

The Effect of Proximity to Pain on Protective Blink Reflexes in Individuals with

Induced Hyperalgesia

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Bachelor of Science Honours

*This thesis is presented in partial fulfilment of the requirements for the degree of*

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**Declaration**

*I declare that this thesis is my own account of my research.*

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## Abstract

The present study explored the notion that proximity to pain increases protective blink reflexes, and that individuals with chronic pain experience an exacerbation of this effect. Chronic pain sufferers experience a heightened sensitivity to pain (hyperalgesia) and body-space perceptual disturbances. Thus, this study predicted that healthy individuals induced with similar symptoms would have stronger blink reflexes when the stimulated wrist was closer to the face. Adopting a between-subjects design, half of the participants ( $N = 14$ ) received high-frequency electrical stimulation (HFS) to induce hyperalgesia while the other half of the participants ( $N = 14$ ) did not. Firstly, participants underwent psychophysical testing on the wrists and forehead to discern baseline sensitivity to stimuli. The HFS condition then completed an electrical detection threshold test to determine the stimulus intensity of the HFS procedure. Following HFS of the wrist, psychophysical testing was repeated to discern changes in sensitivity. Lastly, all twenty-eight participants completed the blink reflex procedure, consisting of a sequence of sixty electrical stimuli applied to the head and the wrist. Independent variables included the position of the wrist (close vs. far), and the site stimulated (head alone, wrist alone, vs. head + wrist together). Dependent variables included pain and sharpness ratings of each stimulus, in addition to the magnitude of the blinks. Results revealed that blink reflexes increased when the wrist was close to the face, confirming the findings of previous literature on proximity to pain. Although the HFS group gave higher sharpness ratings for close stimuli, there was no difference between groups for pain ratings and blinks. Thus, the central premise was not substantiated. Additionally, simultaneous stimulation of the head and the wrist increased all dependent variables; however, this effect did not interact with the proximity effect. Further research should collect a larger sample for more meaningful comparisons between groups.

The Effect of Proximity to Pain on Protective Reflex Responses in Individuals with  
Induced Hyperalgesia

Pain is the most common reason to seek medical help, not only to relieve symptoms but also to reduce the impact of symptoms on quality of life and well-being (Gureje, Von Korff, Simon & Gater, 1998). Chronic pain especially has profound adverse effects on the economic, psychological, and social domains of the individual and the wider community because symptoms can persist for years. In 2001, Blyth and colleagues conducted a chronic pain prevalence study amongst a random sample of adult Australians. Researchers discovered significant links between chronic pain, disability, unemployment benefits, and elevated levels of psychological distress (Blyth et al., 2001). Additionally, five billion dollars per annum in lost productivity as a result of ineffective and absent workdays are due to chronic pain (Van Leeuwen, Blyth, March, Nicholas & Cousins, 2006). Chronic pain is also highly comorbid with mental health conditions including anxiety disorders, depression and post-traumatic stress disorder (Bair, Robinson & Katon, 2003; Outcalt et al., 2015). Mental distress leads to further disability, more intense pain, lower quality of life, and reduces effective treatment response (Kroenke et al., 2013). However, chronic pain conditions remain tough to treat because pain mechanisms are complex. To develop effective treatments, it is essential we understand the abnormal pain mechanisms responsible for the manifestation and maintenance of chronic pain. Successful treatment of chronic pain will reduce the accompanied psychological and financial strain on the individual and society. Thus, further research is necessary to enhance our understanding of chronic pain.

Past research emphasizes that pain perception is not solely dependent upon noxious input; in fact, it is highly subjective and dependent on context. Our emotions,

mental states, and focus of attention all influence the way we experience pain (McCracken, 1997; Quevedo & Coghill, 2007). Past experiences can also bias our expectations of pain (Sambo, Liang, Cruccu & Ianetti, 2012). Chronic pain sufferers are highly sensitive to painful and innocuous stimuli, and based on this experience, they expect most stimuli to have the ability to cause harm. In this sense, chronic pain sufferers judge most stimuli as threatening. Threat perception in turn influences pain perception. Past research revealed high threat values attenuate higher pain intensity levels (Ramírez-Maestre, Esteve & López, 2008). Thus, threat perception is another cognition that can bias pain processing (Wiech, Ploner & Tracey, 2008).

Our appraisal of pain also influences our protective responses. Protective responses in healthy humans typically involve a primitive startle reflex, such as the eye blink, to protect the most vulnerable parts of the body (Yeomans & Frankland, 1995; Bufacci, Liang, Griffin & Iannetti, 2016). Due to threat overestimation, protective responses of chronic pain sufferers are magnified, and often involve protective postures and disuse of their affected limb (Allen, Galer, & Schwartz, 1999; Rho, Brewer, Lamer, & Wilson, 2002). Additionally, body-space representations bias threat perception and protective responses. Startle reflexes intensify when objects close to our body threaten vulnerable body parts (Sambo, Forster, Williams & Ianetti, 2012a). Due to somatosensory disturbances, chronic pain individuals possibly experience an exacerbation of this effect. However, studies have not explored the effect of proximity to pain on protective reflexes in chronic pain. The present study aimed to fill this gap in the current literature. A significant effect would support a link between threat, body perception, pain perception, and protective reflexes. The sections to follow explore these links in more detail.

### **Pain Mechanisms**



It is difficult to understand abnormal pain mechanisms without an understanding of how pain mechanisms work in healthy individuals. In particular, [it is important to understand](#) how pain is detected, transmitted, and maintained by the peripheral nervous system (PNS). The PNS consists of a network of peripheral nerve fibres, and allows for communication between the central nervous system (CNS) and the spinal cord with the rest of the body. There are three types of peripheral nerve fibres: motor, sensory, and autonomic. [One class of](#) sensory afferent fibres (nociceptors) detect potentially harmful stimuli and relay this information to the dorsal horn of the spinal cord, in a process known as nociception (Costigan & Woolf, 2000). [Presenting as](#) myelinated A delta peripheral fibres and unmyelinated C peripheral fibres (Loeser & Melzack, 1999), nociceptors can encode and transduce noxious stimuli to the central nervous system (Snider & McMahon, 1998).

The perception of pain is influenced by stimulus intensity, cognitive expectations, and the amount of stimuli delivered (Weisenberg, 1987; Wiech, Ploner & Tracey, 2008). For instance, paired electrical stimuli delivered up to twenty centimeters apart can elicit greater perceived pain intensity than a single electrical stimulus of equal intensity (Reid, Harvie, Miegel, Spence & Moseley, 2015). Known as spatial summation, this phenomenon involves the activation of a larger number of nociceptive receptive fields. Effectively, spatial summation lowers pain thresholds and increases pain intensities (Price, McHaffie & Larson, 1989; Defrin & Urca, 1996). Although, other research suggests simultaneous stimulation can inhibit nociceptive activity, such that pain thresholds increase and pain intensities decrease (Marchand & Arsenault, 2002; Edwards, Ness, Weigent & Fillingim, 2003). This phenomenon is referred to as diffuse noxious inhibitory controls (Yarnitsky, 2010).

Alternatively, an individual's perception of pain can influence the intensity, duration, and sensation of pain (Woolf, 2004). Previous research asserts that the perception of pain is dependent on genetics, attention, attitudes, and affect (Villemure & Bushnell, 2002). For example, pain is reported as less intense when attention is drawn away from the pain (McCracken, 1997; Quevedo & Coghill, 2007). Additionally, higher levels of anxiety are associated with higher perceived pain intensities and threat values, particularly for ambiguous stimuli (Bishop, 2007; Enck, Benedetti & Schedlowski, 2008). Past experiences, whereby individuals learn the meaning of the word "pain," also influences pain perception. Interestingly, the [nociceptive](#) mechanisms that underlie [different pain](#) experiences are identical among healthy individuals. [However](#), there are clear distortions in nociceptive pain processing [in many people with](#) chronic-pain, [as they](#) feel amplified pain unrestricted to the site of injury.

### **Hyperalgesia**

Hyperalgesia, the most characteristic sensory disturbance of chronic pain, involves a heightened sensitivity to suprathreshold stimuli and a lowered pain threshold (Hardy, Wolff, & Goodell, 1950; Raja, Meyer, James, & Campbell, 1988). Hyperalgesia [presents as](#) two types: primary hyperalgesia and secondary hyperalgesia. Primary hyperalgesia occurs [at](#) the initial site of injury, in response to mechanical and thermal stimuli. Secondary hyperalgesia arises outside the initial area of injury, in response to mechanical stimuli (Raja, Campbell, & Meyer, 1984; Raja et al., 1988). One type of secondary hyperalgesia, known as allodynia, is a heightened sensitivity to [innocuous](#) stimuli (non-painful), such as the sensation of touch (Moseley, 2005; Drummond & Finch, 2010).

Both hyperalgesia and allodynia are manifestations of central sensitisation. Central sensitisation is a hyperactive protective response to noxious input evoked by the

potential risk of further damage, in which pain thresholds fall, and further responses are amplified (Latremolier & Woolf, 2009). At a microscopic level, the excitability and synaptic efficacy of central nociceptive neurons in bulbospinal pathways (which control the spreading of pain from the site of injury to nearby regions) increase. Effects can last for hours after the original stimulus (Costigan & Woolf, 2000; Marinus et al., 2011; Woolf, 2011). Additionally, wide dynamic range nociceptive neurons in the dorsal horn of the spinal cord now respond to both noxious and innocuous stimuli. Simply, an innocuous input may induce pain (Woolf, 2011). New cell properties signify that the intensity and presence of noxious stimuli are no longer essential to trigger noxious sensations (Latremolier & Woolf, 2009). The false perception of pain as a result of neuronal changes demonstrates the role of central neuronal plasticity in the experience of pain. Distortions in pain perception also highlight the responsibility of the central nervous system in the maintenance of chronic pain.

