Mean intensity of secondary hyperalgesia grouped by condition, over time n
= 26',
       y = 'SPARS rating',
       x = 'Time (minutes)')
```




```
# All participants, group data represented by boxplots

#Unblinding condition for graphs
intensity1$condition[intensity1$condition == 'A'] <- 'Threaten site'

intensity1$condition[intensity1$condition == 'B'] <- 'Control site'

ggplot(data = intensity1) +
  aes(x = time,
      y = rating,
      group = interaction(condition, time),
      colour = condition) +
  geom_boxplot(width = 3) +
  geom_point(position = 'jitter') +
  geom_hline(yintercept = 0) +
  guides(colour = guide_legend("Condition"), size = guide_legend("Condition")) +
  labs(title = 'Intensity of secondary hyperalgesia, over time, grouped by condition n = 26
',
       y = 'SPARS rating',
       x = 'Time (minutes)')
```




```
 # Calculate difference between conditions for group boxplots

intensity_wide <- intensity %>%
  group_by(id,
           time) %>%
  spread(key = condition,
         value = pp_rating) %>%
  mutate(site_diff = A-B)

# All participants, group data represented by boxplots
ggplot(data = intensity_wide) +
  aes(x = time,
      y = site_diff,
      group = time) +
  geom_boxplot(width = 2) +
  geom_point(position = 'jitter') +
  geom_hline(yintercept = 0) +
  labs(title = 'The between condition difference in secondary hyperalgesia, over time n = 2
6',
       y = 'Pinprick ratings of condition A minus rating of condition B',
       x = 'Time (minutes)')
```




```
# Collapse all post-baseline ratings for each condition and then compare them using a RM AN
OVA type model. Time is nested within (and fully crossed with) ID.

int_bl %<>% select(-time)
names(int_bl)[names(int_bl) == 'rating'] <- 'baseline_rating'
collapse <- int_fu %>% # resolve each rating relative to baseline
  right_join(int_bl) %>%
  mutate(rating_controlled = rating-baseline_rating) %>%
  select(id,
         time,
         condition,
         rating_controlled) # spread to calculate difference between conditions

# We want to find out whether rating_controlled (for baseline) is predicted by condition.

model_null <- lmer(rating_controlled ~ (1|id),
                   data = collapse)
model_condition <- lmer(rating_controlled ~ condition + (1|id),
                        data = collapse)

anova(model_condition, model_null) # No improvement

## Data: collapse
## Models:
## model_null: rating_controlled ~ (1 | id)
## model_condition: rating_controlled ~ condition + (1 | id)
##                  Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## model_null        3 2492.6 2504.3 -1243.3   2486.6
## model_condition   4 2494.5 2510.1 -1243.2   2486.5 0.1168      1     0.7326

model_pre_condition_crossed <- lmer(rating_controlled ~ condition + (1|id/time),
                                    data = collapse)
summary(model_pre_condition_crossed)

## Linear mixed model fit by REML ['lmerMod']
## Formula: rating_controlled ~ condition + (1 | id/time)
```

```
##    Data: collapse
##
## REML criterion at convergence: 2480.7
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -3.6524 -0.3753  0.0285  0.4071  3.0526
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  time:id  (Intercept)   3.567   1.889
##  id       (Intercept) 134.624  11.603
##  Residual              38.141   6.176
## Number of obs: 364, groups:  time:id, 182; id, 26
##
## Fixed effects:
##             Estimate Std. Error t value
## (Intercept)   7.5119     2.3253   3.231
## conditionB    0.2303     0.6474   0.356
##
## Correlation of Fixed Effects:
##            (Intr)
## conditionB -0.139
```

```
anova(model_pre_condition_crossed, model_null)
```

```
## Data: collapse
## Models:
## model_null: rating_controlled ~ (1 | id)
## model_pre_condition_crossed: rating_controlled ~ condition + (1 | id/time)
##                             Df    AIC    BIC  logLik deviance  Chisq
## model_null                   3 2492.6 2504.3 -1243.3   2486.6
## model_pre_condition_crossed  5 2495.2 2514.7 -1242.6   2485.2 1.4364
##                             Chi Df Pr(>Chisq)
## model_null
## model_pre_condition_crossed      2     0.4876
```

```
# not a significant improvement

# Secondary hyperalgesia intensity is not predicted by condition.


# Assessing whether the individual calibration approach confounded the results (intensity o
f SH)

max_intensity <- intensity %>%
  group_by(id, condition) %>%
  summarise(max_rating = max(pp_rating, na.rm=TRUE))

max_vs_used <-merge(x=max_intensity, y=demo_info[,c(1,10)], by.x = 'id' , by.y = 'id' , all
.x = TRUE)


# First check distributional assumptions for correlation.
# Shapiro-Wilk normality
with(max_vs_used, shapiro.test(Intensity_used)) # P = 0.011
```

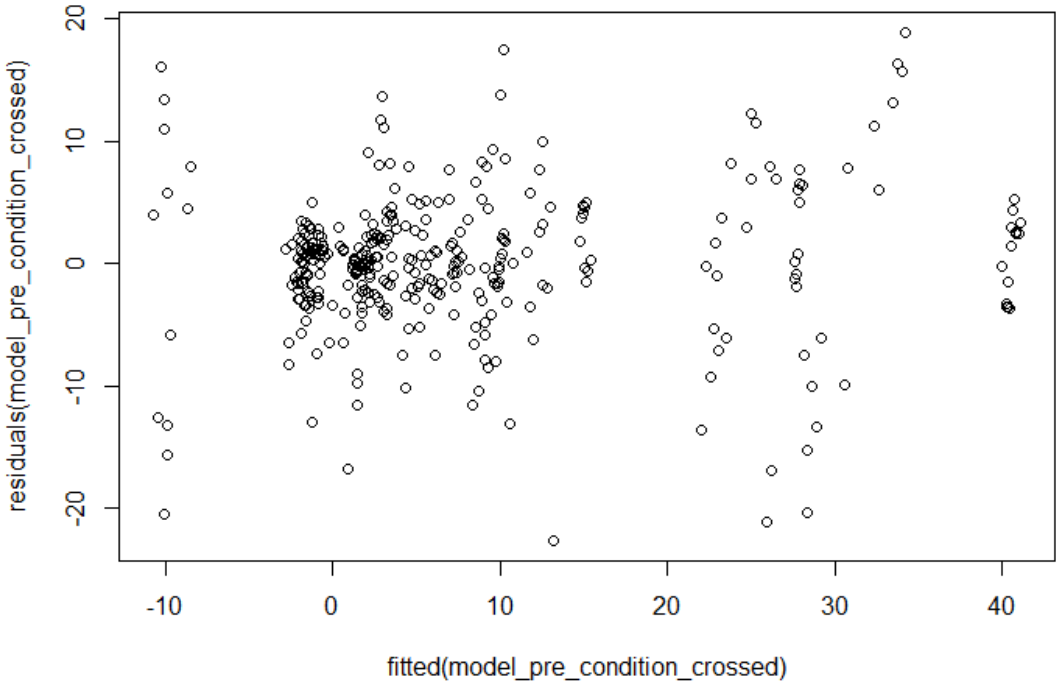
```
##
##  Shapiro-Wilk normality test
##
## data:  Intensity_used
## W = 0.93955, p-value = 0.01074
```

```
correlation <- cor.test(max_vs_used$max_rating, max_vs_used$Intensity_used, method = "spear
man")
correlation # Not significant
```

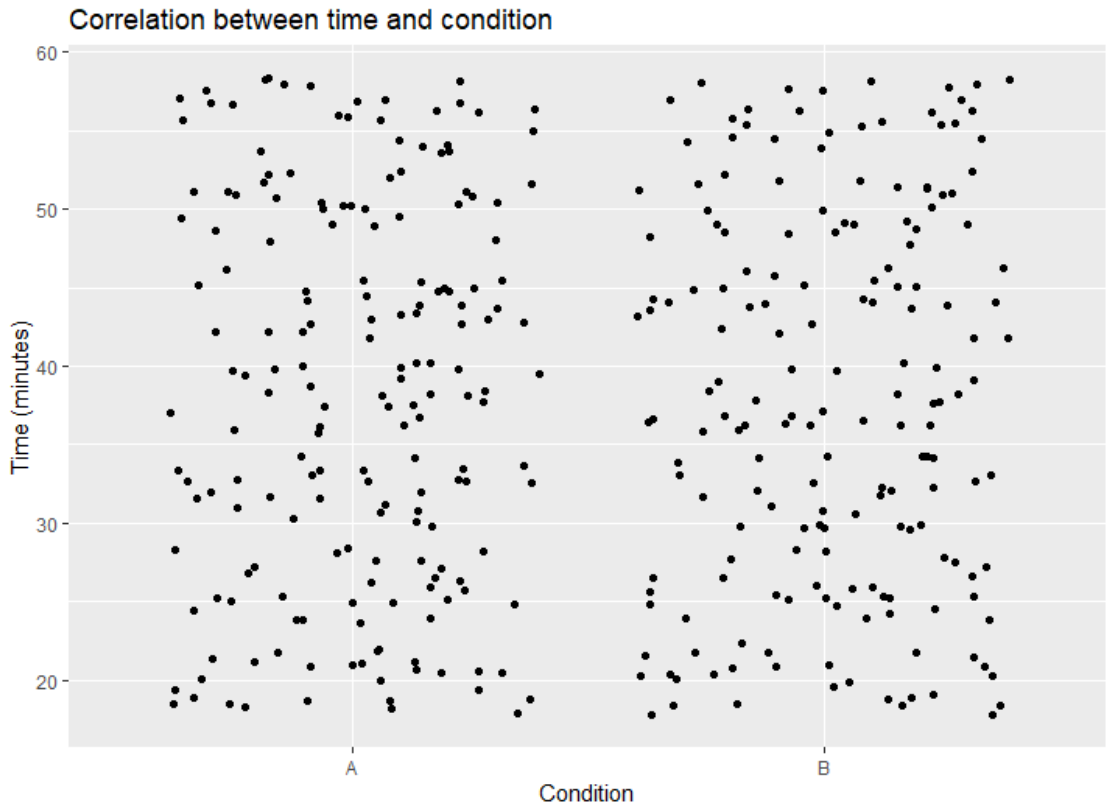
```
##
##  Spearman's rank correlation rho
##
## data:  max_vs_used$max_rating and max_vs_used$Intensity_used
## S = 22496, p-value = 0.7799
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##       rho
## 0.03970626
```

SH: Assessment of model fit

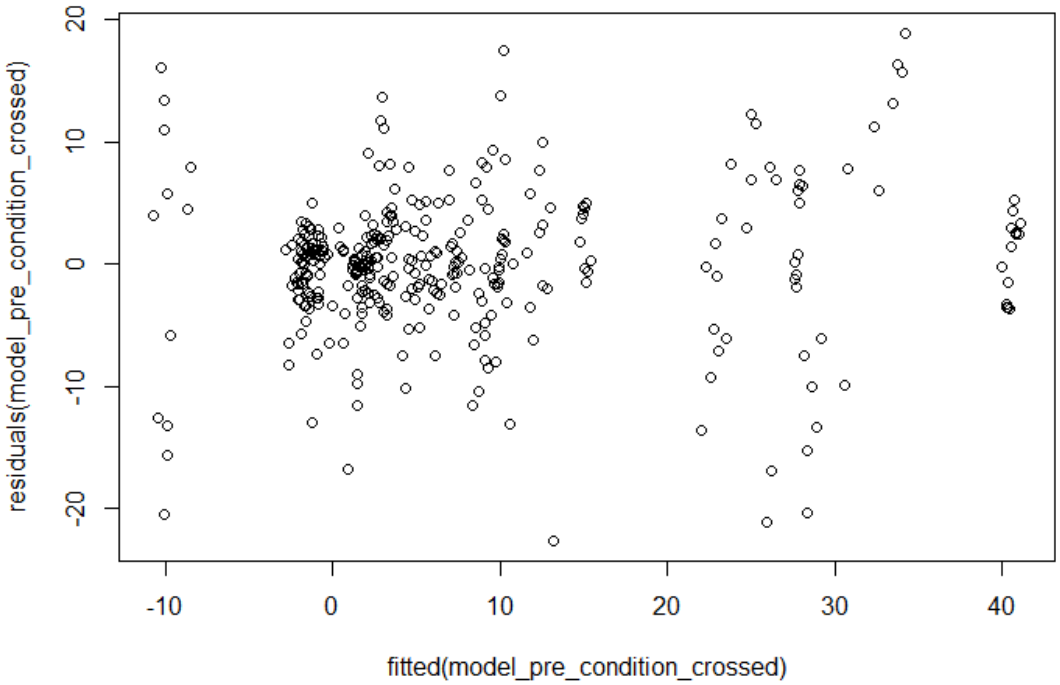
```
# The model is called model_pre_condition_crossed and has the structure: rating_controlled
~ condition + (1|id/time)

