UNDERSTANDING PAIN: HOW IS PAIN PROCESSED IN HEALTHY HUMANS?

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Declaration

This thesis is my own account of my research and contains as its main content work that has not previously been submitted for a degree at any tertiary education institution.

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Abstract

The aim of this thesis was to examine the effects of experimentally-induced limb pain on pain in other remote body sites in a pain-free healthy population. In Study one, we compared the effects of limb-pain induced by high-frequency electrical stimulation (HFS) and ultraviolet B (UVB) on sensitivity to heat, and to sharpness and pressure-pain on the conditioned forearm site, the contralateral control site, and on each side of the forehead in samples of 30 (HFS) and 16 (UVB) healthy participants. Prior to pain induction, sensitivity to heat, sharpness and pressure-pain was similar at the conditioned site and the control site, and between the two sides of the forehead. UVB triggered more intense signs of primary hyperalgesia at the conditioned site than HFS. Secondary hyperalgesia developed after HFS but not UVB, indicating that HFS evoked signs of central sensitisation. Pressure-pain sensitivity decreased on both sides of the forehead with a greater reduction on the ipsilateral side after HFS, but not UVB. Furthermore, electrically-evoked pain at the HFS-conditioned site decreased significantly more during ipsilateral temple cooling than contralateral cooling, whereas pain reduction at the UVB-conditioned site was similar irrespective of the side of forehead that was cooled. Thus, central sensitisation evoked by HFS might also have triggered ipsilateral pain-inhibitory modulation processes in healthy humans.

In Study two, to further delineate pain modulation processes evoked by HFS, we examined sensory changes in the forearm and forehead, and nociceptive blink reflexes elicited by supraorbital electrical stimulation with and without counter-irritation (electrically-evoked pain at the HFS-conditioned site) in 20 healthy
participants before and after HFS conditioning. In line with Study one, secondary hyperalgesia and bilateral and ipsilateral forehead analgesia to pressure-pain developed after HFS conditioning. In general, counter-irritation of the forearm and HFS suppressed pain perception, and inhibited the amplitude of nociceptive blink reflex to supraorbital stimuli. However, in the absence of forearm counter-irritation, HFS facilitated the ipsilateral blink reflex amplitude to supraorbital stimuli delivered ipsilateral to the HFS-conditioned site. Thus, HFS might have triggered hemilateral pain-inhibitory and pain-facilitatory mechanisms simultaneously.

In Study three [53], to determine whether central sensitisation is necessary for triggering this sign of ipsilateral inhibitory pain modulation, we compared the effects of HFS and low frequency electrical stimulation (LFS) in the forearm on sensitivity to pressure-pain in the ipsilateral forehead in samples of 50 (HFS) and 18 (LFS) healthy individuals. LFS was chosen as it triggers only minor sign of central sensitisation. Before conditioning, sensitivity to heat, sharpness, and pressure-pain were similar at the conditioned and the control sites, and between the two sides of the forehead. Pain perception was higher after HFS than LFS, and central sensitisation developed after HFS but not LFS. Nevertheless, pressure-pain sensitivity decreased in the ipsilateral forehead after both forms of electrical stimulation. This decrease was associated with a heightened sensitivity to pressure-pain at the conditioned forearm site, but with a reduced sensitivity to heat in skin surrounding the electrically-conditioned site. Thus, the ipsilateral pain-inhibitory process might have suppressed sensitivity to pressure-pain in the ipsilateral forehead and secondary hyperalgesia to heat.

Evidence from rat studies indicates adrenergic influences descending from the locus coeruleus (LC) in mediating ipsilateral inhibitory pain control via the activation
of inhibitory α₂-adrenoreceptors. Therefore, in the final study (Study four), to determine whether ipsilateral forehead analgesia to HFS is mediated by α₂-adrenoreceptors, we attempted to block their effects with oral administration of yohimbine, an α₂-adrenoreceptor antagonist, in a double-blind placebo-controlled crossover design in a sample of 22 healthy individuals. Sensitivity to heat, sharpness, and pressure-pain at and adjacent to the conditioned and control sites, and on each side of the forehead was assessed at baseline, following drug administration, and after HFS conditioning. Blood pressure, heart rate and electrodermal activity were also measured across these three stages. Nociceptive blink reflexes to supraorbital stimulation were also investigated following drug administration and after HFS conditioning. In addition, the effects of ipsilateral versus contralateral temple cooling on electrically-evoked pain at the HFS-conditioned site were compared. Prior to drug administration, sensitivity to heat, sharpness, and pressure-pain were similar at the conditioned and the control site, and between the two sides of the forehead. In line with our previous studies, in the placebo condition, HFS evoked primary and secondary hyperalgesia in the forearm, ipsilateral forehead analgesia to pressure-pain, and a reduction of electrically-evoked forearm pain during ipsilateral temple cooling. As expected, yohimbine increased blood pressure and electrodermal activity compared to placebo. Yohimbine also enhanced the excitability of the ipsilateral nociceptive blink reflex compared with placebo, consistent with yohimbine facilitating pro-nociceptive effects. Unexpectedly, the development of ipsilateral forehead analgesia to pressure-pain following yohimbine, and a greater reduction of electrically-evoked forearm pain during ipsilateral temple cooling following yohimbine compared to placebo, suggests that yohimbine might have enhanced
analgesia. Thus, non-noradrenergic mechanisms may also be involved in mediating these analgesic effects, in addition to adrenergic influences.