### **Perception**

The human brain perceives space according to egocentric and allocentric frames of reference. Of interest to this study, egocentric frames of reference are observer-dependent. Observers judge space according to the distance between our body and objects in our surroundings as well as the distance between different parts of our body (Legrain, Bultitude, De Paepe & Rossetti, 2012). We perceive nociceptive stimuli according to our frames of reference, as well as different areas of space, such as peripersonal space. The immediate space surrounding the body is known as peripersonal space (Brozzoli, Makin, Cardinali, Holmes & Farnè, 2011). Physical information arising from the body and visual information close to the body modulates the perception of peripersonal space. Depending on the properties of objects found in peripersonal space (i.e. pleasant or harmful), this visual-tactile relationship also guides motor

responses. We approach pleasant objects and avoid harmful objects (Anelli, Nicoletti, Bolzani & Borghi, 2013). Thus, peripersonal representations of the body also guide our responses to nociceptive stimuli.

The relationship between body-space representations and pain processing is also evident in chronic pain sufferers. Distortions in body representations adversely affect pain processing. For example, patients often inaccurately report that their limb is enlarged, constantly on fire, heavy, and swollen (Lewis & Schweinhardt, 2012; Turton et al., 2013). Additionally, pain causes distortions in body representations. Feelings of intense pain often result in significant difficulty in knowing the position of the affected limb (proprioception) (Lewis et al., 2010). Proprioception relies upon body-schema; neural body representations associated with every body part, determined by somatosensory and visual interactions. Thus, distorted proprioception position suggests disturbances to body-schema (caused by central disturbances) contribute to chronic pain symptoms.

Recent research suggests cortical representation deficits cause the distortions in body schema that contribute to perceptual distortions (Holmes & Spence, 2004; Lewis & Schweinhardt, 2012). Body schema is stored in somatosensory cortices, such as the primary somatosensory cortex (S1) (Lewis & Schweinhardt, 2012). Neuroimaging has revealed that in chronic pain, cortical maps (a symbolization of a sensory modality or motor operation) [in somatosensory cortices](#) are drastically altered to form new cortical maps. Cortical reorganisation [reflects](#) perceptual disturbances (Lewis & Schweinhardt, 2012). Previous research revealed that the amount of hyperalgesia and pain felt in the affected limb relates to the extent of cortical reorganisation (Maihöfner, Handwerker, Neundörfer, & Birklein, 2003). Additionally, chronic pain patients in greater pain experience great body perception disturbances than those in less intense pain (Lewis &

Schweinhardt, 2012). Therapy for patients can also reverse changes in [somatotopic maps in S1](#). Following one year of therapy, patients [with](#) a reduction in mechanical hyperalgesia also displayed recovery from cortical reorganization (Maihofner et al., 2004).

Evidently, cortical reorganisation contributes to both body perception disturbances and nociceptive distortions. Why? Somatosensory areas (SI and the secondary somatosensory cortex (SII)) also process nociceptive inputs (Legrain, Iannetti, Plaghki & Mouraux, 2011). The strength of neural responses in SI and SII strongly and positively correlate with the perceived intensity of pain. Therefore, central disturbances in somatosensory processing contribute to nociceptive and perceptual distortions in chronic pain patients (Drummond & Finch, 2014). Ultimately, nociception and body perception are linked.

### **Protection & Neglect**

In order to survive, it is important for organisms to avoid harmful stimuli. When threatened by noxious stimuli protective reflexes are adopted to promote self-protection. Protective reflexes are mediated by our nociceptors, which are excited by the sensation of pain (Snider & McMahon, 1998; Costigan & Woolf, 2000). When nociceptors detect the existence of harmful stimuli, they alert somatosensory systems to stimulate antagonistic muscles [to protect the body](#). However, a startle reflex occurs before voluntary protective action is executed (Yeomans & Frankland, 1995). Startle reflexes aim to protect the most vulnerable parts of the body (such as the [head](#) and the [eyes](#)), and are comprised of biological responses such as rapid head movement, neck movement, and the eye blink reflex. Startle research is useful in the examination of sensitisation, habituation, anxiety, and pain (Davis, Gendelman, Tischler, & Gendelman, 1982; Berg

& Balaban, 1999). Furthermore, it provides insight into the neural pathways behind the provocation and regulation of startle responses (Berg & Balaban, 1999).

Recently, the mechanisms that mediate startle responses are gaining attention in the scientific world. One such mechanism is peripersonal space representations, under the premise that it allows us to detect potential threats surrounding our body and execute defensive action. Exploring this notion, Cooke et al. (2003) examined the effect of electrical microstimulation of the ventral intraparietal area of the parietal lobe and the pre-central gyrus (i.e. primary motor cortex) of the monkey brain. Visuospatial neurons within these regions respond to objects touching or near the body surface. Thus, the responses of these neurons are affected by changes in peripersonal space (Graziano & Cooke, 2005). Electrical stimulation [of this part of the brain elicited defensive responses](#), including squinting, blinking, and grimacing. Most recently, a human sample confirmed the link between peripersonal space and startle. Electrical stimulation of the wrist when in peripersonal space elicited stronger protective blink reflexes than when the wrist was stimulated outside peripersonal space (Sambo et al., 2012a). Why? Threat perception influences nociceptive processing. The most threatening stimuli are noxious stimuli located in peripersonal space (Holmes & Spence, 2004). This suggests that the regions associated with peripersonal space also mediate top-down facilitation of protective blink reflexes.

In chronic pain, distorted peripersonal representations elicit extreme protective responses to noxious and innocuous input. Specifically, misrepresentation of the affected limb and impaired spatial perception of stimuli surrounding it causes sufferers to adopt neglect-like behaviours (Legrain et al., 2012). Affected individuals implement a protective posture to shelter their affected limb from further harm (Allen et al., 1999; Rho et al., 2002). Actions become slighter and less frequent. Additionally, the limb can

feel foreign; patients are unaware of its position and guided attention is necessary to move it (Galer & Jensen, 1999). Eventually, protective disuse can occur. The presence of neglect-like symptoms in chronic pain supports the notion that representations of peripersonal space determine protective responses to nociceptive information (Legrain et al., 2012). However, distinct spatial representation impairments in chronic pain are yet to be explored.

### **The Nociceptive Blink Reflex**

Measurement of the nociceptive blink reflex is the most common method used to understand of the plasticity of startle (Davis & Heninger, 1972). Facilitated by subcortical circuits, the blink reflex is the involuntary closure of the eyelids aimed at protecting the eyes and consists of an early ipsilateral R1 component, followed by bilateral R2 and R3 components (Ellrich, Bromm, & Hopf, 1997; Vo & Drummond, 2013). The R2 component is also referred to as a local nociceptive reflex because it can be elicited by painful stimuli (Ellrich et al., 1997). The nociceptive blink reflex is a non-invasive measure of activity in and excitability of trigeminal pain pathways, and can be manipulated by a variety of stimulus modulations (e.g. intensity, duration, type) (Giffin, Katsarava, Pfundstein, Ellrich, & Kaube, 2003). Electrical stimulation of the supraorbital nerve, a branch of the frontal nerve that extends to the forehead, upper eyelid, scalp, and frontal sinus, elicits R2 (Ellrich et al., 1997; Vo & Drummond, 2013). Electrical stimulation of the median nerve on the hand also evokes a hand-blink reflex (Sambo, Forster, Williams & Iannetti, 2012b). Surface electromyographic (EMG) recording electrodes attached to the orbicularis oculi muscle (found surrounding the eye), measure action potentials produced by the blink reflex (Berg & Balaban, 1999; Blumenthal et al., 2005). In comparison to other methods, EMG is more advanced at detecting blink activity and is unobtrusive (Davis & Heninger, 1972; Blumenthal et al.,

2005). Thus, stimulating the supraorbital nerve and recording from the orbicularis oculi muscle allows us to explore the trigeminal nerve and its brainstem connections.

Ultimately, we can understand the nociceptive nature of the blink reflex (Giffin et al., 2003).

### **High Frequency Electrical Stimulation**

Understanding the abnormal pain mechanisms underlying chronic pain requires central sensitization to be replicated in healthy humans. Noxious conditioning of nociceptive fibres in the skin elicits central sensitization (Woolf, 2011). High-frequency electrical stimulation (HFS) effectively conditions nociceptive fibres (Vo & Drummond, 2013). HFS can induce both central and peripheral sensitisation at the site of conditioning (primary hyperalgesia) and adjacent areas (secondary hyperalgesia). The skin becomes hypersensitive to heat and pressure-pain at the primary site, and sharp stimuli at primary and secondary sites (Vo & Drummond, 2013). Additionally, ipsilateral (same side as conditioned site) forehead analgesia to pressure-pain occurs, suggesting there are pain inhibitory mechanisms that hinder nociceptive activity in the spinal cord. Attempting to delineate excitatory and inhibitory pain modulation processes, changes to the ipsilateral and contralateral blink reflex following HFS conditioning of the forearm were examined (Vo & Drummond, 2013). HFS conditioning increased the magnitude of the R2 component of the blink reflex (Vo & Drummond, 2014). Thus, facilitatory and inhibitory processes coexist, thereby implicating the involvement of supraspinal mechanisms (Vo & Drummond, 2014). This finding establishes an association between protective responses and nociceptive processing that is relevant to chronic pain.