# Assumption 1: linearity
# Plot fitted values against residual values, for fixed factor 'condition'.

plot(fitted(model_pre_condition_crossed), residuals(model_pre_condition_crossed))
```



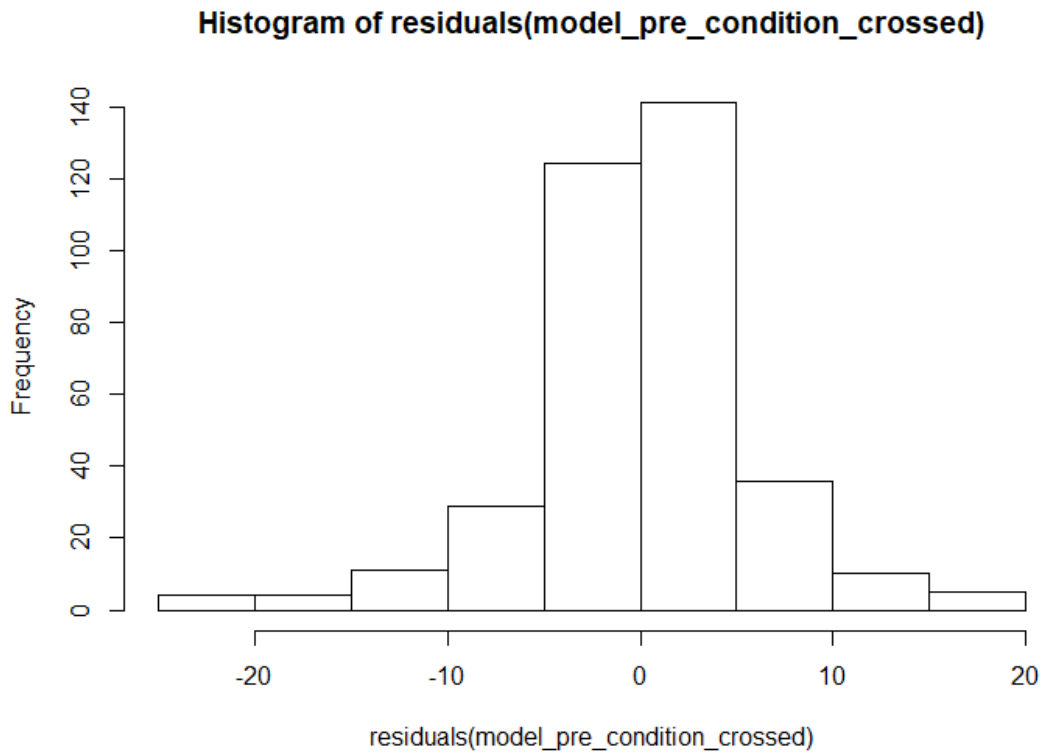

```
## Interpretation: increased density in a blob on the left, but no obvious linear or curvil
inear pattern. We deemed the assumption to have been upheld.

# Assumption 2: absence of collinearity
# Check for correlation between time and condition

ggplot(data = collapse) +
  aes(x = condition,
      y = time) +
  geom_jitter() +
  geom_smooth() +
  labs(title = 'Correlation between time and condition',
       x = 'Condition',
       y = 'Time (minutes)')
```



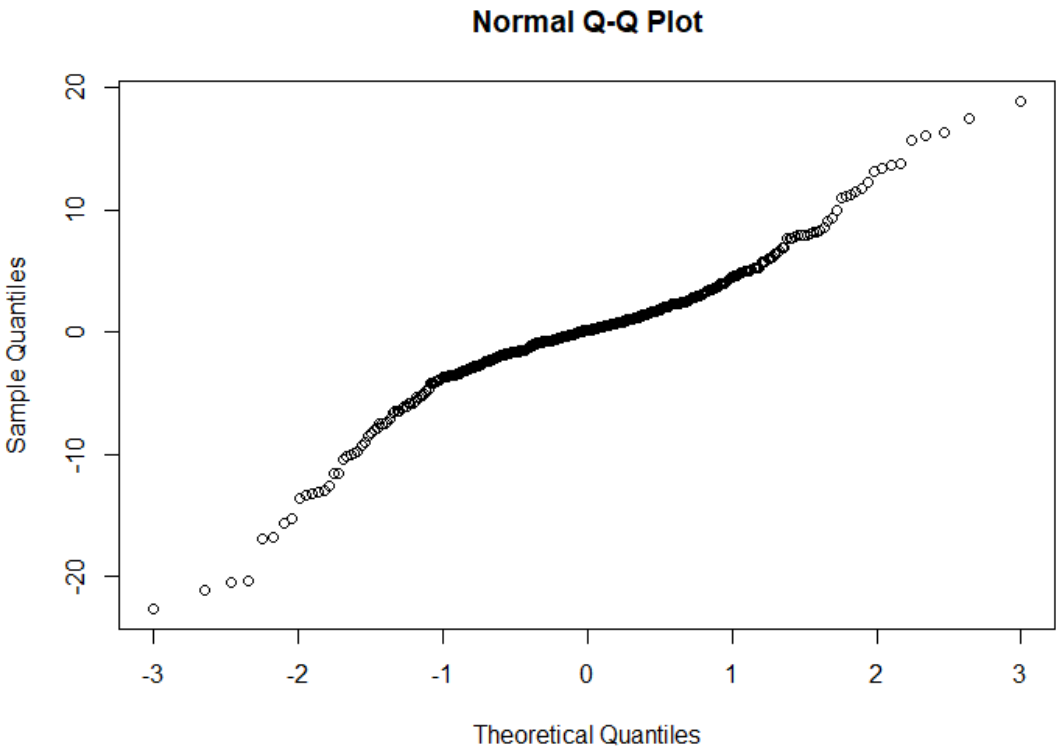

```
# Interpretation: no collinearity. Assumption was deemed to have been upheld.

# Assumption 3: Homoscedasticity, i.e. equal variance across the range of predicted values.
plot(fitted(model_pre_condition_crossed), residuals(model_pre_condition_crossed))
```



```r
# Interpretation: increased density in a blob on the left (i.e. more data points here), but the range of maximum and minimum values seems consistent across the x-axis. Assumption was deemed to have been upheld.

# Assumption 4: Normally distributed residuals
# Plot residuals
hist(residuals(model_pre_condition_crossed))
```




```r
qqnorm(residuals(model_pre_condition_crossed))
```



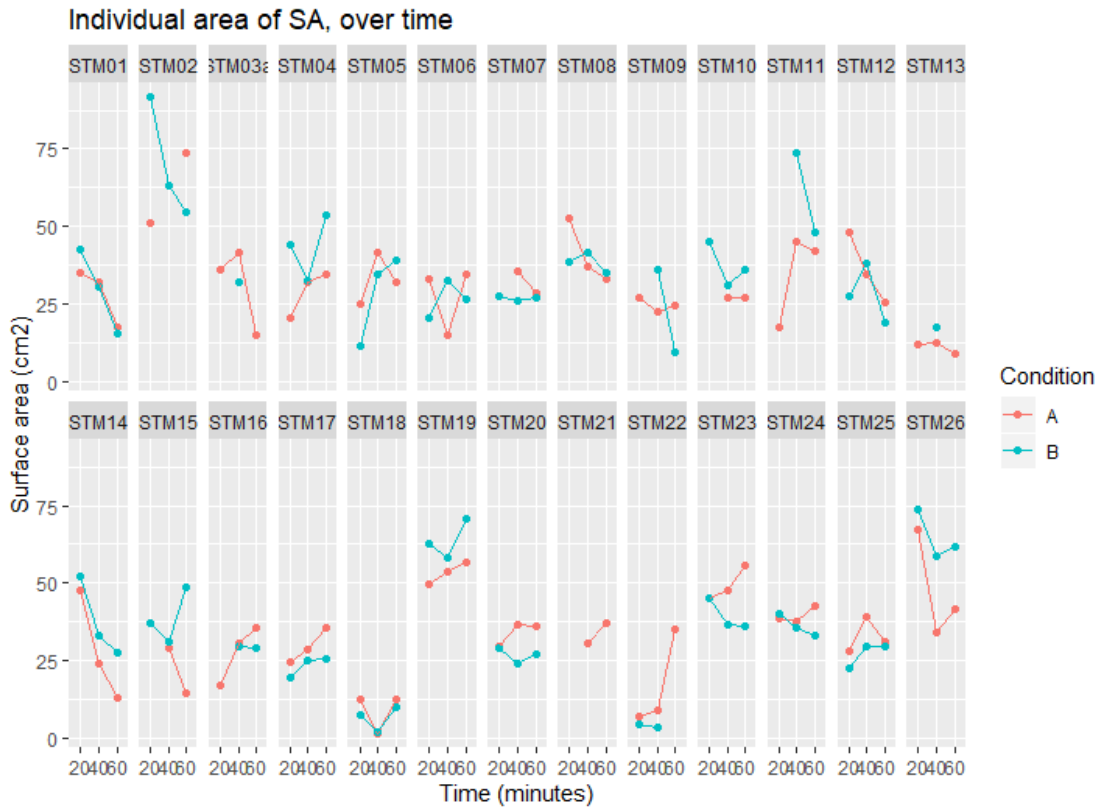

```
# Interpretation: Q-Q plot shows extremely minor deviation from the diagonal reference line
. Histogram shows acceptable distribution. Assumption was deemed to have been upheld.
```

Secondary outcome: surface area of secondary hyperalgesia

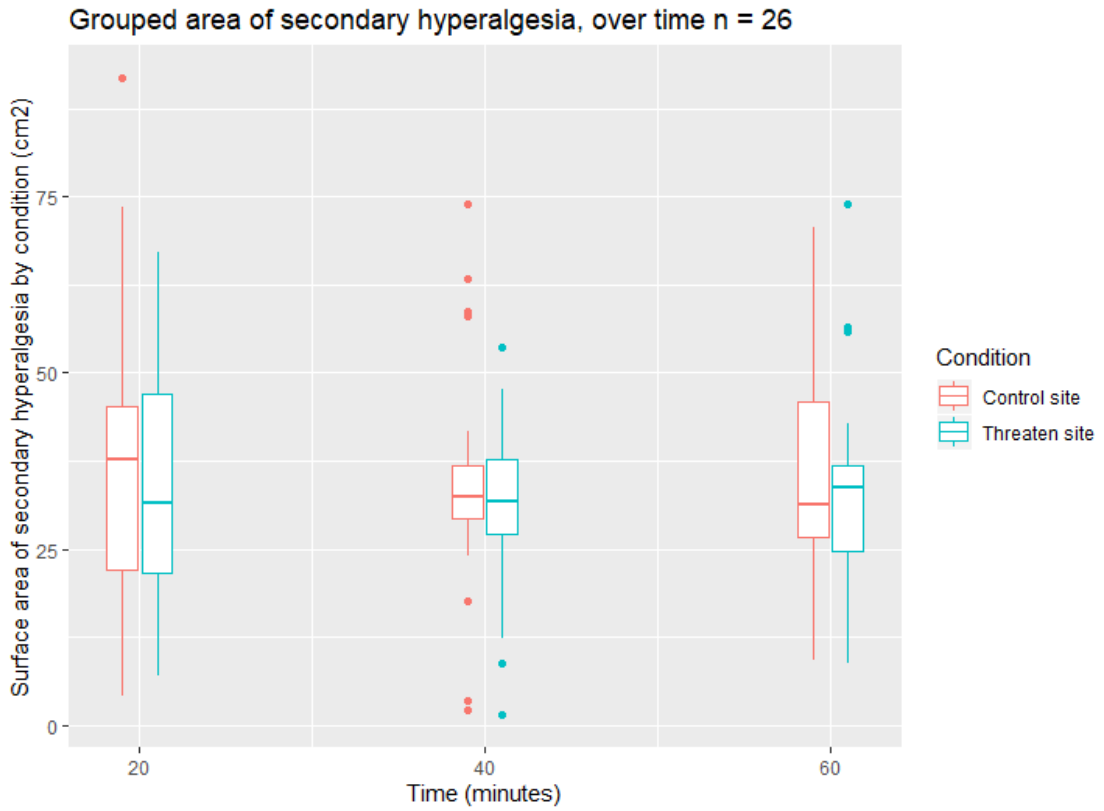
We are interested in determining the surface area of secondary hyperalgesia on each arm, and the comparison between arms.