Together, these findings indicate that in healthy humans noxious stimulation with HFS may trigger ipsilateral inhibitory modulation processes, which could be mediated both by noradrenergic and non-adrenergic mechanisms. Further investigation of ipsilateral inhibitory modulation processes is important, as this process may be disrupted in conditions such as complex regional pain syndrome.
Publications

Refereed Articles


Submitted Articles

Vo L, Drummond PD: Involvement of α2-adrenoceptors in inhibitory and facilitatory pain modulation processes.

These articles are reproduced in the thesis in their full, original state. This accounts for a certain degree of repetition and inconsistencies in reference style.
For my parents
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Finally, this thesis is also a tribute to my aunt, Cô Tư, who passed away in June 2013. A strong believer in values of education, she fought hard to ensure my sisters and I continued our education during some difficult times we had in our birth country. I would not be where I am today without her.
Table of Contents

Abstract iii
Publications vii
Acknowledgements ix
List of Tables xii
List of Figures xiii

Chapter 1 Normal Pain Processing 1

1.1. Pain 1

1.2. Nociception 2

1.3. Gate Control 4

1.4. Diffuse Noxious Inhibitory Controls (DNIC) 5

1.5. Stress-induced analgesia (SIA) 7

1.6. Stress-induced hyperalgesia (SIH) 9

1.7. Baroreflex-mediated Hypoalgesia 10

1.8. Coeruleospinal Pain Modulation 11

1.9. Peripheral sensitisation and inflammation 13

1.10. Central sensitisation 15
1.11. Cognitive and emotional influences on pain 17

Chapter 2 Human Experimental Pain Models 52
2.1. The Cold Pressor Test 52
2.2. Capsaicin 54
2.3. Ultraviolet-B (UVB) radiation 55
2.4. Electrical stimulation 56
2.5. Approach 59

Chapter 3 Study 1 High frequency electrical stimulation concurrently induces central sensitisation and ipsilateral inhibitory pain modulation 74

Chapter 4 Study 2 Coexistence of ipsilateral pain-inhibitory and facilitatory processes after high-frequency electrical stimulation 110

Chapter 5 Study 3 Analgesia to pressure-pain develops in the ipsilateral forehead after high- and low-frequency electrical stimulation of the forearm 142

Chapter 6 Study 4 Involvement of α2-adrenoceptors in inhibitory and facilitatory pain modulation processes 171

Chapter 7 Conclusions 225
7.1. Summary of all studies 225
7.2. Implications for non-CRPS chronic pain and CRPS 231
List of Tables

Tables from Study Three

Table 1  Experimental procedure 165

Table 2  Association between hyperalgesia in the forearm after electrical stimulation and a greater reduction in sensitivity to pressure-pain on the ipsilateral than contralateral side of the forehead 166

Table 3  Regression analysis predicting greater analgesia to pressure-pain in the ipsilateral than contralateral forehead after electrical stimulation 167

Tables from Study Four

Table 1  Experimental Procedure 213

Table 2  Differences between the yohimbine (Y) and placebo (P) conditions 214

Table S1  Cold pain rating ± S.E. in the temple during temple cooling 215
List of Figures

Figures from Study One

Figure 1  Sensitivity to heat, sharpness and pressure were assessed at a site in the ventral forearm conditioned by HFS or UVB radiation (the Primary Area) and in adjacent skin (the Secondary Area). During HFS, five 1-s bursts of electrical stimulation (100 Hz, 2 ms pulse width, at 10 times the electrical detection threshold up to a maximum of 8 mA) were delivered from the conditioning probe with a 9-second rest between bursts. Sensitivity to heat, sharpness and pressure were re-assessed at each site 10 minutes later.

Figure 2  Mean sensitivity ± S.E. to (a) heat; (b) pinprick; (c) von Frey’s monofilament; and (d) pressure-pain in the Primary and Secondary Areas before and after conditioning with HFS or UVB. Sensitivity to each stimulus increased significantly in the Primary Area after HFS- and UVB conditioning (* p< .05 and ** p<.01 compared with values before conditioning). However, heat sensitivity in Primary Area was greater after UVB- than HFS-conditioning (# p < .05), indicating that primary hyperalgesia was greater
after UVB- than HFS conditioning. In contrast, sharpness ratings increased in the Secondary Area after HFS conditioning (consistent with central sensitisation) but not after UVB conditioning. In addition, PPT decreased in the Secondary Area after UVB (* p < .05 compared with values before conditioning).