### **This Study**



This study [was](#) primarily interested in the impact of peripersonal space perception and threat estimation on pain perception and protective reflexes in individuals with induced hyperalgesia. Although past research examined the effect of pain on defensive behaviours in monkeys (Graziano & Cooke, 2005), and the effect of proximity to pain on blink reflexes in healthy individuals (Sambo et al., 2012a; Sambo et al., 2012b), the effect of proximity to pain on involuntary pain modulation processes in individuals with chronic pain [has not yet been explored](#). The present study was also interested in the effects of spatial summation on peripersonal representations and protective reflexes in healthy individuals with hyperalgesia. Research suggests that stimulating a larger surface area through simultaneous head and wrist stimulation can either induce spatial summation (Price et al., 1989; Defrin & Urca, 1996; Reid et al., 2015) or inhibit the activity of nociceptive neurons (Edwards et al., 2003; Yarnitsky, 2010). Previous studies on proximity to pain separately stimulated the head and the wrist (Sambo et al., 2012a; Sambo et al., 2012b). Thus, it remains unknown whether the proximity between two distinct stimuli either increases or inhibits spatial summation and if this effect intensifies protective reflexes in chronic pain.

If this link is found between peripersonal space, nociceptive processing and protective reflexes, it will implicate more specific brain regions in the modulation of disrupted pain and perceptual processes in chronic pain. The disrupted neurophysiological mechanisms that mediate pain perception and space perception presently remain unspecified, meaning [that](#) chronic-pain patients are often inadequately treated (Allen et al., 1999). I [hoped that the](#) results of this study [would stimulate the development of](#) neuropsychological and behavioural treatments to alleviate specific symptoms, by targeting the brain regions behind its maintenance.

### **Aims and Hypotheses**

Thus, this study aimed to assess the relationship between peripersonal space representations, pain perception, and defensive action by examining the effect of proximity to pain on protective reflex responses. Healthy participants were electrically stimulated on the wrist and on the ipsilateral forehead to induce nociceptive blink reflexes. Manipulating the distance between the stimulated wrist and the forehead, and examining protective blink reflex responses and verbal pain ratings allowed us to determine effects of proximity. Approximately half of the participants were exposed to HFS conditioning of their wrist in order to replicate signs of central sensitization present in chronic pain.

First, it was hypothesized that blink reflex responses to wrist stimulation would be greater when the wrist was close to the face than when it was further away. As stated previously, objects situated in peripersonal space are of the most immediate threat, particularly if near a vulnerable body part (Holmes & Spence, 2008). Thus, I expected stronger protective responses to noxious stimuli that threatened the safety of the eye. I tested this by comparing blink reflex responses to wrist stimulation (close) vs. wrist stimulation (far) without any nociceptive supraorbital stimulation.

Second, it was hypothesized that blink reflex responses and pain ratings to simultaneous wrist and supraorbital stimuli would be greater when the wrist was close to the face than when it was further away. As previously mentioned, stimulating separate body areas elicits spatial summation, which intensifies nociceptive activity (Price et al., 1989; Reid et al., 2015). Thus, I predicted stronger protective blink reflexes when noxious stimuli threatened a larger surface area. I tested this by comparing blink reflex responses and pain ratings to wrist stimulation (close) + ipsilateral supraorbital stimuli vs. wrist stimulation (far) + ipsilateral supraorbital stimuli.

Third, it was hypothesized that blink reflex responses, and pain and sharpness ratings to supraorbital stimuli without simultaneous wrist stimulation would be greater when the wrist was close to the eye than further away. Based on the first and second hypotheses, I expected stronger blink reflexes when both noxious stimuli approached peripersonal space and spatial summation occurred. I tested this by comparing blink reflex responses and pain ratings to wrist (close) + ipsilateral supraorbital stimuli vs. wrist (far) + ipsilateral supraorbital stimuli.

Lastly, it was hypothesized that blink reflex responses, pain ratings and sharpness ratings would be greater when the wrist was conditioned by HFS. Due to the somatosensory and perceptual disturbances present in chronic pain, I predicted that induced hyperalgesia would exacerbate the effects of proximity and simultaneous stimulation. I expected that HFS conditioning would significantly interact with proximity and the site stimulated, wherein responses would be greater following simultaneous stimulation when the wrist was close and HFS conditioning had occurred. I tested this by comparing the blink reflex responses and self-reports of a control group without HFS and an experimental group with HFS.

## Method

### Participants

Participants ( $N = 28$ , 53.57% females) aged between 18 and 61 years ( $M = 28.29$ ,  $SD = 12.50$ ) consisted of psychology students who enrolled through the Research Participant Portal and individuals from the general population that volunteered through convenience sampling. Psychology students received one-hour worth of research credit, and external volunteers received a free coffee voucher redeemable at Aroma.

Participants were randomly assigned to either the control condition ( $N = 14$ , 57.14%

females) or experimental condition ( $N = 14$ , 50% females). Exclusion criteria included individuals who suffer from chronic pain, CRPS, epilepsy, any psychiatric disorder, or are currently taking any medication, pregnant or breastfeeding, have a pacemaker or any other implanted device.

### **Design**

This study adopted a between-subjects design consisting of random assignment of the participants to the control or experimental condition. The control condition did not include HFS conditioning before the blink reflex procedure. The experimental condition consisted of HFS conditioning before the blink reflex procedure (see Appendix A). There were six sets of stimuli conditions for the blink reflex procedure (see Appendix B). Each condition consisted of 10 electrical stimuli; 60 stimuli altogether. Independent variables consisted of “wrist position” (close or far away) and “site stimulated” (head alone, wrist alone, or head + wrist together). Dependent variables were pain ratings, sharpness ratings, and the magnitude of the R2 component of the blink reflex (R2 AUC). To manipulate the distance between the wrist and the forehead, participants moved their wrist “far-away” (resting their arm on the arm-rest) or brought their wrist “close” to their face (approximately 10 cm away from their forehead) upon verbal instruction by the researcher. Previous research has defined the boundary of peripersonal space as between 20cm and 40cm from the face; however, the area closest to the face is the highest-risk area (Sambo & Iannetti, 2013).

### **Apparatus**

#### **Von Frey monofilament.**

The Von Frey monofilament was used to monitor changes in sensitivity to mild and moderate mechanical stimulation caused by HFS. The Von Frey monofilament tests the sensitivity of receptive fields at test sites, and consists of an interchangeable spring

mechanism that exerts a force of 10g on one end (mild), and exerts a force of 40g on the other end (moderate) (Neuro-pen, Owen Mumford, USA). The difference between pain and sharpness ratings before HFS and after HFS reflected changes in sensitization.

#### **Algometer.**

An algometer with an 8 mm diameter rubber tip was used to measure pressure-pain sensitivity (i.e. the force that elicits a PPT) on all test sites (FDX, Wagner Instruments, USA). Readings from an algometer are a valid measure of pressure-pain thresholds (Kinser, Sands & Stone, 2009). Application at a consistent rate (100g per second) provides reliability. The force applied when a participant felt pain was their PPT.

#### **Electrical detection threshold (EDT).**

EDT was used to determine the lowest intensity of an electrical stimulus that the participant could detect. Through the method of limits for two descending and ascending series of single pulses at 2-ms pulse width, the geometric mean of the four stimulus intensity levels was calculated as the participant's EDT.

#### **High-frequency electrical stimulation (HFS).**

The ventral wrist was conditioned with HFS to stimulate hyperalgesia. A constant current stimulator (DS7A; Digitimer, Welwyn Garden City, UK) generated electrical stimuli. A purpose-built electrode consisting of 24 copper pins with 0.2 mm diameter tips attached to a 2 cm x 3cm Perspex block delivered stimuli. The HFS procedure consisted of 5 bursts of electrical stimulation (100 Hz, 2-ms pulse width, 20 times EDT up to a maximum of 8mA) lasting 1 second, with a 9 second break between each. These particular properties are known to activate nociceptive fibres (Inui, Tran, Hoshiyama & Kakigi 2002). A ground plate (3.0 cm x 3.5 cm) was placed 1 cm from the purpose-built electrode to complete the electrical circuit.

**Electromyographic bio-potential amplifier (EBPA).**

During the blink reflex procedure, the EBPA (Biopac Systems, Inc., USA) amplified the electromyography signals originating from the orbicularis oculi muscles. These signals were also digitised by an MP150 Biopac Systems receptor at a sampling rate of 2,000 Hz (Biopac Systems, Inc., USA).

**Acqknowledge.**

Acqknowlegde software collected and recorded blink reflex data. It allowed the researcher to deliver stimuli manually and displayed the electromyographic waveforms on the computer monitor after delivery (Biopac Systems, Inc., USA). Using Acqknowledge, the electromyographic waveforms were then filtered to remove frequencies below 20 Hz and attenuate levels between 49.5 and 50.5 Hz.

**Blink reflex stimuli.**

A concentric electrode attached to the ipsilateral supraorbital region delivered ten monopolar square-wave electrical stimuli (20 Hz, 3ms delay, 10 V) at a current intensity of 2 mA. Stimuli had a 0.5 ms pulse duration and an inter-pulse interval of 5ms, consisting of a 3-pulse train. Triple-pulse stimuli are best suited because they increase feelings of pain and promote the R2 area under the curve (AUC) (Griffin et al., 2004). A concentric electrode attached to the ventral wrist, 2 cm proximal to the radiocarpal joint, elicited 3-pulse train electrical stimuli of the same physical properties to the medial nerve of the wrist.

**Blinky Bill.**

The computer program “Blinky Bill” analysed blink data; it calculated the integrated amplitude (i.e. AUC (V/s)) of the ipsilateral and contralateral R2 component of the blink reflex. R2 AUC was measured over a period of 60 ms, beginning 27 ms and ending 87ms after stimulus onset (Ellrich & Treede, 1998).

**Procedure**

The experiment took place in the Pain Laboratory on the Murdoch University campus (temperature maintained at  $21 \pm 1^\circ\text{C}$ ). Upon participant arrival, random assignment to either the control condition (no HFS) or the experimental condition (HFS) occurred. The laterality of the wrist tested was counterbalanced across participants to minimise order effects. Next, an information sheet detailing the procedures involved was given to participants (see Appendix C). Provided they were comfortable with the information provided; participants gave their written informed consent (see Appendix D). Seated in a comfortable armchair, next, the experimenter (GM) cleansed the participant's test sites (the wrists, forehead, and the eyelids under both eyes) with a pumice stone and alcohol wipe, to minimise skin electrical resistance.