```
SA <- master_data %>%
  select(group,
         id,
         time,
         phase,
         site,
         modality,
         rating) %>%
  filter(phase == 'test_sa')


SA %<>% mutate(condition = case_when(
  group == '1' & site == 'right' ~ 'A',
  group == '1' & site == 'left' ~ 'B',
  group == '2' & site == 'right' ~ 'B',
  group == '2' & site == 'left' ~ 'A')) %>%
  select(-group,
         -site,
         -phase)

# replace ND with 0

SA$rating[SA$rating == 'ND'] <- 0
names(SA)[names(SA) == 'rating'] <- 'point1'
names(SA)[names(SA) == 'modality'] <- 'radial_line'
```

```r
SA %<>% group_by(id, time) %>%
  mutate(point2 = case_when(radial_line == 'A' ~ lead(point1, 5),
                            radial_line == 'B' ~ lag(point1, 2),
                            radial_line == 'C' ~ lag(point1, 2),
                            radial_line == 'D' ~ lag(point1, 2),
                            radial_line == 'E' ~ lead(point1, 7),
                            radial_line == 'F' ~ lag(point1, 2),
                            radial_line == 'G' ~ lag(point1, 2),
                            radial_line == 'H' ~ lag(point1, 2))) %>%
  ungroup()

SA$point1 <- as.numeric(SA$point1)
SA$point2 <- as.numeric(SA$point2)
# Now calculate surface area for each triangle
library(REdaS)
sinangle <- sin((deg2rad(45)))

sinangle

## [1] 0.7071068

SA %<>% mutate(triangle = (point1*point2*sinangle)/2)

# Now calculate SA for each id, site and time

SA %<>% select(-radial_line,
                      -point1,
                      -point2)

SA %<>% group_by(id,
                 time,
                 condition) %>%
  summarise(SA = sum(triangle)) %>%
  ungroup()

SA_plot <- ggplot(data = SA) +
  aes(x = as.factor(time),
      y = SA,
      colour = condition,
      group = condition) +
  geom_point() +
  geom_line(aes(colour = condition)) +
  facet_wrap(~ id,
             nrow = 2) +
  guides(colour = guide_legend("Condition"), size = guide_legend("Condition")) +
  labs(x = 'Time (minutes)',
       y = 'Surface area (cm2)',
       title = 'Individual area of SA, over time')
SA_plot
```




```r
#Unblinding condition for graphs
SA$condition[SA$condition == 'A'] <- 'Threaten site'

SA$condition[SA$condition == 'B'] <- 'Control site'


# All participants, group data represented by boxplots
ggplot(data = SA) +
  aes(x = time,
      y = SA,
      group = interaction(time,condition),
      colour = condition) +
  geom_boxplot(width = 4) +
  scale_x_continuous(breaks = seq(20,60,20)) +
  guides(colour = guide_legend("Condition"), size = guide_legend("Condition")) +
  labs(title = 'Grouped area of secondary hyperalgesia, over time n = 26',
       y = 'Surface area of secondary hyperalgesia by condition (cm2)',
       x ='Time (minutes)')
```

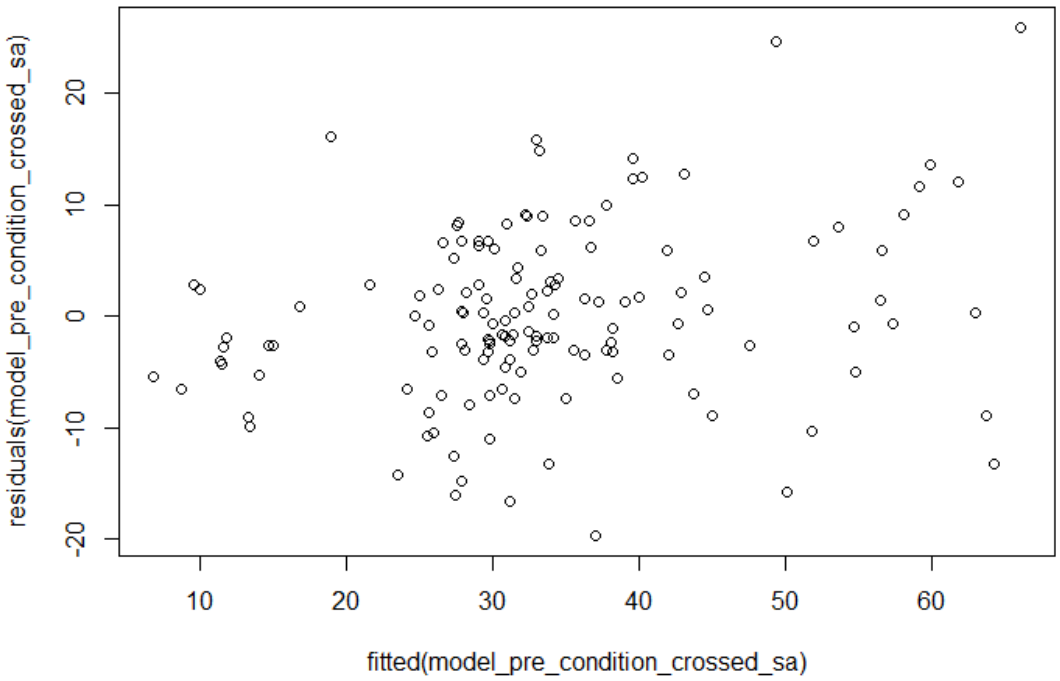



```
# We are interested in a main effect of condition on area of secondary hyperalgesia

model_null_sa <- lmer(SA ~ (1|id),
                      data = SA)
model_condition_sa <- lmer(SA ~ condition + (1|id),
                           data = SA)
anova(model_condition_sa, model_null_sa) # Condition does not predict area of secondary hyp
eralgesia

## Data: SA
## Models:
## model_null_sa: SA ~ (1 | id)
## model_condition_sa: SA ~ condition + (1 | id)
##                    Df    AIC    BIC  logLik deviance Chisq Chi Df
## model_null_sa       3 1107.4 1116.2 -550.70   1101.4
## model_condition_sa  4 1108.1 1119.9 -550.07   1100.1  1.27      1
##                    Pr(>Chisq)
## model_null_sa
## model_condition_sa     0.2598

model_pre_condition_crossed_sa <- lmer(SA ~ condition + (1|id/time),
                                       data = SA)

anova(model_pre_condition_crossed_sa, model_condition_sa)

## Data: SA
## Models:
## model_condition_sa: SA ~ condition + (1 | id)
## model_pre_condition_crossed_sa: SA ~ condition + (1 | id/time)
##                                Df    AIC    BIC  logLik deviance  Chisq
## model_condition_sa              4 1108.1 1119.9 -550.07   1100.1
## model_pre_condition_crossed_sa  5 1108.2 1122.9 -549.09   1098.2 1.9442
##                                Chi Df Pr(>Chisq)
## model_condition_sa
## model_pre_condition_crossed_sa      1     0.1632
```

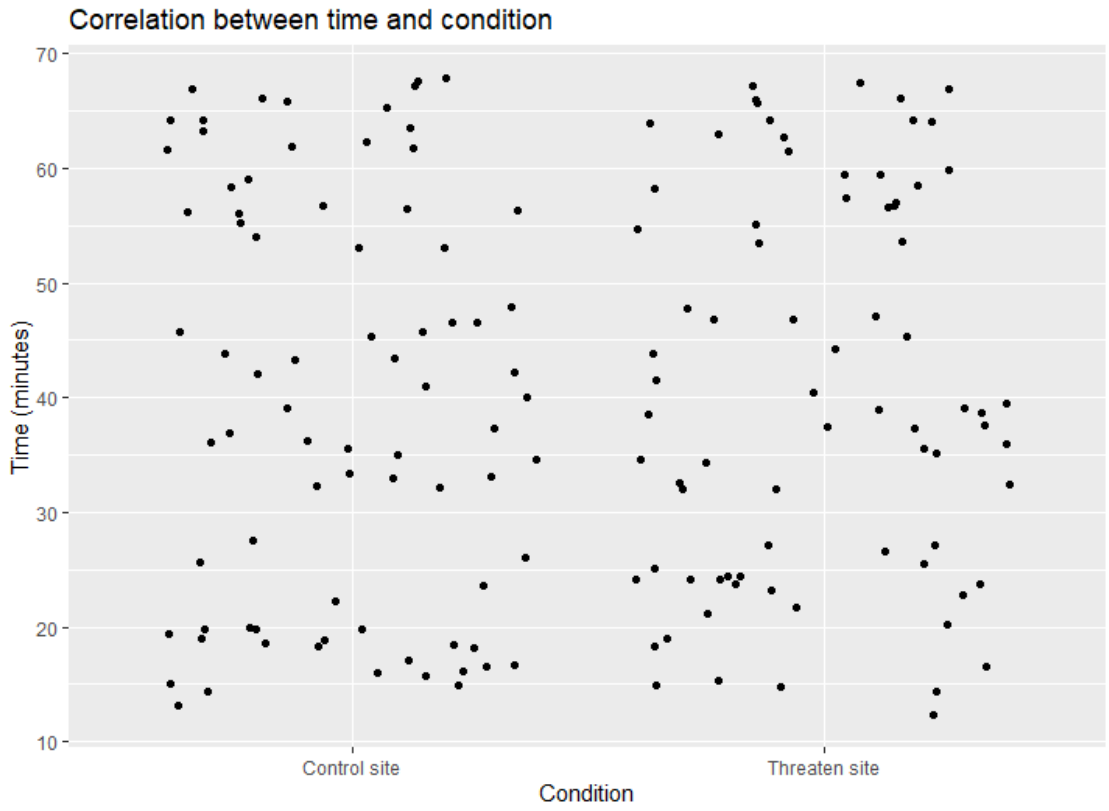
```
# not a significant improvement

# Secondary hyperalgesia surface area is not predicted by condition.
```

SA: Assessment of model fit

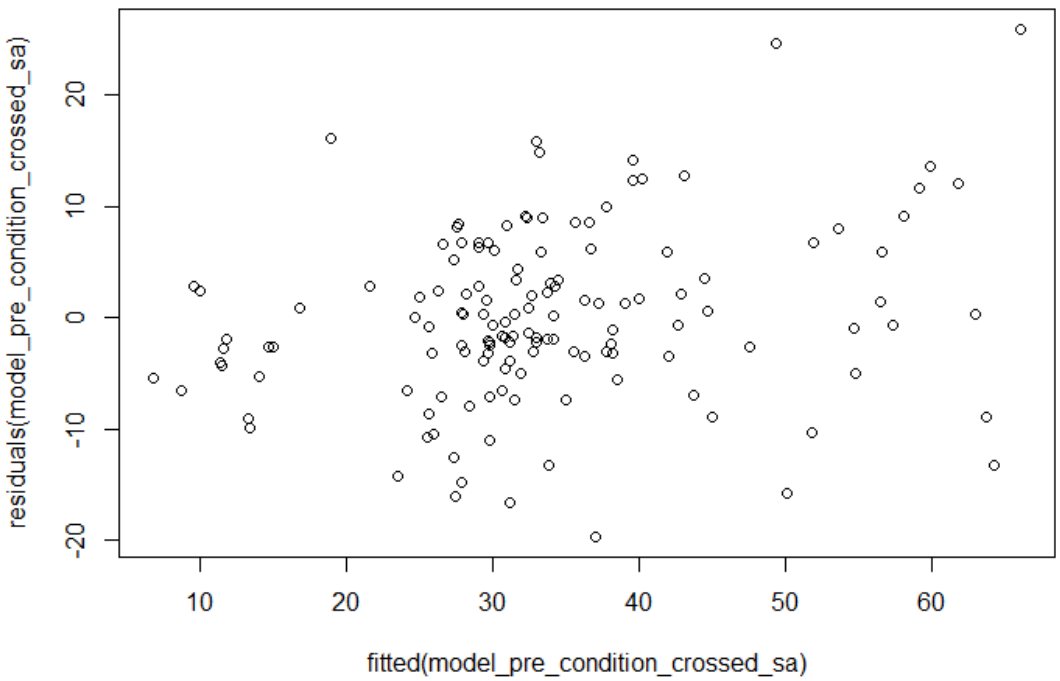
```
# The model is called model_pre_condition_crossed_sa and has the structure: SA ~ condition
+ (1|id/time)

# Assumption 1: linearity
# Plot fitted values against residual values, for fixed factor 'condition'.

plot(fitted(model_pre_condition_crossed_sa), residuals(model_pre_condition_crossed_sa))
```



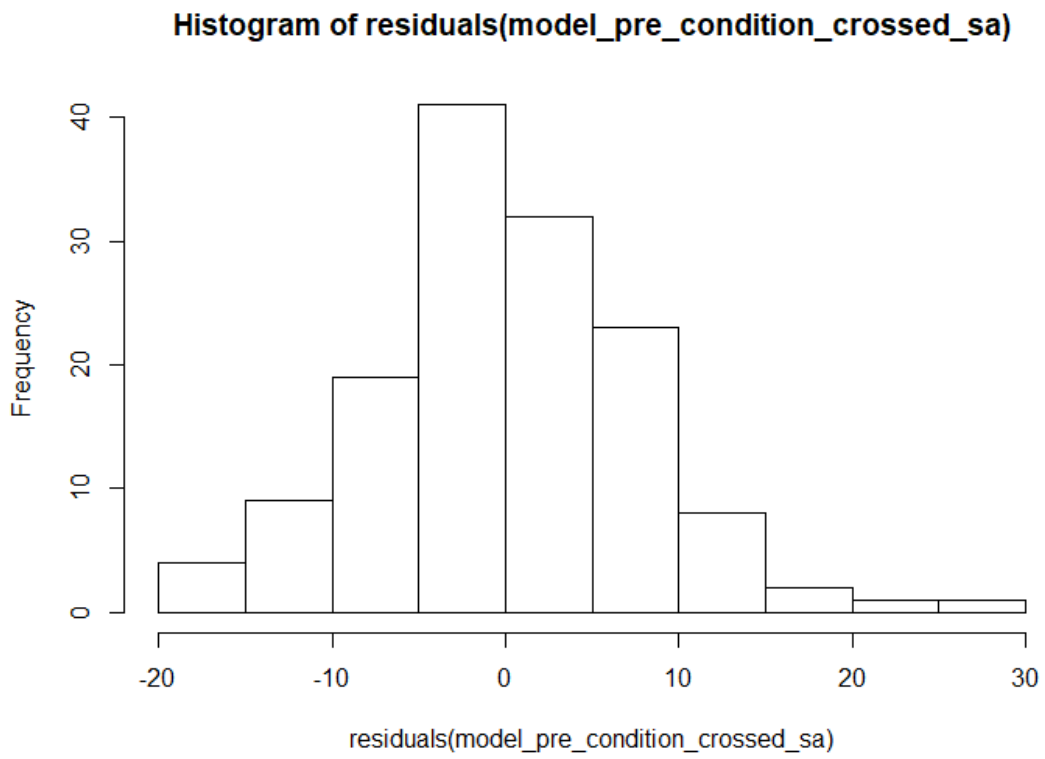

```
# Interpretation: No obvious linear or curvilinear pattern. Assumption was deemed to have b
een upheld.

# Assumption 2: absence of collinearity
# Check for correlation between time and condition

ggplot(data = SA) +
  aes(x = condition,
      y = time) +
  geom_jitter() +
  geom_smooth() +
labs(title = 'Correlation between time and condition',
       x = 'Condition',
       y = 'Time (minutes)')
```



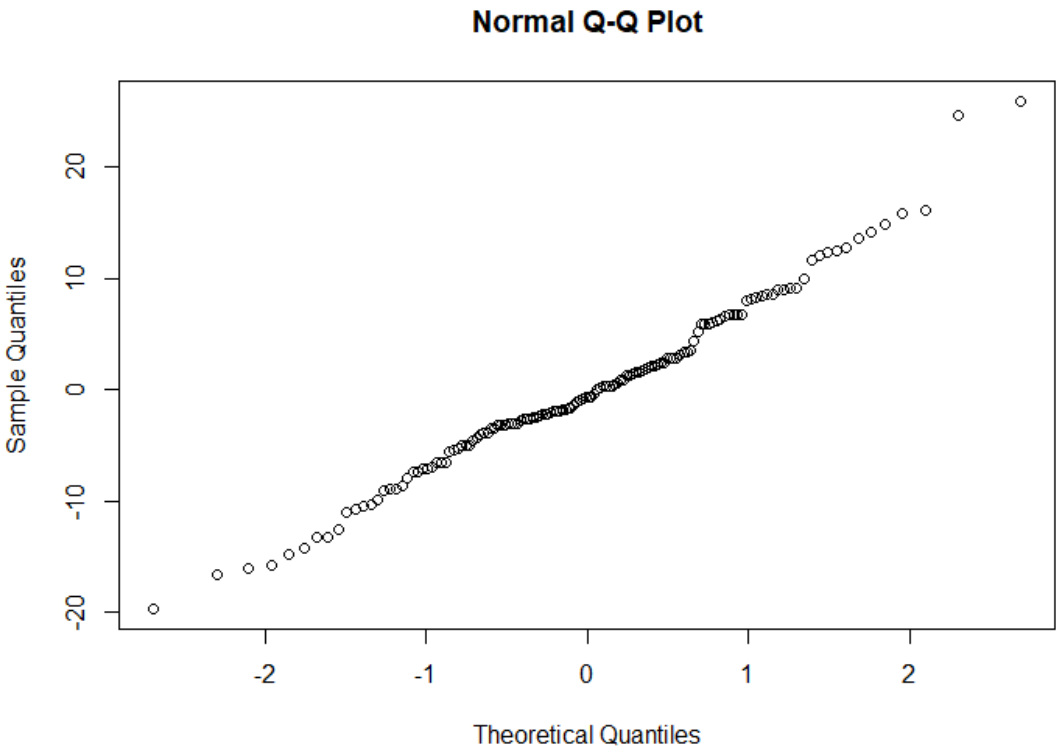

```
# Interpretation: no collinearity. Assumption was deemed to have been upheld.

# Assumption 3: Homoscedasticity, i.e. equal variance across the range of predicted values.
plot(fitted(model_pre_condition_crossed_sa), residuals(model_pre_condition_crossed_sa))
```



```
## Interpretation: slightly increased density in a blob in the middle (i.e. more data point
s here). The range of max and min values seems slightly lower on the left than on the right
.


# Assumption 4: Normally distributed residuals
# Plot residuals
hist(residuals(model_pre_condition_crossed_sa))
```




```
qqnorm(residuals(model_pre_condition_crossed_sa))
```




```
# Interpretation: Q-Q plot and histogram show normal distribution. Assumption was deemed to have been upheld.
```

Exploratory outcomes:

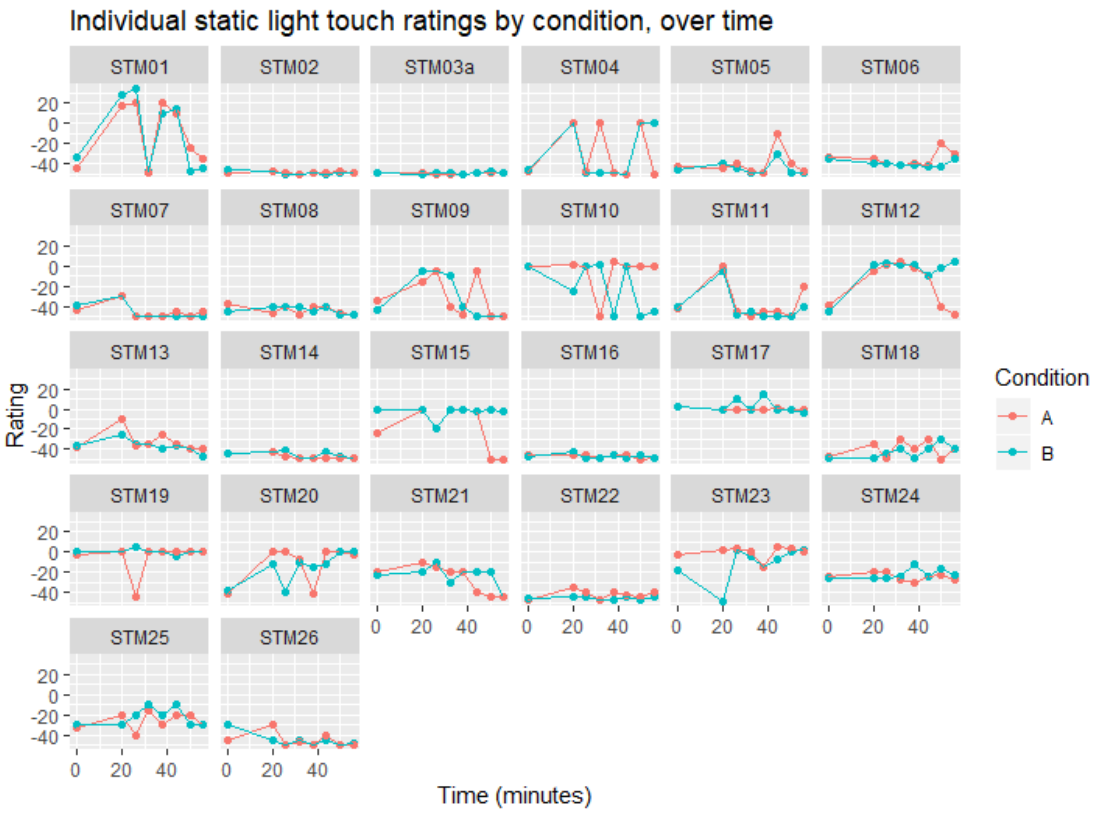
Sensory outcomes

We are interested in:

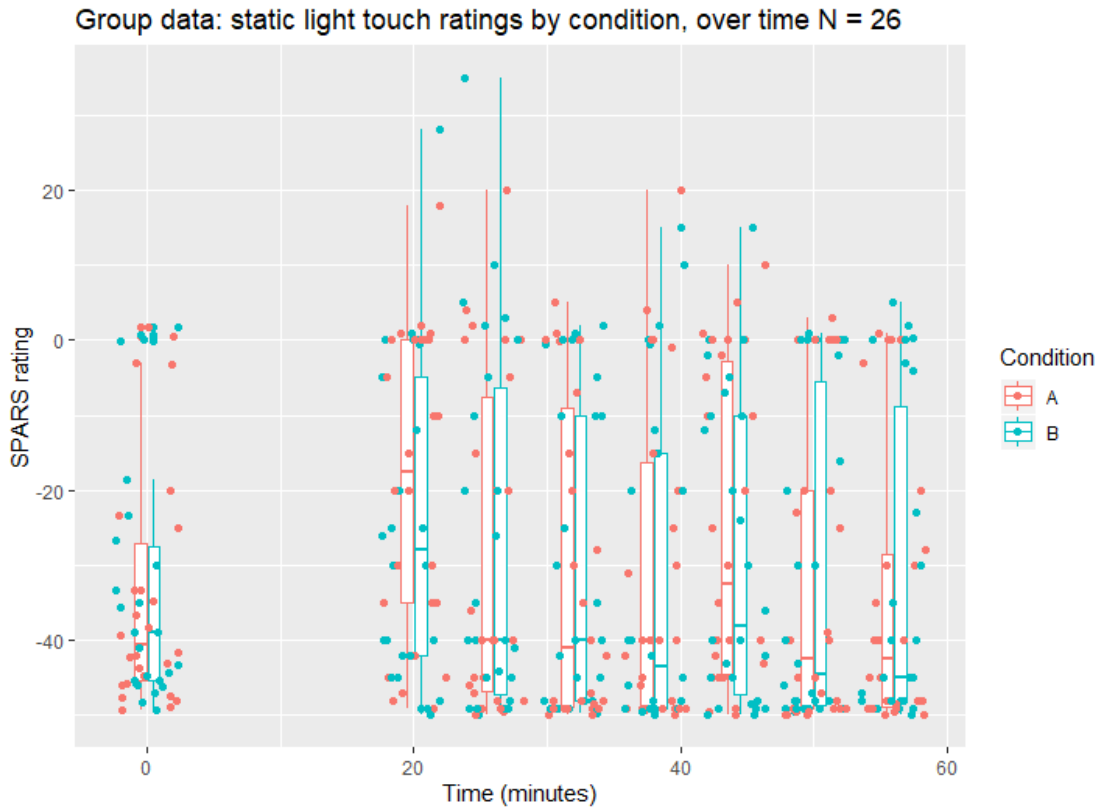
- Determining the intensity of static light touch on each arm, and the comparison between arms.
- Determining the intensity of dynamic light touch on each arm, and the comparison between arms.
- Determining the intensity of e-stim on each arm, and the comparison between arms.