**Psychophysical tests.**

A series of psychophysical tests were used to measure the participants' sensitivity to mild and moderate pain, mild and moderate sharpness, and pressure-pain. The experimenter tested sensitivity in the ventral area of both wrists (primary area) and each side of the forehead. Sensitivity was also measured 1cm distal to the primary area (secondary area). The laterality of the wrist and the side of the forehead tested first was counterbalanced across participants. The researcher applied each stimulus in runs alternating between the primary and the secondary areas, and the sides of the forehead. Participants were required to verbally rate levels of pain and sharpness following each stimulus on a scale ranging from 0 to 10, in which 0 indicated 'no pain/no sharpness' and 10 indicated 'most extreme pain/extremely sharp' (see E for data recording sheet). Before conducting psychophysical testing, participants were trained to give accurate verbal ratings of pain and sharpness. First, the 10g Von Frey monofilament was applied perpendicular to the skin surface of test sites so that it bent for one second. Second, the

40g Von Frey monofilament was applied perpendicular to the skin surface of test sites for 2 seconds. Lastly, the algometer was applied perpendicular to the skin of test sites at 100g per second until the participant reported the first sign of pain.

#### **EDT.**

Then, the experimental group completed the EDT. From a starting intensity of 0.1mA, single pulses subtly increased in steps of 0.1mA until the participant perceived the stimulus, and then decreased in steps of 0.05mA until it was no longer perceived. This method was repeated to gain an average threshold.

#### **HFS.**

Next, the experimental group underwent HFS conditioning of the ventral wrist. The experimenter randomised the laterality of the wrist across participants. Participants rated pain, sharpness, and unpleasantness (0-10 scale) following each burst of electrical stimulation. The experimenter re-administered psychophysical tests to the test sites five minutes following HFS conditioning to measure changes in sensitivity.

#### **Nociceptive blink reflex preparation.**

Following first round psychophysical testing (control group) or second round psychophysical testing (experimental group), participants underwent preparation for the blink reflex procedure. The experimenter attached one stimulating electrode with an adhesive ring to the supraorbital region on the ipsilateral side of the forehead and one stimulating electrode with an adhesive ring to the participant's wrist. A headband secured the electrode on the forehead, while a Velcro arm strap secured the electrode on the wrist. Four Cleartrode electrodes (ConMed Corporation, USA) were attached to the orbicularis oculi muscles below the eyes, and one ground electrode was placed behind the ipsilateral ear.

#### **Standardisation of wrist stimuli.**



Before beginning the blink reflex procedure, standardisation of wrist stimuli occurred. The participant had to verbally rate the pain intensity (from 0 to 10) of a series of wrist stimuli that increased in intensity (starting at 1mA) in steps of 0.5mA until the participant gave a moderate pain rating of 5. The intensity that delivered a “5” pain rating was the intensity used for the remainder of the experiment. Alternatively, if a pain level of “5” was not reached for a participant, a maximum intensity of 15 mA was applied to the wrist for the remainder of the experiment. The black box used to alter intensities capped out at a maximum intensity of 15mA. Thus, stimuli ranged from a minimum of 1mA to a maximum of 15 mA.

### **Nociceptive Blink Reflex.**

Lastly, participants completed the blink reflex procedure. The series of sixty stimuli were in the same randomised order for each participant (see Appendix F for stimuli order). Each stimulus was followed by a fifteen-second interval to allow the participant to give pain and sharpness ratings and to minimise habituation. Participants rated the pain and sharpness associated with each stimulus on a scale of 0 to 10, same as the psychophysical tests, with 0 representing no pain and no sharpness, and 10 representing extreme pain and sharpness. For simultaneous stimulation, participants were asked to report pain and sharpness for the head, and then pain and sharpness for the wrist.

## **Statistical Analyses**

### **Psychophysical Tests**

Changes in sensitivity to mild pain/sharpness, moderate pain/sharpness, and pressure-pain in the forehead before HFS conditioning and after HFS conditioning were examined [in](#) repeated measures analysis of variances. Within-subjects variables were

time (before, after) and side (ipsilateral, contralateral). This analysis determined whether sensitivity changes occurred as a result of HFS. ANOVA was also conducted to measure changes in sensitivity in the wrists before and after HFS conditioning. Within-subjects variables were time (before, after), side (ipsilateral, contralateral), and site (primary, secondary).

### **Pain and Sharpness Ratings**

Changes in mean pain and sharpness ratings to stimuli were examined separately in 2 x 2 x 2 mixed design analyses of variance with HFS conditioning (HFS, no HFS) as the between-groups factor, and wrist position (close, far) and site stimulated (head alone, head + wrist or wrist alone, head + wrist) as within-groups factors. Each ANOVA was conducted separately for supraorbital stimuli (head alone, head + wrist) and wrist stimuli (wrist alone, head + wrist).

### **R2 AUC**

Changes in mean R2 AUC were examined in a mixed design analysis of variance with HFS conditioning (HFS, no HFS) as the between-subjects factor and site stimulated (head alone, arm alone, head + arm), wrist position (close, far) and laterality of supraorbital stimulation (ipsilateral, contralateral) as within-subjects factors. Additional mixed design analysis of variance was conducted to assess R2 AUC percentage changes between each condition (see Appendix G for results).

## Results

### Tests of ANOVA Assumptions

Due to electrical contamination, the participants that felt a stimulus in the neck were excluded from the following analyses. We believed this additional stimulus would adversely affect pain ratings, sharpness ratings, and R2 AUC. The effects we found when we included the neck-group ( $N = 11$ ) in our sample confirm our decision (see Appendices H to K). Due to small sample size ( $N = 28$ ), the Shapiro-Wilk [statistic](#) was used to test the ANOVA assumption of normality. This test indicated that only four R2 variables were moderately violated. However, Tabachnick and Fidell (2007) suggest going ahead with ANOVA because results are robust against violations of the normality assumption. Visual inspection of box-plots revealed there were no major outliers for [any](#) variable. Levene's Test of Equality of Error Variances was used to assess the homogeneity of variance assumption for all variables. To ensure homogeneity of variance, scores should not have a significance level less than .05 (Tabachnick & Fidell, 2007). Homogeneity of variance was minimally violated in a few instances [but the](#) majority of variables did not violate this assumption, thus ANOVA was continued.

The Sphericity assumption was not applicable for pain rating analyses and sharpness rating analyses, because each factor only contained two levels. However, ANOVA of R2 AUC had a within-subjects factor with more than two levels (site stimulated), thus Mauchly's Test of Sphericity was used to assess violations of sphericity. The assumption of sphericity was violated ( $p < .05$ ) threefold. To produce a valid F-ratio and reduce Type I error rate, degrees of freedom were altered using the Huynh-Feldt Epsilon.

**EDT and HFS**

The EDT ranged from .18 mA to 1.36 mA ( $M = .64$ ,  $SD = .37$ ), with subsequent calculated HFS intensities ranging from 3.6 mA to 8 mA ( $M = 7.20$ ,  $SD = 1.45$ ). The mean pain rating for HFS was 6.25 ( $SD = 1.40$ ), while mean sharpness rating was 6.51 ( $SD = 1.48$ ), and mean unpleasantness rating was 7.48 ( $SD = 1.05$ ).

**Changes to Mild and Moderate Sensitivities after HFS**

For mild sharpness applied to the wrist, ANOVA observed a significant main effect of time,  $F(1, 8) = 5.33$ ,  $p = .05$ ,  $\eta^2 = .40$ , with higher sharpness ratings before HFS ( $M = 2.78$ ,  $SD = 1.87$ ) and lower sharpness ratings after HFS ( $M = 2.14$ ,  $SD = 1.35$ ) indicating sensitivities to stimuli decreased (see Figure 1). A significant main effect for time was also found for ratings of head stimuli,  $F(1, 8) = 9.09$ ,  $p = .017$ ,  $\eta^2 = .53$ , with higher sharpness ratings before HFS ( $M = 2.39$ ,  $SD = 1.34$ ) than after HFS ( $M = 1.56$ ,  $SD = 1.36$ ). For moderate sharpness applied to the wrist, ANOVA demonstrated a significant interaction between side and time,  $F(1, 8) = 5.26$ ,  $p = .05$ ,  $\eta^2 = .40$ , with higher ratings on the ipsilateral side before HFS, and lower ratings on the contralateral side after HFS. Thus, sensitivities decreased indicating that HFS did not have the desired effect on sensitivities to moderate sharpness. ANOVA did not reveal significant main effects or interactions between variables for pain ratings and PPT (see Appendices L and M for results).

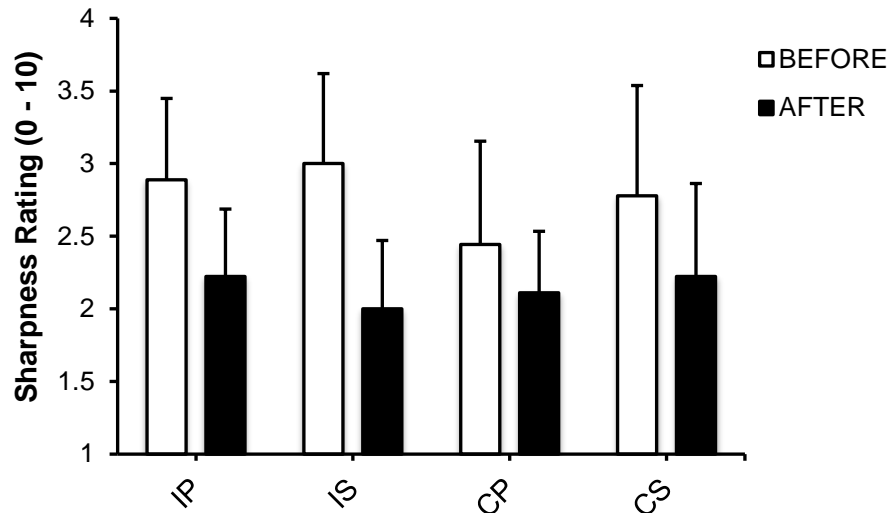


Figure 1. Mean sharpness ratings in response to mild sharpness applied to the wrist before HFS and after HFS. Error bars denote standard error (IP = ipsilateral-primary; IS = ipsilateral-secondary, CP = contralateral-primary; CS = contralateral-secondary).