```r
## Static light touch

slt <- master_data %>%
  select(group,
         id,
         time,
         phase,
         site,
         modality,
         rating) %>%
  filter(modality == 'VFF') %>%
  filter(phase != 'orientation') %>%
  mutate(time = if_else(phase == 'baseline', time*-1L, time))

slt %<>% mutate(condition = case_when(
  group == '1' & site == 'right' ~ 'A',
  group == '1' & site == 'left' ~ 'B',
  group == '2' & site == 'right' ~ 'B',
  group == '2' & site == 'left' ~ 'A')) %>%
  select(-group,
         -site)
slt$rating <- as.numeric(slt$rating)

slt_bl <- slt %>% filter(phase == 'baseline') %>%
  group_by(id, condition) %>%
  summarise(rating = mean(rating)) %>%
  ungroup() %>%
  mutate(time = 0) %>% ungroup()

slt_fu <- slt %>% filter(phase != 'baseline') %>% ungroup () %>%
  select(-phase,
         -modality)

slt <- rbind(slt_bl, slt_fu)

slt_wide <- slt %>%
  group_by(id) %>%
  spread(key = condition,
         value = rating)

#Determining the mean (SD) anxiety rating per condition
mean(slt_wide$A, na.rm = TRUE)

## [1] -29.26619

sd(slt_wide$A, na.rm = TRUE)

## [1] 20.4084

mean(slt_wide$B, na.rm = TRUE)

## [1] -29.58072

sd(slt_wide$B, na.rm = TRUE)

## [1] 20.78741

# For interest, plot the pilot data

ggplot(data = slt) +
  aes(x = time,
      y = rating,
      group = condition,
      colour = condition) +
  facet_wrap(~ id) +
  geom_point() +
```

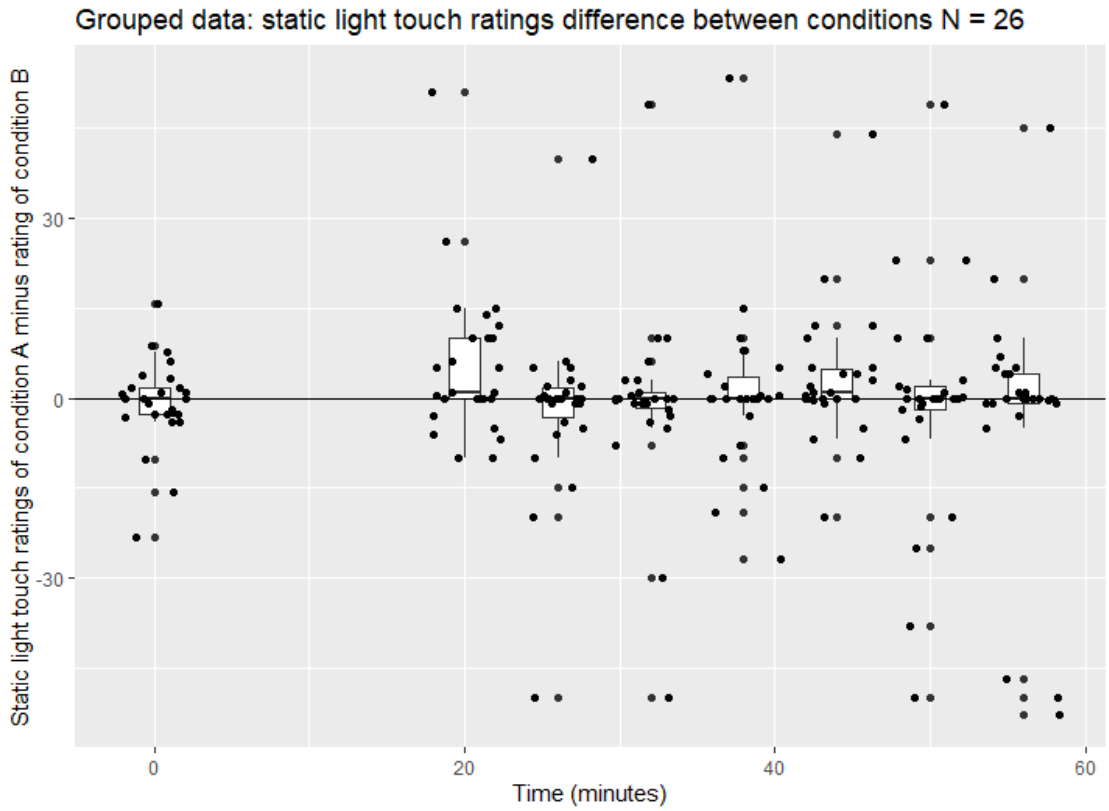
```
  geom_line() +
  guides(colour = guide_legend("Condition"), size = guide_legend("Condition")) +
  labs(title = 'Individual static light touch ratings by condition, over time',
       y = 'Rating',
       x = 'Time (minutes)')
```




```
# All participants, group data represented by boxplots
ggplot(data = slt) +
  aes(x = time,
      y = rating,
      group = interaction(condition, time),
      colour = condition) +
  geom_boxplot(width = 2) +
  geom_point(position = 'jitter') +
  guides(colour = guide_legend("Condition"), size = guide_legend("Condition")) +
  labs(title = 'Group data: static light touch ratings by condition, over time N = 26',
       y = 'SPARS rating',
       x = 'Time (minutes)')
```




```
 # Calculate diff between conditions for group boxplots

slt_wide <- slt %>%
  group_by(id,
           time) %>%
  spread(key = condition,
         value = rating) %>%
  mutate(site_diff = A-B)

# All participants, group data represented by boxplots
ggplot(data = slt_wide) +
  aes(x = time,
      y = site_diff,
      group = time) +
  geom_boxplot(width = 2) +
  geom_point(position = 'jitter') +
  geom_hline(yintercept = 0) +
  labs(title = 'Grouped data: static light touch ratings difference between conditions N =
26',
       y = 'Static light touch ratings of condition A minus rating of condition B',
       x = 'Time (minutes)')
```




```
# Collapse all post-baseline ratings for each condition and then compare them using a RM AN
OVA type model. Time is nested within (and fully crossed with) ID.

slt_bl %<>% select(-time)
names(slt_bl)[names(slt_bl) == 'rating'] <- 'baseline_rating'
collapse_slt <- slt_fu %>% # resolve each rating relative to baseline
  right_join(slt_bl) %>%
  mutate(rating_controlled = rating-baseline_rating) %>%
  select(id,
         time,
         condition,
         rating_controlled) # spread to calculate difference between conditions

# We want to find out whether rating_controlled (for baseline) is predicted by condition.

model_null_slt <- lmer(rating_controlled ~ (1|id),
                   data = collapse_slt)
model_condition_slt <- lmer(rating_controlled ~ condition + (1|id),
                       data = collapse_slt)
anova(model_condition_slt, model_null_slt) # No improvement

## Data: collapse_slt
## Models:
## model_null_slt: rating_controlled ~ (1 | id)
## model_condition_slt: rating_controlled ~ condition + (1 | id)
##                       Df    AIC    BIC  logLik deviance  Chisq Chi Df
## model_null_slt         3 3013.4 3025.1 -1503.7   3007.4
## model_condition_slt    4 3014.5 3030.1 -1503.3   3006.5 0.8949      1
##                       Pr(>Chisq)
## model_null_slt
## model_condition_slt       0.3442

model_pre_condition_crossed_slt <- lmer(rating_controlled ~ condition + (1|id/time),
                                    data = collapse_slt)

anova(model_pre_condition_crossed_slt, model_null_slt)
```

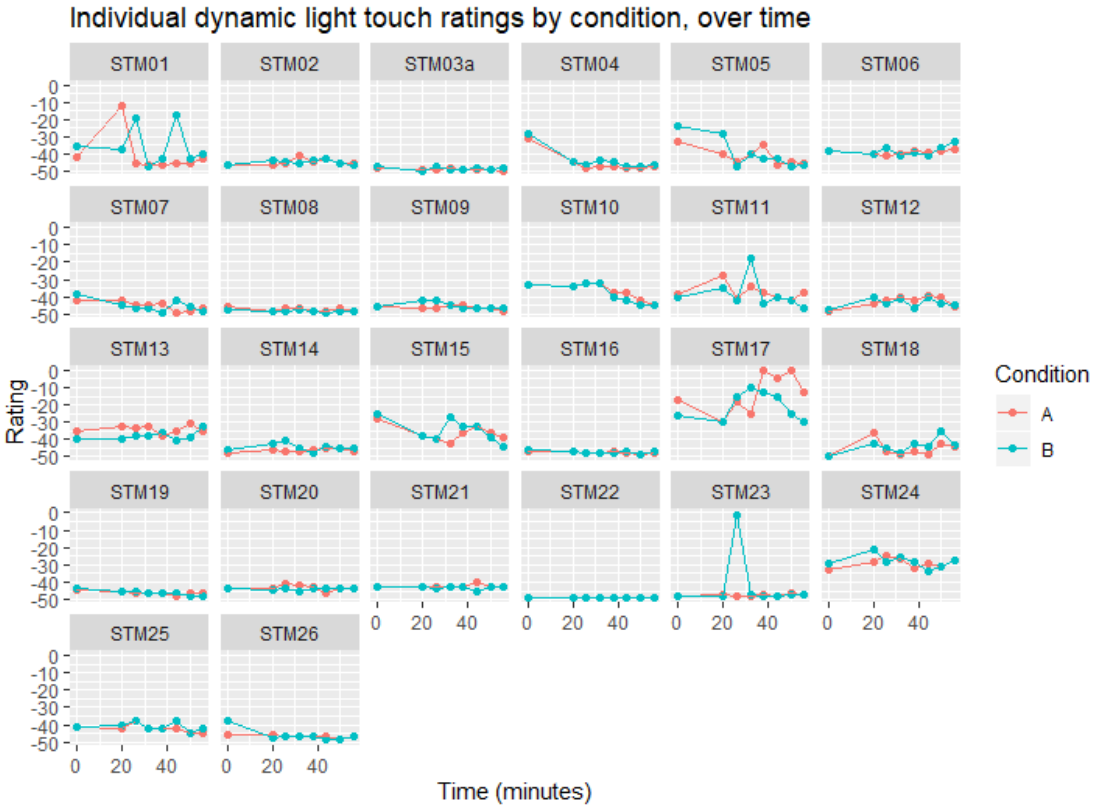
```
## Data: collapse_slt
## Models:
## model_null_slt: rating_controlled ~ (1 | id)
## model_pre_condition_crossed_slt: rating_controlled ~ condition + (1 | id/time)
##                                 Df    AIC    BIC  logLik deviance  Chisq
## model_null_slt                   3 3013.4 3025.1 -1503.7   3007.4
## model_pre_condition_crossed_slt  5 2997.1 3016.6 -1493.6   2987.1 20.307
##                                 Chi Df Pr(>Chisq)
## model_null_slt
## model_pre_condition_crossed_slt      2  3.893e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Significant improvement. Condition predicts static light touch ratings but only in fully
crossed model.

# Dynamic light touch

dlt <- master_data %>%
  select(group,
         id,
         time,
         phase,
         site,
         modality,
         rating) %>%
  filter(modality == 'CW' | modality == 'BR') %>%
  filter(phase != 'orientation') %>%
  mutate(time = if_else(phase == 'baseline', time*-1L, time))

dlt %<>% mutate(condition = case_when(
  group == '1' & site == 'right' ~ 'A',
  group == '1' & site == 'left' ~ 'B',
  group == '2' & site == 'right' ~ 'B',
  group == '2' & site == 'left' ~ 'A')) %>%
  select(-group,
         -site)
dlt$rating <- as.numeric(dlt$rating)

dlt_bl <- dlt %>% filter(phase == 'baseline') %>%
  group_by(id, condition) %>%
  summarise(dlt_rating = mean(rating)) %>%
  ungroup() %>%
  mutate(time = 0) %>% ungroup()

dlt_fu <- dlt %>% filter(phase != 'baseline') %>% ungroup() %>%
  group_by(id,
           time,
           condition) %>%
  summarise(dlt_rating = mean(rating)) %>%
  ungroup()

dlt <- rbind(dlt_bl, dlt_fu)


dlt_wide <- dlt %>%
  group_by(id) %>%
  spread(key = condition,
         value = dlt_rating)

#Determining the mean (SD) dlt rating per condition
mean(dlt_wide$A, na.rm = TRUE)
```

```
## [1] -41.70841
```

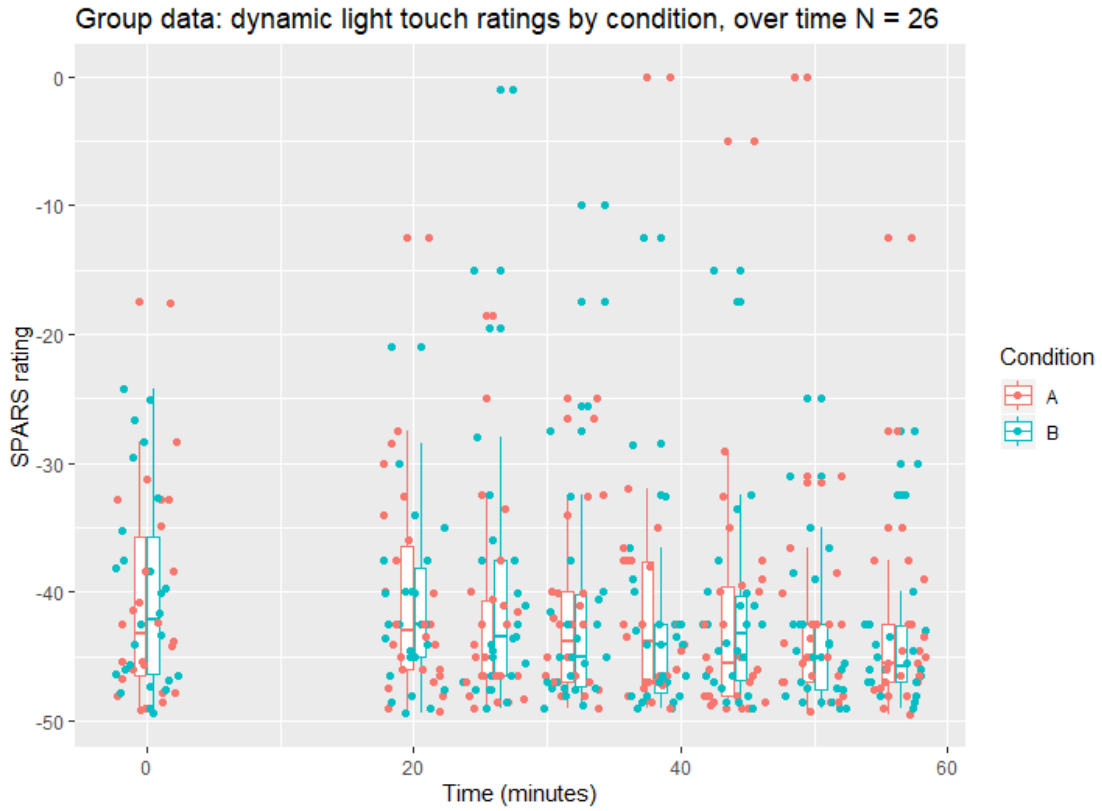
```
sd(dlt_wide$A, na.rm = TRUE)
```

```
## [1] 8.393769

mean(dlt_wide$B, na.rm = TRUE)

## [1] -41.41579

sd(dlt_wide$B, na.rm = TRUE)

## [1] 8.308582

# For interest, plot the data

ggplot(data = dlt) +
  aes(x = time,
      y = dlt_rating,
      group = condition,
      colour = condition) +
  facet_wrap(~ id) +
  geom_point() +
  geom_line() +
  guides(colour = guide_legend("Condition"), size = guide_legend("Condition")) +
  labs(title = 'Individual dynamic light touch ratings by condition, over time',
       y = 'Rating',
       x = 'Time (minutes)')
```

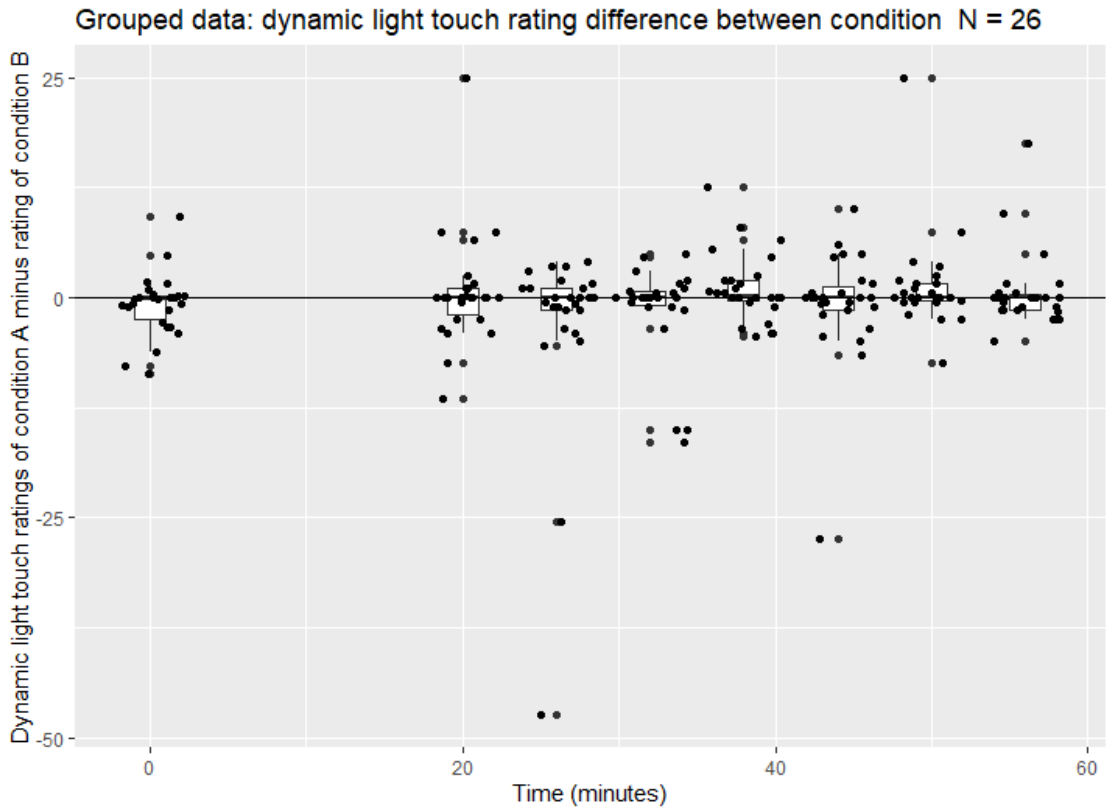



```
# All participants, group data represented by boxplots
ggplot(data = dlt) +
  aes(x = time,
      y = dlt_rating,
      group = interaction(condition, time),
      colour = condition) +
  geom_boxplot(width = 2) +
  geom_point(position = 'jitter') +
  guides(colour = guide_legend("Condition"), size = guide_legend("Condition")) +
  labs(title = 'Group data: dynamic light touch ratings by condition, over time N = 26',
```

```
       y = 'SPARS rating',
       x = 'Time (minutes)')
```



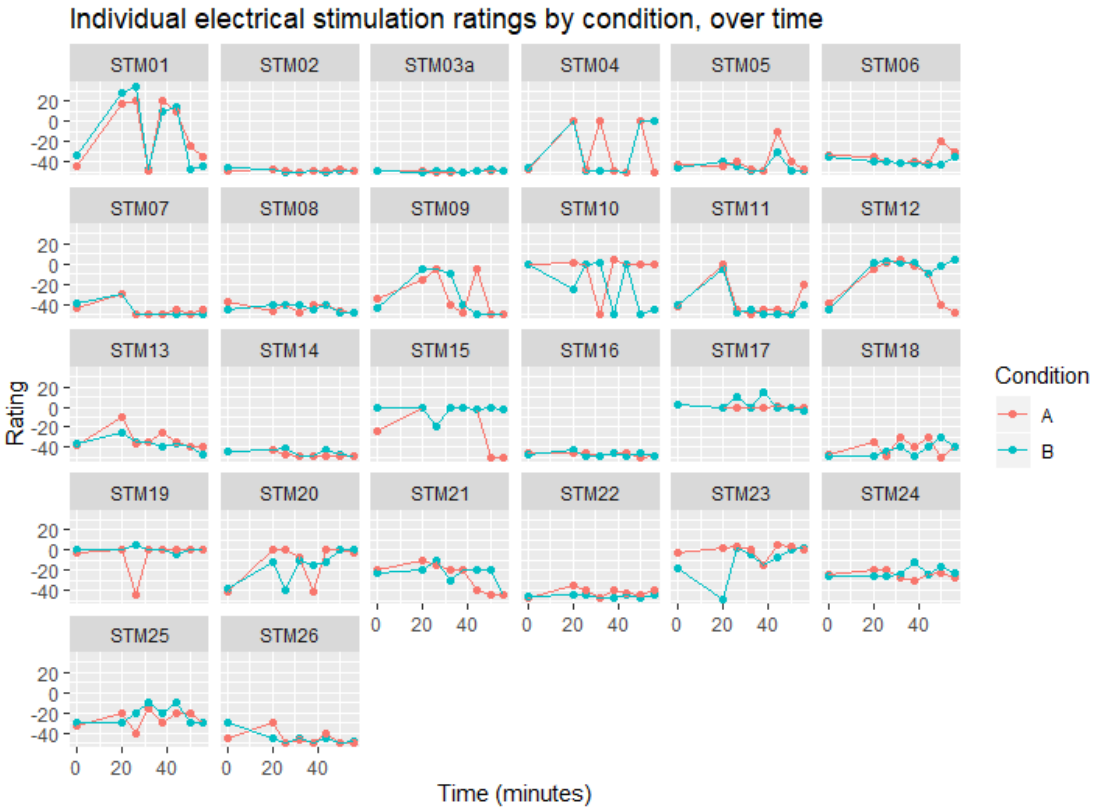

```
 # Calculate difference between conditions for group boxplots

dlt_wide <- dlt %>%
  group_by(id,
           time) %>%
  spread(key = condition,
         value = dlt_rating) %>%
  mutate(site_diff = A-B)

# All participants, group data represented by boxplots
ggplot(data = dlt_wide) +
  aes(x = time,
      y = site_diff,
      group = time) +
  geom_boxplot(width = 2) +
  geom_point(position = 'jitter') +
  geom_hline(yintercept = 0) +
  labs(title = 'Grouped data: dynamic light touch rating difference between condition  N =
26',
       y = 'Dynamic light touch ratings of condition A minus rating of condition B',
       x = 'Time (minutes)')
```



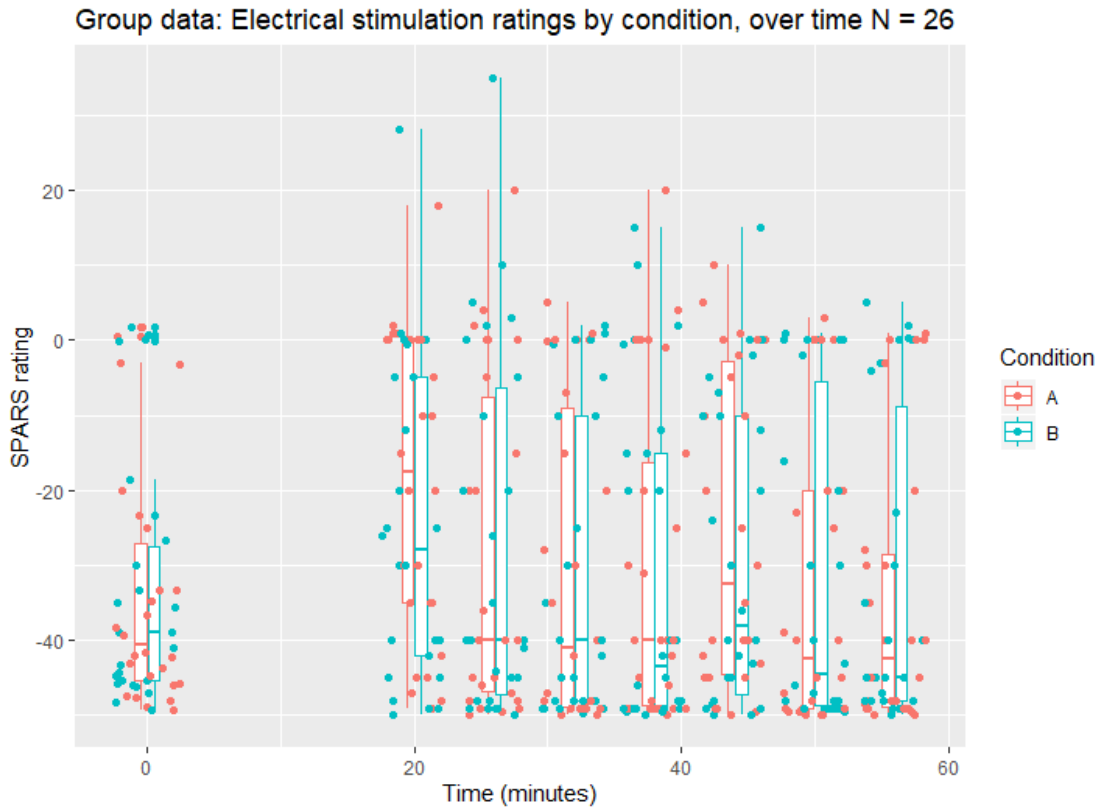

```
# Collapse all post-baseline ratings for each condition and then compare them using a RM AN
OVA type model. Time is nested within (and fully crossed with) ID.

dlt_bl %<>% select(-time)
names(dlt_bl)[names(dlt_bl) == 'dlt_rating'] <- 'baseline_rating'
collapse_dlt <- dlt_fu %>% # resolve each rating relative to baseline
  right_join(dlt_bl) %>%
  mutate(rating_controlled = dlt_rating-baseline_rating) %>%
  select(id,
         time,
         condition,
         rating_controlled) # spread to calculate difference between conditions

# We want to find out whether rating_cotrolled (for baseline) is predicted by condition.

model_null_dlt <- lmer(rating_controlled ~ (1|id),
                   data = collapse_dlt)
model_condition_dlt <- lmer(rating_controlled ~ condition + (1|id),
                       data = collapse_dlt)
anova(model_condition_dlt, model_null_dlt) # Condition predicts DLT rating

## Data: collapse_dlt
## Models:
## model_null_dlt: rating_controlled ~ (1 | id)
## model_condition_dlt: rating_controlled ~ condition + (1 | id)
##                     Df    AIC    BIC  logLik deviance  Chisq Chi Df
## model_null_dlt       3 2280.3 2292.0 -1137.2   2274.3
## model_condition_dlt  4 2280.7 2296.3 -1136.3   2272.7 1.6108      1
##                     Pr(>Chisq)
## model_null_dlt
## model_condition_dlt     0.2044

model_pre_condition_crossed_dlt <- lmer(rating_controlled ~ condition + (1|id/time),
                                   data = collapse_dlt)

anova(model_pre_condition_crossed_dlt, model_null_dlt) # not better
```

```
## Data: collapse_dlt
## Models:
## model_null_dlt: rating_controlled ~ (1 | id)
## model_pre_condition_crossed_dlt: rating_controlled ~ condition + (1 | id/time)
##                                 Df    AIC    BIC  logLik deviance  Chisq
## model_null_dlt                   3 2280.3 2292.0 -1137.2   2274.3
## model_pre_condition_crossed_dlt  5 2281.3 2300.8 -1135.7   2271.3 2.9775
##                                 Chi Df Pr(>Chisq)
## model_null_dlt
## model_pre_condition_crossed_dlt      2     0.2257