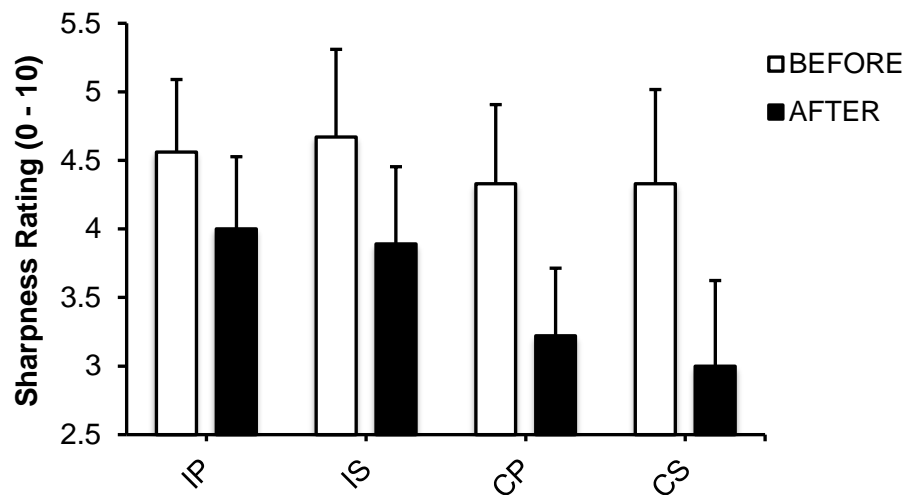


Figure 2. Mean sharpness ratings in response to moderate sharpness applied to the wrist before HFS and after HFS. Error bars denote standard error.

### Pain Ratings [to Supraorbital Stimuli](#)

Participants with HFS conditioning gave higher pain ratings across all four conditions [than participants who did not receive HFS](#) (see Figure 3). However, for both HFS and No HFS groups there was no difference in pain ratings between single site stimulation and simultaneous site stimulation, and between close and far wrist positions. ANOVA revealed a main effect of the position of the wrist,  $F(1, 27) = 4.23, p = .05, \eta^p$

$\eta^2 = .14$ , with pain ratings increasing when the arm was far away ( $M = 2.91$ ,  $SD = 1.73$ ) in comparison to when it was up close ( $M = 2.80$ ,  $SD = 1.71$ ). However, all interaction effects were insignificant.

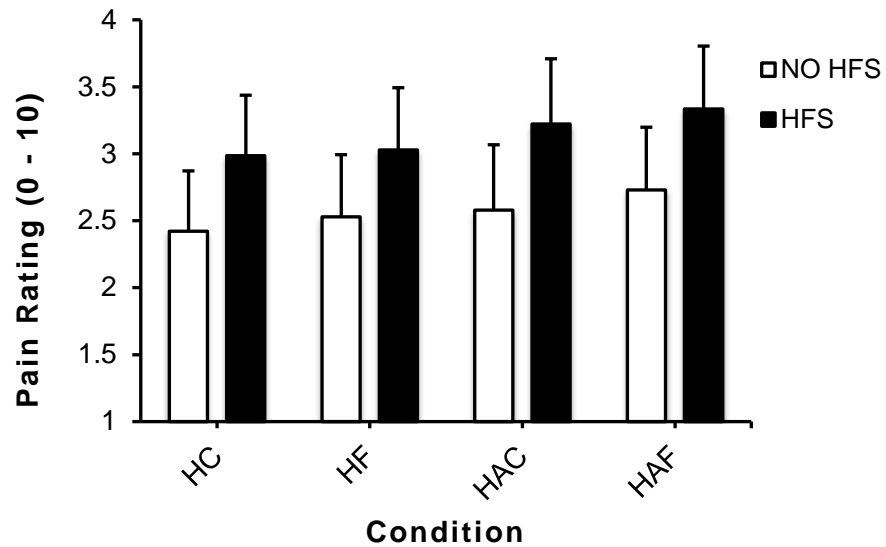


Figure 3. Mean pain ratings to supraorbital stimulation with HFS ( $N = 14$ ) and without HFS ( $N = 14$ ) conditioning. Error bars denote standard error (HC = head when wrist is close; HF = head far; HAC = head + arm close; HAF = head + arm far).

### Pain Ratings of Wrist Stimuli

Participants without HFS conditioning gave higher pain ratings across all four conditions (see Figure 4). Pain ratings were higher following simultaneous stimulation compared to wrist only stimulation. ANOVA supported this observation, revealing a main effect of site stimulated,  $F(1, 27) = 9.89$ ,  $p = .004$ ,  $\eta p^2 = .276$ , with higher pain ratings when both the head and wrist were stimulated simultaneously ( $M = 2.60$ ,  $SD = 1.49$ ), as opposed to the wrist alone ( $M = 2.20$ ,  $SD = 1.49$ ). However, all interaction effects were insignificant. Additionally, no between-subjects effect for HFS was found, indicating there was no difference in pain ratings between conditions.

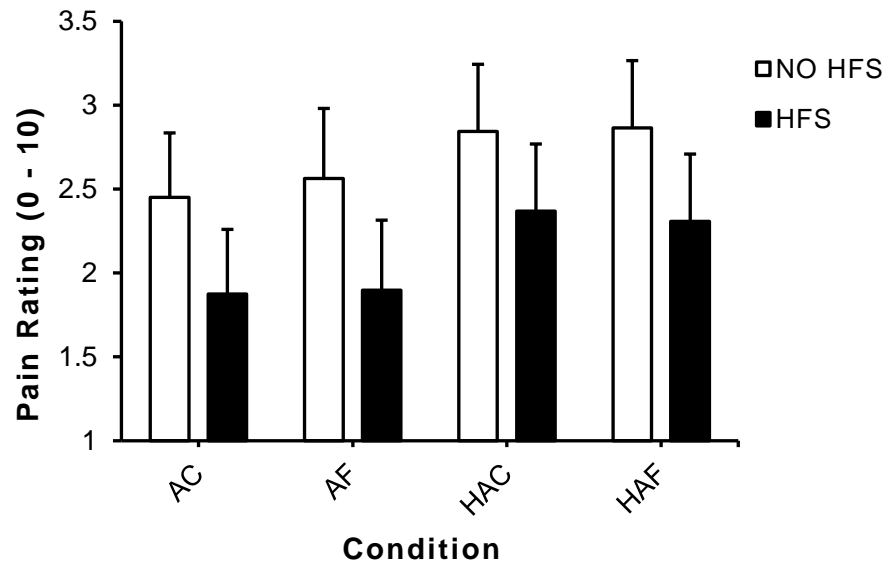


Figure 4. Mean pain ratings of wrist stimulation with HFS ( $N = 14$ ) and without HFS ( $N = 14$ ) conditioning. Error bars denote standard error (AC = arm close; AF = arm far; HAC = head + arm close; HAF = head + arm far).

### Sharpness Ratings of Supraorbital Stimuli

Participants demonstrated higher sharpness ratings when the wrist was far away, irrespective of HFS conditioning, and higher sharpness ratings across all four conditions within the HFS group (see Figure 5). Indeed, ANOVA revealed a significant main effect of position,  $F(1, 27) = 9.33, p = .005, \eta p^2 = .26$ , with higher sharpness ratings when the wrist was far away ( $M = 3.93, SD = 2.05$ ) compared to when the wrist was close ( $M = 3.74, SD = 2.05$ ). However, there was no main effect for HFS conditioning and all interaction effects were insignificant.

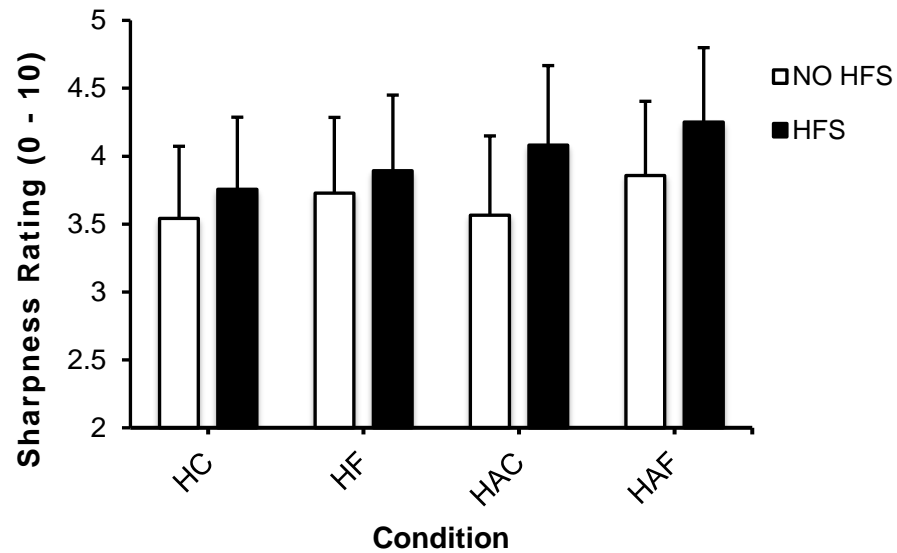


Figure 5. Mean sharpness ratings to supraorbital stimuli with HFS ( $N = 14$ ) and without HFS ( $N = 14$ ) conditioning. Error bars denote standard error.