# Condition predicts dynamic light touch ratings.

# Electrical stimulation

estim <- master_data %>%
  select(group,
         id,
         time,
         phase,
         site,
         modality,
         rating) %>%
  filter(modality == 'VFF') %>%
  filter(phase != 'orientation') %>%
  mutate(time = if_else(phase == 'baseline', time*-1L, time))

estim %<>% mutate(condition = case_when(
  group == '1' & site == 'right' ~ 'A',
  group == '1' & site == 'left' ~ 'B',
  group == '2' & site == 'right' ~ 'B',
  group == '2' & site == 'left' ~ 'A')) %>%
  select(-group,
         -site)
estim$rating <- as.numeric(estim$rating)

estim_bl <- estim %>% filter(phase == 'baseline') %>%
  group_by(id, condition) %>%
  summarise(rating = mean(rating)) %>%
  ungroup() %>%
  mutate(time = 0) %>% ungroup()

estim_fu <- estim %>% filter(phase != 'baseline') %>% ungroup () %>%
  select(-phase,
         -modality)

estim <- rbind(estim_bl, estim_fu)

estim_wide <- estim %>%
  group_by(id) %>%
  spread(key = condition,
         value = rating)

#Determining the mean (SD) anxiety rating per condition
mean(estim_wide$A, na.rm = TRUE)

## [1] -29.26619

sd(estim_wide$A, na.rm = TRUE)

## [1] 20.4084

mean(estim_wide$B, na.rm = TRUE)

## [1] -29.58072
```

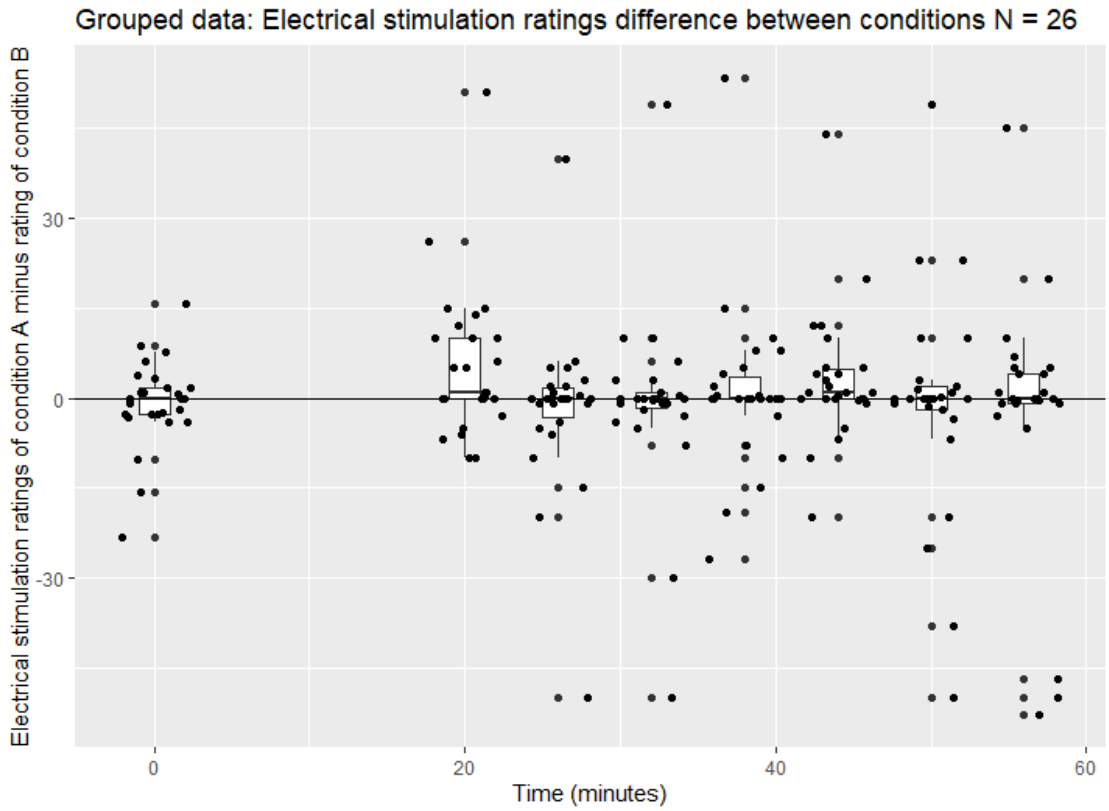
```r
sd(estim_wide$B, na.rm = TRUE)

## [1] 20.78741

# For interest, plot the data

ggplot(data = estim) +
  aes(x = time,
      y = rating,
      group = condition,
      colour = condition) +
  facet_wrap(~ id) +
  geom_point() +
  geom_line() +
  guides(colour = guide_legend("Condition"), size = guide_legend("Condition")) +
  labs(title = 'Individual electrical stimulation ratings by condition, over time',
       y = 'Rating',
       x = 'Time (minutes)')
```




```r
# All participants, group data represented by boxplots
ggplot(data = estim) +
  aes(x = time,
      y = rating,
      group = interaction(condition, time),
      colour = condition) +
  geom_boxplot(width = 2) +
  geom_point(position = 'jitter') +
  guides(colour = guide_legend("Condition"), size = guide_legend("Condition")) +
  labs(title = 'Group data: Electrical stimulation ratings by condition, over time N = 26',
       y = 'SPARS rating',
       x = 'Time (minutes)')
```




```
 # Calculate difference between conditions for group boxplots

estim_wide <- estim %>%
  group_by(id,
           time) %>%
  spread(key = condition,
         value = rating) %>%
  mutate(site_diff = A-B)

# All participants, group data represented by boxplots
ggplot(data = estim_wide) +
  aes(x = time,
      y = site_diff,
      group = time) +
  geom_boxplot(width = 2) +
  geom_point(position = 'jitter') +
  geom_hline(yintercept = 0) +
  labs(title = 'Grouped data: Electrical stimulation ratings difference between conditions
N = 26',
       y = 'Electrical stimulation ratings of condition A minus rating of condition B',
       x = 'Time (minutes)')
```




```
# Collapse all post-baseline ratings for each condition and then compare them using a RM AN
OVA type model. Time is nested within (and fully crossed with) ID.

estim_bl %<>% select(-time)
names(estim_bl)[names(estim_bl) == 'rating'] <- 'baseline_rating'
collapse_estim <- estim_fu %>% # resolve each rating relative to baseline
  right_join(estim_bl) %>%
  mutate(rating_controlled = rating-baseline_rating) %>%
  select(id,
         time,
         condition,
         rating_controlled) # spread to calculate difference between conditions

# We want to find out whether rating_cotrolled (for baseline) is predicted by condition.

model_null_estim <- lmer(rating_controlled ~ (1|id),
                   data = collapse_estim)
model_condition_estim <- lmer(rating_controlled ~ condition + (1|id),
                      data = collapse_estim)
anova(model_condition_estim, model_null_estim) # No improvement

## Data: collapse_estim
## Models:
## model_null_estim: rating_controlled ~ (1 | id)
## model_condition_estim: rating_controlled ~ condition + (1 | id)
##                        Df    AIC    BIC  logLik deviance  Chisq Chi Df
## model_null_estim        3 3013.4 3025.1 -1503.7   3007.4
## model_condition_estim   4 3014.5 3030.1 -1503.3   3006.5 0.8949      1
##                        Pr(>Chisq)
## model_null_estim
## model_condition_estim     0.3442

model_pre_condition_crossed_estim <- lmer(rating_controlled ~ condition + (1|id/time),
                                  data = collapse_estim)

anova(model_pre_condition_crossed_estim, model_null_estim)
```

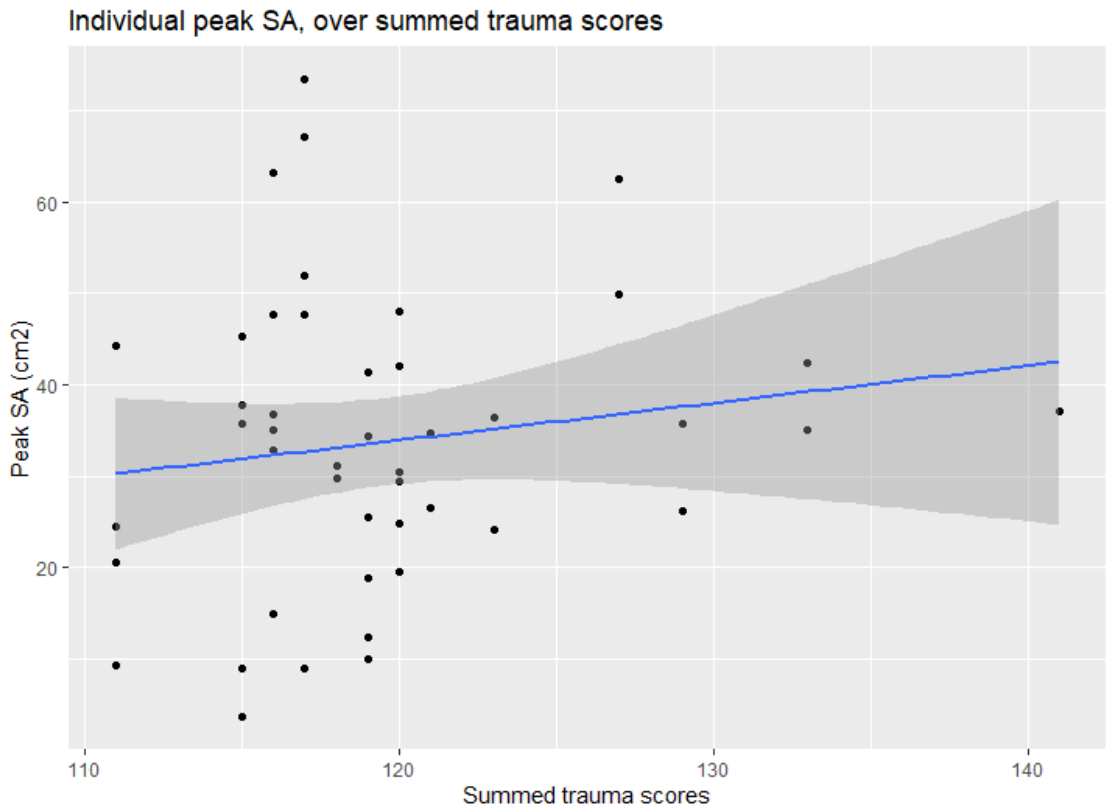
```
## Data: collapse_estim
## Models:
## model_null_estim: rating_controlled ~ (1 | id)
## model_pre_condition_crossed_estim: rating_controlled ~ condition + (1 | id/time)
##                                  Df    AIC    BIC  logLik deviance  Chisq
## model_null_estim                  3 3013.4 3025.1 -1503.7   3007.4
## model_pre_condition_crossed_estim  5 2997.1 3016.6 -1493.6   2987.1 20.307
##                                  Chi Df Pr(>Chisq)
## model_null_estim
## model_pre_condition_crossed_estim      2  3.893e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# Significant improvement... condition predicts Electrical stimulation ratings but only in
fully crossed model.
```

Childhood and recent trauma scores

We are interested in exploring the relationship between trauma history and area of SA.

```
cidi <- master_data[ , grep('cidi', colnames(master_data))]
cidi$cidi_total <- rowSums(cidi)
ctq <- master_data[ , grep('ctq', colnames(master_data))]
ctq$ctq_total <- rowSums(ctq)

trauma <- cbind(master_data$id, cidi, ctq)
names(trauma)[names(trauma) == 'master_data$id'] <- 'id'
trauma %<>% mutate(trauma = cidi_total + ctq_total) %>%
  select(id,
         trauma)
trauma <- unique(trauma)

SA_trauma <- SA %>% right_join(trauma)

# We need to identify the best time point from which to draw the SA data for each participa
nt. We will do this by identifying the time of peak SH intensity individually, and then use
the SA time point closest to that time.