### Sharpness Ratings of Wrist Stimuli

Analysis of the means identified higher sharpness ratings for the no HFS group compared to the HFS group, and higher ratings for simultaneous stimulation compared to wrist stimulation alone (see Figure 6). ANOVA supported this observation, revealing a significant main effect of site stimulated,  $F(1, 27) = 6.84, p = .015, \eta^2 = .21$ , with higher sharpness ratings reported when the wrist and head were stimulated



simultaneously ( $M = 3.26$ ,  $SD = 1.85$ ), as opposed to when the wrist was stimulated alone ( $M = 2.90$ ,  $SD = 1.82$ ). A significant interaction effect between position of the wrist and HFS conditioning was found,  $F(1, 27) = 6.58$ ,  $p = .016$ ,  $\eta p^2 = .20$ , with the HFS group giving higher sharpness ratings when the wrist was close to the face ( $M = 2.90$ ,  $SD = 2.52$ ) compared to far away ( $M = 2.78$ ,  $SD = 2.59$ ), and the No HFS group giving higher sharpness ratings when the wrist was far away ( $M = 3.37$ ,  $SD = 2.59$ ), compared to when it was close ( $M = 3.24$ ,  $SD = 2.52$ ) (see Figure 7).

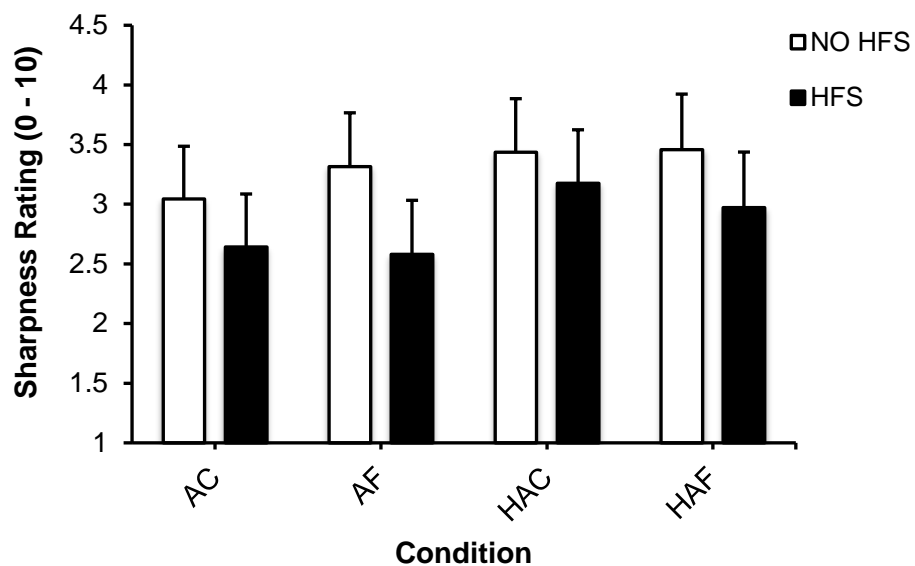


Figure 6. Mean sharpness ratings of wrist stimuli with HFS ( $N = 14$ ) and without HFS conditioning ( $N = 14$ ). Error bars denote standard error.

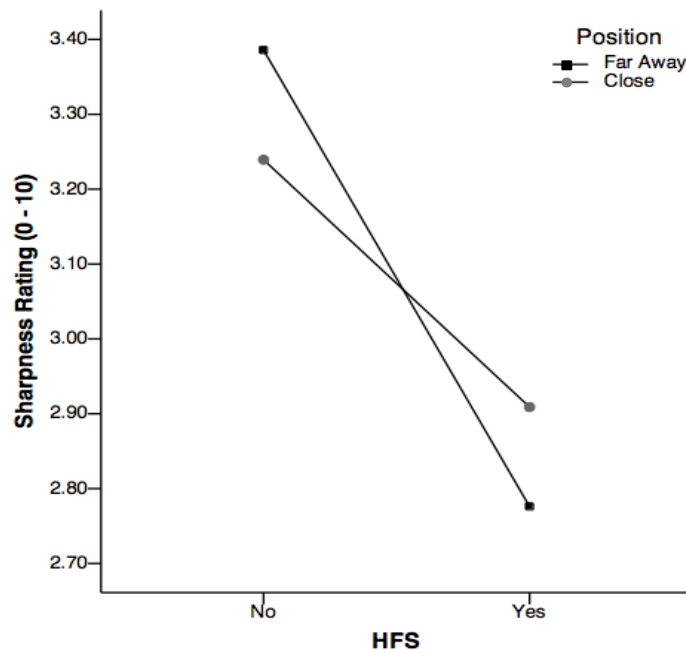
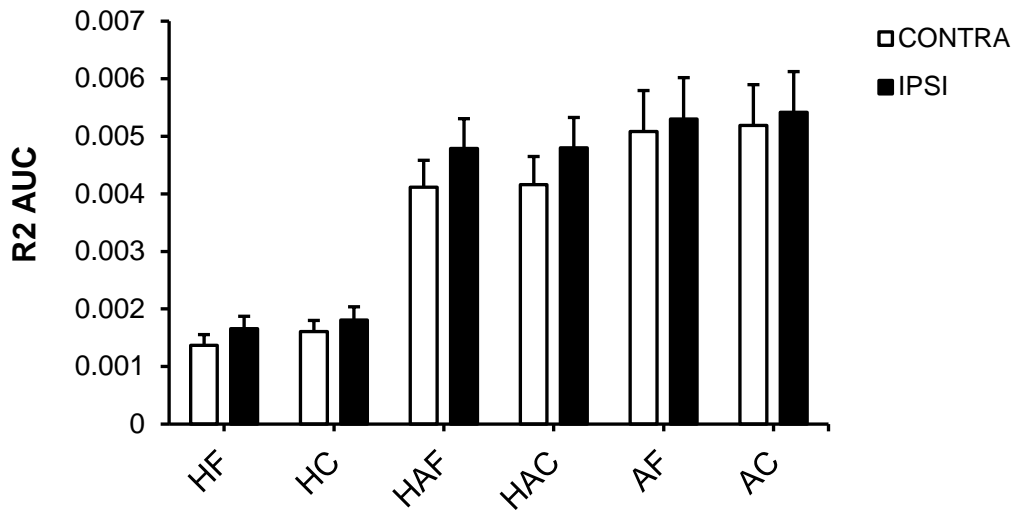


Figure 7. Interaction between HFS conditioning and position of the wrist.

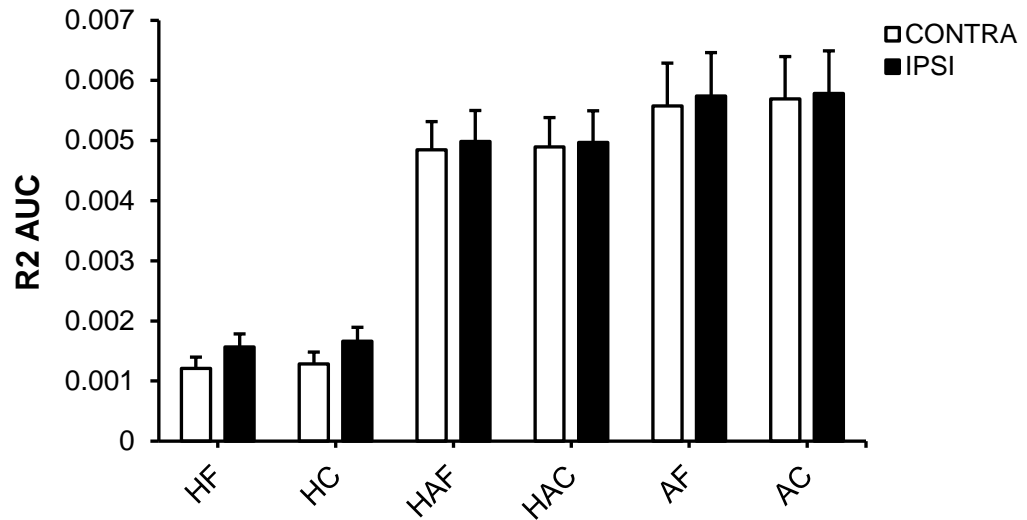
### Raw R2 AUC Response to Supraorbital and Wrist Stimuli

For both [the](#) no-HFS and HFS group blinks were greatest when the wrist was stimulated alone, whilst blinks were significantly smallest when the head was [stimulated](#) alone (see Figures 8 & 9). ANOVA discovered a significant main effect of site stimulated,  $F(2, 26) = 66.28, p < .001, \eta^2 = .72$ , with R2 AUC being greater when head + wrist were stimulated together ( $M = .0047, SD = .0017$ ) than when the head was stimulated alone ( $M = .0015, SD = .0007$ ). Additionally, a main effect of position was revealed,  $F(1, 27) = 11.79, p = .002, \eta^2 = .31$ , with R2 AUC being greater when the wrist was close ( $M = .0039, SD = .0014$ ) than when the wrist was far away ( $M = .0038, SD = .0014$ ). A significant interaction between site stimulated and position was demonstrated,  $F(2, 26) = 3.64, p = .033, \eta^2 = .12$ . Within-subjects simple contrasts indicated that this significance only lies between the head alone condition and the head + wrist condition (i.e. no significant interaction between head alone and wrist alone),

$F(1,27) = 5.01, p = .034, \eta p^2 = .16$ , with R2 AUC being greater for both head alone stimulation and head + wrist stimulation when the wrist was close ( $M = .0016, SD = .0007; M = .0047, SD = .0017$ ) compared to far away ( $M = .0014, SD = .0007; M = .0046, SD = .0018$ ). However, no significant main effect for HFS was reported.



*Figure 8.* Mean contralateral and ipsilateral R2 AUC responses to head and wrist stimuli conditions for the no-HFS group ( $N = 14$ ). Error bars denote standard error.



*Figure 9.* Mean contralateral and ipsilateral R2 AUC responses to head and wrist stimuli conditions for the HFS group ( $N = 14$ ). Error bars denote standard error.

However, the interaction between position and site stimulated looked very slight albeit non-existent (see Figures 8 and 9). We predicted that this significant interaction might be a result of the difference between contralateral blinks and ipsilateral blinks. Thus, we conducted an ANOVA to further investigate this interaction. We examined the significance of position on R2 AUC responses to head alone stimuli and head + wrist stimuli (the conditions involved in the significant interaction), however, instead of examining ipsilateral averages and contralateral averages, we calculated the average of combined ipsilateral and contralateral R2 AUC values. For head alone, the observed

result was a significant main effect of position,  $F(1, 27) = 17.65, p < .001, \eta p^2 = .40$ , with greater R2 AUC when the arm was close ( $M = .0016, SD = .0007$ ), compared to far away ( $M = .0014, SD = .0007$ ) (see Figure 10). However, there was a non-significant effect of position for the head + wrist condition,  $F(1, 27) = .28, p = .604, \eta p^2 = .01$  (see Figure 11). Post-hoc comparison using a paired samples t-test with the Bonferroni adjusted alpha level of .005 (.05/10) supported these findings, demonstrating a significant difference between HC and HF ( $t(27) = 4.08, p < .001$ ), and a non-significant difference between HAC and HAF ( $t(27) = .54, p = .60$ ).

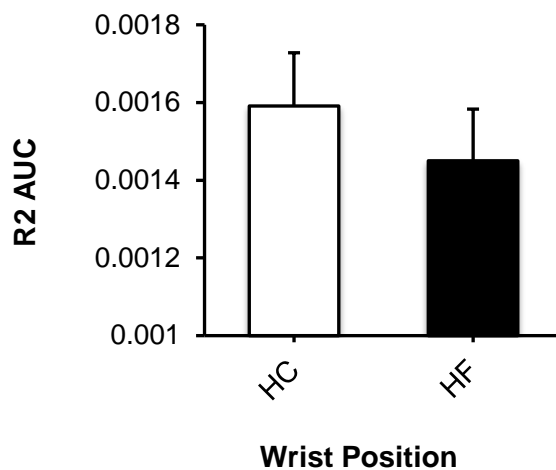


Figure 10. Mean R2 AUC responses to head alone stimuli when the wrist was close and far away.

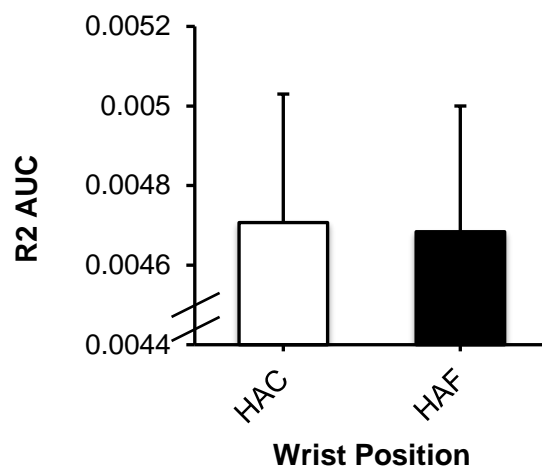


Figure 11. Mean R2 AUC responses to head + wrist stimuli when the wrist was close and far away.

## Discussion

The current study [aimed](#) to assess the relationship between peripersonal space representations, pain, and defensive action by examining the effect of proximity to pain on threat perception and protective reflex responses. Uniquely, this study sought to determine whether this relationship [was](#) exacerbated [by HFS conditioning to mimic chronic pain symptoms](#). This premise was assessed through a nociceptive blink reflex procedure, in which the magnitude (R2 AUC) of participants' blinks to electrical stimulation was measured when the [stimulated](#) wrist was far away and up close.

### Hypothesis One

Firstly, it was hypothesised that blink reflex responses and pain and sharpness ratings to stimulation [would](#) be greater when the wrist [was](#) close to the face compared to [further](#) away. Objects situated in peripersonal space garner a higher level of perceived threat because they have the ability to harm the most vulnerable parts of the body (i.e. the head and the eyes) (Holmes & Spence, 2004). Therefore, highly threatening objects (noxious stimuli applied to the wrist when close to the face) should induce intense protective reflexes to defend these parts from harm (Bufacci et al., 2016). Thus, I expected [to find](#) a significant main effect of wrist position, with higher means for the close conditions (in essence; close > far away).

In support of this hypothesis, raw means showed that R2 AUC differed significantly for the position of the wrist, in which blinks were significantly greater when the wrist was close compared to far away. These findings are consistent with the research conducted by Sambo et al. (2012a), who discovered that human blink reflexes elicited by hand stimulation increase in magnitude when the hand is inside peripersonal

space surrounding the face. However, the magnitude of blink reflexes elicited by supraorbital nerve stimulation was not affected by the position of the hand (Sambo et al., 2012a). Our study adds that the position of the wrist indeed affects blink reflexes elicited by supraorbital stimulation. The magnitude of blink reflexes elicited by [supraorbital](#) stimulation alone significantly increased when the wrist was close. The moderating effect of position on blink reflexes elicited by supraorbital stimuli suggests that the top-down modulation of the blink reflex involves the facilitation of facial motor neurons that innervate the orbicularis oculi muscle.

Alternatively, blink reflexes may have intensified because participants expected to feel pain on the hand when it was close to the face (Sambo et al., 2012b). Due to the randomized blink sequence, participants were unaware whether they would receive an electrical stimulus to the wrist, to the head or to both the head and the wrist [in any given trial](#). Participants were also unaware whether stimuli to the wrist would occur when the arm was far away or close. However, in anticipation of pain, participants may have tried to predict what type of stimulus was next, and thus interpreted the wrist being close to the face as an indication that the wrist would be stimulated. Predicting high stimulus probability may have ultimately increased the perceived threat of the stimulus, and subsequent protective blink reflex responses (Sambo et al., 2012b).

Against our hypothesis, mean pain and sharpness ratings of head stimuli were significantly greater when the wrist was far away. Cognitive expectations and ambiguity surrounding the perceived threat of impending stimuli can [explain](#) why this occurred. Expecting high levels of pain can increase perceived pain intensity levels beyond the real nociceptive intensity (Weisenberg, 1987; Wiech et al., 2008). Moreover, high anxiety levels due to the uncertainty surrounding the next stimulus can result in ambiguous stimuli being interpreted as more threatening (Bishop, 2007; Enck,

Benedetti & Schedlowski, 2008). High anxiety levels can also extend the boundaries of defensive peripersonal space (Sambo & Iannetti, 2013). Thus, a higher anxiety level, thereby increasing the area of peripersonal space, also increases defensive responses to objects typically outside defensive boundaries (Sambo & Iannetti, 2013). In this study, negative psychosocial context may have induced anxiety and increased the threat of ambiguous stimuli and peripersonal space margins. In order to control for anxiety and maximize the internal validity of the study, it is suggested that further research incorporate anxiety assessment before experimental testing. Attention also explains why head stimuli had higher pain ratings when the arm was far away. Perhaps when the arm was far away, participants were able to focus on head stimuli without being distracted by the vision of their arm in peripersonal space. Additionally having the arm up close may have felt like a protective position, thereby decreasing its overall “threat” levels and decreasing blinks.

### **Hypothesis Two**

Secondly, I hypothesised that blink reflex responses and pain and sharpness ratings would be greater following simultaneous stimulation than when either site was stimulated alone. I predicted that simultaneous stimulation would trigger spatial summation based on the premise that stimulating a larger surface area would decrease sensory thresholds and increase perceived pain intensity of the stimuli. Greater responses would occur because paired electrical stimuli activate a larger number of nociceptive receptive fields (Reid et al., 2015). Thus, we expected to find a significant main effect of site stimulated (in essence; head + wrist > head alone, wrist alone). In support of this hypothesis, results showed a significant increase in pain and sharpness ratings of wrist stimuli following simultaneous stimulation. Additionally, R2 AUC for simultaneous head and wrist stimulation was significantly greater than R2 AUC for



head alone stimulation. Our results are in alignment with previous research, which found that an increase in the size of the stimulated surface area intensified nociceptive activity and decreased sensory thresholds (Marchand & Arsenault, 2002; Reid et al., 2015). Changes in pain thresholds are indicative of spatial summation.

However, results failed to demonstrate a significant increase in pain ratings and sharpness ratings of head stimuli for simultaneous stimulation. It is possible that the wrist stimulus was notably more intense (greater pain and sharpness) than the head stimuli, causing an inhibition of responses to the less intense stimulus. Indeed, we found a significant difference between head alone stimulation and simultaneous stimulation (in which the latter demonstrated greater blink reflexes). However, observation of the R2 AUC raw means and percentages through t-tests revealed that blink reflexes to wrist alone stimulation were significantly larger than blink reflexes to both simultaneous stimulation and head alone stimulation. Past literature on diffuse noxious inhibitory controls (DNIC) attempts to explain this phenomenon. DNIC is a pain modulatory mechanism in which a noxious stimulus impedes the pain generated by another spatially distant but weaker stimulus (Edwards, Ness, Weigent & Fillingim, 2003). It specifically consists of the inhibition of nociceptive neurons and wide dynamic range neurons. Evidence suggests that failure of DNIC is a reliable predictor of chronic pain in previously healthy individuals (Edwards, Ness, Weigent & Fillingim, 2003; Yarnitsky, 2010). Ultimately, results suggest that inhibitory and excitatory mechanisms affected spatial summation.

Additionally, previous studies have not specifically examined the effect of simultaneous supraorbital and medial nerve stimulation on the nociceptive blink reflex. Lack of significant research on this topic resulted in the design of a new framework. Thus our attempt to develop an appropriate research design required a degree of trial

and error, which could contribute to our non-significant results. However, our study has successfully established itself as the foundation for further research into this topic.