# Considering that secondary hyperalgesia needs first to be verified before the surface are
a becomes a meaningful outcome, we first identified the time at which each participant show
ed the peak SH intensity (mean of both conditions). We then used that individualised time p
oint for each participant and identified the SA of SH at that time point, using that as the
peak (relevant) SA for that participant.

intensity_time <- intensity %>% group_by(id, time) %>%
  summarise(mean_int = mean(pp_rating)) %>%
  filter(time != 0) %>%
  ungroup() %>%
  group_by(id) %>%
  filter(mean_int == max(mean_int))

# Now identify SA at those time point, using the df 'intensity_time'. Adjust values to clos
est applicable SA assessment times - i.e. 20 or 26 (int) to 20 mins, 32 or 38 or 44 to 40 m
ins, 50 or 56 to 60 mins. # Complete manually

SA_at_peak <- SA_trauma %>%
  filter(id == 'STM01' & time == 20 |
           id == 'STM02' & time == 40 |
           id == 'STM03a' & time == 60 |
           id == 'STM04' & time == 20 |
           id == 'STM05' & time == 40 |
           id == 'STM06' & time == 60 |
           id == 'STM07' & time == 40 |
           id == 'STM08' & time == 60 |
           id == 'STM09' & time == 60 |
```

```
           id == 'STM10' & time == 20 |
           id == 'STM11' & time == 60 |
           id == 'STM12' & time == 60 |
           id == 'STM13' & time == 60 |
           id == 'STM14' & time == 20 |
           id == 'STM15' & time == 20 |
           id == 'STM16' & time == 40 |
           id == 'STM17' & time == 20 |
           id == 'STM18' & time == 60 |
           id == 'STM19' & time == 20 |
           id == 'STM20' & time == 40 |
           id == 'STM21' & time == 20 |
           id == 'STM22' & time == 40 |
           id == 'STM23' & time == 40 |
           id == 'STM24' & time == 40 |
           id == 'STM25' & time == 60 |
           id == 'STM26' & time == 20 ) # for STM20 the mean_int was the same at time point
s 38 and 50. Therefore adjusted time point to 40 minutes because 40 is closer to 38 than 50
is to 60.


names(SA_at_peak)[names(SA_at_peak) == 'SA'] <- 'peak_SA'

ggplot(data = SA_at_peak) +
  aes(x = trauma,
      y = peak_SA) +
  geom_point() +
  geom_smooth(method = 'lm')+
  labs(title = 'Individual peak SA, over summed trauma scores',
       y = 'Peak SA (cm2)',
       x = 'Summed trauma scores')
```




```
# First check distributional assumptions for correlation.
# Shapiro-Wilk normality test for peak surface area
with(SA_at_peak, shapiro.test(peak_SA)) # P = 0.483
```

```
##
##  Shapiro-Wilk normality test
##
## data:  peak_SA
## W = 0.97644, p-value = 0.483

# Pearsons correlation

correlation <- cor.test(SA_at_peak$trauma, SA_at_peak$peak_SA, method= "pearson")
correlation # P = 0.307 therefore no significant relationship between summed trauma scores
and peak SA.

##
##  Pearson's product-moment correlation
##
## data:  SA_at_peak$trauma and SA_at_peak$peak_SA
## t = 1.0339, df = 43, p-value = 0.307
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  -0.1443881  0.4296389
## sample estimates:
##       cor
## 0.1557468
```

Post-hoc analysis

Post-hoc analysis Post-hoc analyses were performed on the mean (range) of the Pain Catastrophising Scale and the 10-item Connor-Davidson Resilience Scale. These analyses were not initially planned nor described in this study's protocol. These analyses were performed for exploratory purposes to inform the development of future research questions.

```
#PCS

 PCS_results_only <- read_delim('C:/Users/Gill/Desktop/Formal data analysis Jan 2020/Gill_f
ormal_data_HFS_ST_080120/PCS_results_only.csv',
    ';', escape_double = FALSE, trim_ws = TRUE)

pcs <- PCS_results_only[-c(1,2),] # removing participants STM01 and STM02 because they are
missing data for question 8

pcs_range <- pcs %>%
  select(-SUBJ_ID)

pcs_range$pcs_total <- rowSums(pcs_range)

#Determining the overall mean PCS scores among the 24 participants
mean(pcs_range$pcs_total)

## [1] 31

#Determining the range
range(pcs_range$pcs_total)

## [1] 14 50

#Post-hoc: CD-RISC-10

cdrisc <- master_data[ , grep('cdrisc', colnames(master_data))]

cdrisc<- unique(cdrisc)

cdrisc$cdrisc_total <- rowSums(cdrisc)
```

```r
# Determining the mean
mean(cdrisc$cdrisc_total)

## [1] 40.80769

#Determining the range
range(cdrisc$cdrisc_total)

## [1] 32 48



## Session information


```r
sessionInfo()

## R version 3.6.1 (2019-07-05)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 17763)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_South Africa.1252
## [2] LC_CTYPE=English_South Africa.1252
## [3] LC_MONETARY=English_South Africa.1252
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_South Africa.1252
##
## attached base packages:
## [1] grid      stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] REdaS_0.9.3     gridExtra_2.3   lme4_1.1-21     Matrix_1.2-17
##  [5] readxl_1.3.1    magrittr_1.5    forcats_0.4.0   stringr_1.4.0
##  [9] dplyr_0.8.3     purrr_0.3.3     readr_1.3.1     tidyr_1.0.0
## [13] tibble_2.1.3    ggplot2_3.2.1   tidyverse_1.2.1
##
## loaded via a namespace (and not attached):
##  [1] tidyselect_0.2.5 xfun_0.10        reshape2_1.4.3   splines_3.6.1
##  [5] haven_2.1.1      lattice_0.20-38  colorspace_1.4-1 vctrs_0.2.0
##  [9] generics_0.0.2   htmltools_0.4.0  yaml_2.2.0       rlang_0.4.1
## [13] nloptr_1.2.1     pillar_1.4.2     glue_1.3.1       withr_2.1.2
## [17] modelr_0.1.5     plyr_1.8.4       lifecycle_0.1.0  munsell_0.5.0
## [21] gtable_0.3.0     cellranger_1.1.0 rvest_0.3.4      evaluate_0.14
## [25] labeling_0.3     knitr_1.25       broom_0.5.2      Rcpp_1.0.2
## [29] scales_1.0.0     backports_1.1.5  jsonlite_1.6     hms_0.5.2
## [33] digest_0.6.22    stringi_1.4.3    cli_1.1.0        tools_3.6.1
## [37] lazyeval_0.2.2   crayon_1.3.4     pkgconfig_2.0.3  zeallot_0.1.0
## [41] ellipsis_0.3.0   MASS_7.3-51.4    xml2_1.2.2       lubridate_1.7.4
## [45] minqa_1.2.4      assertthat_0.2.1 rmarkdown_1.16   httr_1.4.1
## [49] rstudioapi_0.10  boot_1.3-22      R6_2.4.0         nlme_3.1-140
## [53] compiler_3.6.1
```
```

Appendix 31: Semi-structured interview

ID code	Site receiving the HFS under a condition of threat	Semi-structured interview response
STM01	Left	No data[13]
STM02	Left	"I was worried that skin would be affected on both arms not really affect by what it said on the computer screen, stimulations to each arm felt equal so was equally concerned."
STM03	Right	Participant reported to *'agree'* that at the time of receiving the HFS, they were concerned that it would cause damage to their skin on the **right** arm "because of what the computer screen said. I was not expecting that type of pain". Participant reported to *'strongly agree'* that at the time of receiving the HFS on their **left** arm, they felt anxious because the "left arm felt a lot more painful and my fingers moved so felt anxious".
STM04	Left	Participant reported to *'agree'* that at the time of receiving the HFS, they were concerned that it would cause damage to their skin on the **right** arm because the "right was more painful. My right arm is more important to me than my left (dominance)". Participant reported concern was "not really due to what the computer screen said".
STM05	Left	Participant reported to *'agree'* that at the time of receiving the HFS, they were concerned that it would cause damage to their skin on the **right** arm because "[the HFS] started with right arm. It was more of a shock, so I was more concerned. Participant reported to *'strongly agree'* that at the time of receiving the HFS, they were concerned that it would cause damage to their skin on the **left** arm "based on what the computer said, and it felt more pain on that side".
STM06	Right	"Right arm felt more painful than left. However, I was equally anxious because of pain intensity".
STM07	Left	"I wasn't too concerned about skin damage. I trusted enough precautions had been taken." Participant reported to *'agree'* that at the time of receiving the HFS on their **right** arm, they felt anxious because "of the anticipation". "Maybe I was a bit more anxious about the right arm."

[13] The researchers in this current study only decided to conduct the semi-structured interview after the first participant had been assessed.

STM08	Right	"The anticipation of the HFS made me feel anxious. I wasn't too concerned about the rating on the screen. I trusted it wasn't going to damage the skin."
STM09	Left	Participant reported to *'agree'* that at the time of receiving the HFS, they were concerned that it would cause damage to their skin on the **right** arm because "I felt from the beginning that the left arm was more sensitive". Participant reported to *'strong agree'* that at the time of receiving the HFS on both their **right and left** arm, they felt anxious because "the anticipation of when the stimulus would happen made me feel really anxious".
STM10	Right	"Expecting the pain was going to increase after each stimulation made me concerned that the increase may be causing damage and associated anxiety."
STM11	Right	Participant reported to *'agree'* that at the time of receiving the HFS, they were concerned that it would cause damage to their skin on the **right** arm "based on what it said on the screen I felt concerned about damage". Participant reported to *'agree'* that at the time of receiving the HFS on their **left** arm, they felt anxious because "it was more painful on the left, so I was more anxious".
STM12	Right	Participant reported to *'disagree'* that at the time of receiving the HFS, they were concerned that it would cause damage to their skin on both the **right and left** arm because "at the beginning of the study, the informed consent said there shouldn't be any harm or damage. I trusted that". Participant reported to *'agree'* that at the time of receiving the HFS on both their **right and left** arm because "anticipation of pain, and unexpected pain. I was worried whether the next [HFS train] would be better than one before".
STM13	Left	"I was concerned about what it said on the screen, which obviously made me anxious".
STM14	Left	"I trusted that the researchers wouldn't put me through anything dangerous. I had forgotten one [arm] was more fragile by the time we started baseline testing after the questionnaires."
STM15	Right	"The intensity and anticipation of stimulation was anxiety provoking".
STM16	Right	Participant reported to *'agree'* that at the time of receiving the HFS, they were concerned that it would cause damage to their skin on both the **right and left** arm because "I felt equally concerned that there could be skin damage to both arms, even based on what the

		computer screen said. It was more just the overall risk that there could be damage".
STM17	Right	Participant reported to *'strongly disagree* that at the time of receiving the HFS, they were concerned that it would cause damage to their skin on both the **right and left** arm because "the stimulation trains were too short to cause damage". Participant reported *'neutral'* that at the time of receiving the HFS on both their **right and left** arm because "waiting for the next stimulation made me feel anxious".
STM18	Right	Participant reported to *'disagree'* that at the time of receiving the HFS on both their **right and left** arm, they felt anxious because "I was more nervous about the anticipation of the stimulus than what was on the screen".
STM19	Left	Participant reported to *'strongly disagree* that at the time of receiving the HFS, they were concerned that it would cause damage to their skin on their **left** arm because "of what the computer screen said". Participant reported to *'strongly agree'* that at the time of receiving the HFS on both their **right and left** arm, they felt anxious because "of anticipation between trains and how intense the pain was".
STM20	Right	"I have worked with UCT human ethics before. I know they are strict. I was slightly concerned about the right because of what the screen said but not enough to 'agree' but [the right] felt a bit stranger than left arm. I believed the skin examination, but I was slightly interested as to why you were using an otoscope."
STM21	Left	Participant reported to *'disagree'* that at the time of receiving the HFS, they were concerned that it would cause damage to their skin on their **left** arm because "I wasn't too concerned. Maybe a small hint of doubt about left arm, but not enough to think it would cause tissue damage".
STM22	Left	"More the discomfort that made it feel uncomfortable"
STM23	Left	"I was equally concerned but not enough to be totally convinced there could be tissue damage. But this did make me feel anxious."
STM24	Right	Participant reported *'neutral'* that at the time of receiving the HFS, they were concerned that it would cause damage to their skin on both their **right and left** arm because "I trusted the controlled environment, even though the screens said one arm was more fragile". Participant reported to *'agree'* that at the time of receiving the HFS on both their **right and left** arm, they felt anxious because "I felt generally anxious just because of pain intensity".

STM25	Right	Participant reported to *'agree'* that at the time of receiving the HFS, they were concerned that it would cause damage to their skin on their **left** arm because "it felt more painful on the left. Participant reported to *'strongly disagree'* and '*neutral'* that at the time of receiving the HFS on their **right and left** arm, respectively, they felt anxious because "I was anxious about the next train. Also [I was anxious about] what the screen said".
STM26	Left	Participant reported to *'strongly disagree'* that at the time of receiving the HFS, they were concerned that it would cause damage to their skin on both their **right and left** arm because "I trusted the controlled environment". Participant reported *'neutral'* that at the time of receiving the HFS on both their **right and left** arm, they felt anxious because "of not knowing what to expect, and the anticipation between trains".