### **Hypothesis Three**

Thirdly, I hypothesised that blink reflex responses and pain and sharpness ratings would be greater in response to simultaneous stimulation when the wrist was close to the face. Based on a combination of our first and second hypotheses, I predicted that an effect of proximity and an effect of simultaneous stimulation would interact to influence the strength of protective reflexes positively. Theoretically, as noxious stimuli approached peripersonal space (increasing threat) and spatial summation occurred via simultaneous stimulation (increasing pain sensitivities), protective reflexes would increase. Supportive results would reveal an interplay between peripersonal space, threat detection, spatial summation, and defensive reflexes. Thus, I expected to find a significant interaction between the position of the wrist and site stimulated for pain ratings, sharpness ratings, and R2 AUC (in essence; head + wrist close > all other conditions). Initial findings demonstrated that raw R2 AUC significantly increased when the wrist was close and there was simultaneous stimulation, in comparison to when the wrist was far away and when the head was stimulated alone. However, results were unable to clarify the source of this interaction in post hoc analyses. Additionally, there were no significant interactions between position and site stimulated for pain or sharpness ratings to supraorbital or wrist stimuli. These results do not support the findings of previous research relevant to hypothesis two and hypothesis three. Applying noxious input to spatially separate areas of the body can increase the total amount of central nociceptive neurons stimulated and increase perceived pain intensities (otherwise known as spatial summation) (Price et al., 1989). The combined effects of

spatial summation and an invasion of peripersonal space should increase the magnitude of protective blink reflexes in an attempt to survive further harm.

Lack of an interaction between wrist proximity and simultaneous stimulation might be due to problems with attention. Some participants in our study reported difficulty differentiating between supraorbital stimuli and wrist stimuli when the wrist was close to their face. Participants were unable to divide their attention between the two stimuli, and subsequent pain and sharpness ratings were unusually low for supraorbital stimuli. In a few instances, participants failed to perceive the noxious stimulus to the head at all (i.e. failed to report pain and sharpness). Previous research has revealed that attention mediates pain intensity, wherein greater attention to noxious input increases perceived pain intensity and less attention decreases pain intensity (McCracken, 1997). This association is particularly evident in chronic pain patients. Most relevant to our results, one study demonstrated when participants were required to divide their attention between two noxious stimuli spatial summation and pain intensities were inhibited (Quevedo & Coghill, 2007). Thus, attention can change the spatial assimilation of nociceptive input to improve the response to noxious stimuli applied to more important or vulnerable body parts. In this way, failing to find a significant interaction between proximity and simultaneous stimulation provided great insight into endogenous pain inhibitory mechanisms.

#### **Hypothesis Four**

Lastly, I hypothesised that blink reflex responses and pain and sharpness ratings to stimulation would be greater following HFS conditioning of the wrist. I expected to find a significant between-subjects effect of HFS conditioning, in which the HFS group would display significantly greater R2 AUC, pain ratings, and sharpness ratings compared to the no-HFS group. Additionally, I predicted there would be a significant

interaction between HFS, position and site-stimulated, in which HFS conditioning would exacerbate the effects of position and site stimulated. Indeed, results indicated that HFS significantly increased sharpness ratings of wrist stimuli when the wrist was close, while lack of HFS conditioning significantly increased sharpness ratings of wrist stimuli when the wrist was far away. Thus, induced primary hyperalgesia of the wrist allowed participants to interpret noxious input as sharper (i.e. more threatening) when situated in peripersonal space.

The interaction between HFS and wrist position partially supports the notion that disturbed nociceptive processing and peripersonal distortions (i.e. somatosensory disturbances) in individuals with chronic pain amplify threat perception. The increased sharpness ratings for HFS participants imply that HFS was effective, while increased sharpness ratings when the wrist was close imply that somatosensory disturbances heightened threat perception. Previous findings support both a facilitatory and an inhibitory effect of HFS, in which hyperalgesia develops at primary and secondary sites while pain is inhibited elsewhere (Vo & Drummond, 2013; Vo & Drummond, 2014). Specifically, HFS can elicit central sensitization in the conditioned forearm while also inducing pressure-pain analgesia in the ipsilateral forehead (Vo & Drummond, 2013). These effects outline the role of central disturbances to somatosensory processing and pain modulation in the formation and maintenance of chronic pain symptoms (Drummond & Finch, 2014). Somatosensory disturbances also contribute to distortions of body and space perception (Holmes & Spence, 2004; Lewis & Schweinhardt, 2012). Therefore, our result partially supports these findings and the accompanied prediction.

However, results failed to demonstrate significant differences between the HFS and no-HFS groups for pain ratings, R2 AUC, or sharpness ratings to supraorbital stimuli. Recent research has also shown an increase in the excitability of the nociceptive

blink reflex (R2 AUC) in the ipsilateral forehead following HFS conditioning (Vo & Drummond, 2014). Thus, I was unable to completely confirm that disturbed nociceptive processing and peripersonal distortions positively affect threat perception and subsequent protective responses (i.e. blink reflexes). The absence of significant differences can be attributed to the ineffectiveness of HFS to mimic chronic pain symptoms in participants. As explained earlier, primary and secondary hyperalgesia to light touch, moderate pain and blunt pressure indicate the effectiveness of HFS (Vo & Drummond, 2013; Vo & Drummond, 2014). However, psychophysical tests suggest that results failed to replicate these effects. Psychophysical tests did not reveal significant increases in sensitivity to mild pain, moderate pain, and blunt pressure following HFS conditioning. In fact, following HFS sensitivity to mild and moderate pain decreased (i.e. pain thresholds increased). However, lack of a significant sample of participants with induced chronic pain symptoms made it difficult to compare the effect of proximity to pain on protective blink reflexes in our simulation of chronic pain.

These events may occur for a variety of reasons. Firstly, it is possible that participants changed the way they rated pain and sharpness following HFS conditioning. During the first round of psychophysical testing before HFS, participants were told to compare stimuli to their most extreme experience of pain and rate stimuli accordingly. However, participants may have compared second round psychophysical tests to the HFS they just received. This frame of reference would be an easier comparison for participants because the recent memories are easier to retrieve. If this is the case, participants should have been told to stick to their original frame of reference during the second round of psychophysical testing. Secondly, the algometer used to measure sensitivity changes to pressure-pain failed to provide an accurate reading on five occasions (it repeatedly lost power after a certain force was applied) during the

psychophysical testing that followed HFS conditioning. Thus I was only able to compare the mean PPT before HFS and after HFS of nine participants. Lack of a broad cross-section may contribute to why PPT tests did not observe significant changes in sensitivity to pressure-pain following HFS.

### **Limitations**

A major limitation of this study was faulty equipment. As stated in the methods, I aimed to standardise the intensity of the electrical stimuli applied to the wrist across participants. Therefore, I applied a level “5” pain rating requirement with a maximum intensity of 15mA. However, most participants with high pain thresholds did not achieve a pain rating of 5. On average, wrist stimuli had an intensity of 13.25 mA (SD = 3.73) and produced an average pain rating of 2.89 (SD = 1.45). Consequentially, an unstandardized intensity for wrist stimuli may have increased the variability of pain and sharpness ratings, and EMG responses. As previously mentioned, eleven participants were excluded from the study due to electrical activity in the ground electrode attached to their neck. Ultimately, participants commented that these additional stimuli made it difficult for them to distinguish between all three stimuli (head, wrist, and neck); therefore, they were unsure how to rate each stimulus. The inability to differentiate between stimuli jeopardised the collection of accurate pain ratings and sharpness ratings for each site stimulated. In fact, data analysis including the eleven participants had profound effects on the non-contaminated results (see Appendices H to K). Perhaps with the inclusion of these participants the greater sample size might have produced more significant results.

Another limitation of this study was the use of self-report measures. Although participants were trained to give accurate self-reports, these measures are subjective; they depend on the individual’s experiences and are affected by mood. For example,

one participant compared electrical stimuli to her experience of childbirth; the most extreme pain she had felt. Subsequently, her pain threshold was very high in comparison to other participants who did not have a notably painful experience. To account for this, we attempted to standardise pain and sharpness ratings by achieving a pain level of “5” for all participants; however, this was unsuccessful. Nevertheless, the objective measure of blink reflexes was able to compensate this limitation.

### **Conclusion**

The present study examined the association between nociception, protective action, and peripersonal space by exploring the influence of proximity to pain on threat sensitivity and blink reflex responses. Specifically, it investigated whether individuals with chronic pain symptoms experience heightened effects of proximity. Although not all three dependent variables demonstrated the same effects, our findings partially supported our hypotheses. Firstly, our results revealed that proximity to pain indeed induced strong protective reflexes and that this effect was present for not only noxious wrist stimuli but also noxious supraorbital stimuli. Secondly, simultaneous stimulation increased the perceived threat of noxious input, thereby triggering excitatory mechanisms of spatial summation. Lack of significant results for pain and sharpness ratings for supraorbital stimuli imply that inhibitory pain mechanisms were also involved. Thirdly, although we failed to find a relationship between proximity and simultaneous stimulation, our results provided great insight into endogenous pain inhibitory mechanisms. Specifically, we discovered that attention changes the spatial assimilation of nociceptive input to improve the response to noxious stimuli applied to vulnerable body parts. Lastly, disturbed nociceptive processing and peripersonal distortions present in individuals with chronic pain positively affect threat perception.

However, further research is necessary to account for the lack of significant results for pain ratings and R2 AUC. Overall, we suggest that additional research attempts to account for cognitive expectations and ambiguity surrounding the perceived threat of impending stimuli. Researchers can control these confounds by incorporating anxiety assessments before testing. Ultimately, the current study was able to support the presence of a protective peripersonal space that is essential for detection of threat and survival and provided insight into the coexistence of excitatory and inhibitory pain modulation mechanisms.



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