Figure 3  
Pressure-pain thresholds ± S.E. in the ipsilateral and contralateral forehead before conditioning, after conditioning, during electrically-evoked pain at the treated forearm site, and during electrically-evoked pain at the control site following HFS- and UVB conditioning. Ipsilateral and contralateral PPTs increased significantly after HFS conditioning (* p < .05 and ** p < .001 compared with values before conditioning). PPT ipsilateral to the HFS-conditioned site decreased significantly when electrical stimuli were applied to the HFS control site (# p < .05 compared with values during test-site stimulation). However, PPTs did not change significantly after UVB conditioning.

Figure 4  
The effect of ipsilateral and contralateral temple cooling on electrically-evoked pain at (a) the HFS-treated site; (b) the HFS control site; (c) the UVB-
treated site; and (d) the UVB control site. Ice was applied to each temple for 30 seconds. Pain ratings decreased significantly at treated and control sites during the indicated time intervals compared with a control task when ice was not applied (* p < .05). In addition, decreases in pain at the HFS-treated site (a) were significantly greater during ipsilateral than contralateral temple cooling during the final 10 seconds of cooling and for the following 5 seconds (# p < .05). Error bars represent standard errors.

Figure 5  Hypothesized effects of HFS and UVB conditioning on pain modulation. (a) UVB conditioning induced signs of peripheral sensitisation (signified by the red star in the dorsal root ganglion – DRG), but did not evoke spontaneous pain, central sensitisation or activity in descending pain-facilitatory or inhibitory pathways (represented by the dashed lines). (b) HFS conditioning induced signs of peripheral and central sensitisation in ascending pain-projection pathways (shown in red). This may have triggered activity in descending ipsilateral pain facilitatory pathways (responsible for central sensitisation, shown in yellow) and hemilateral pain inhibitory pathways (responsible for ipsilateral analgesia, shown in blue). (c) Counter-irritation from noxious stimulation of
either temple inhibited pain evoked by electrical stimulation of the UVB-conditioned site (inhibitory influences shown in light blue). (d) In addition to this bilateral influence, noxious stimulation of the temple ipsilateral to HFS conditioning may have evoked activity in an ipsilateral descending pain-inhibitory pathway (shown in dark blue), augmenting the analgesic effect of counter-irritation.

Figures from Study Two

Figure 1  Unrectified and rectified waveforms of a blink reflex. Latency was measured in milliseconds from the onset of the three-pulse left-sided stimulus to the point where the amplitude of the signal began to change noticeably from background noise. In addition, the R2 rectified area under the curve (AUC) was measured between 27 and 87 ms after stimulus onset.

Figure 2  Mean sensitivity ± S.E. to (a) heat; (b) pinprick; (c) von Frey’s monofilament; and (d) pressure-pain in the Primary and Secondary Areas at baseline and after HFS conditioning. Sensitivity to pinprick and von Frey’s monofilament increased significantly in the Primary and the Secondary Areas after HFS
conditioning, and sensitivity to pressure-pain increased significantly in the Primary Area (* p< .05 compared to baseline values).

Figure 3 Mean sensitivity ± S.E. to (a) heat; (b) pinprick; (c) von Frey’s monofilament; and (d) pressure-pain in the ipsilateral and contralateral forehead at baseline, after the first set of blink reflexes, after HFS conditioning, and after the second set of blink reflexes. Sensitivity to heat, pinprick and von Frey’s monofilament remained stable throughout on both sides of the forehead. In contrast, ipsilateral and contralateral pressure-pain thresholds (PPTs) increased significantly after HFS conditioning and after the second set of blink reflexes (# p < .01 compared with their respective PPT’s at baseline), with a greater increase on the ipsilateral side (* p < .001 compared with PPT on the contralateral side after HFS conditioning and after the second set of blink reflexes).

Figure 4 Pain ratings ± S.E. to supraorbital stimuli with and without electrically-evoked forearm pain at baseline and after HFS conditioning. a. Pain ratings decreased after HFS conditioning, both in the presence and absence of electrically-evoked forearm pain (** p =
.007). b. Both before and after HFS conditioning, electrically-evoked forearm pain reduced sensitivity to supraorbital stimuli (* p = .046). However, sensitivity to supraorbital stimulation did not differ between the ipsilateral and contralateral forehead.

Figure 5  Mean R2 onset latencies ± S.E. Before HFS conditioning, R2 onset latencies were similar irrespective of electrically-evoked forearm pain. However, after HFS conditioning, R2 onset latency decreased significantly in the presence of counter-irritation (* p < .01 compared with all other conditions).

Figure 6  Mean R2 area under the curve (AUC) ± S.E. for blink reflexes ipsilateral to both the HFS-treated site and supraorbital stimuli (R2ii), ipsilateral to the HFS-treated site but contralateral to supraorbital stimuli (R2ic), contralateral to the HFS-treated site but ipsilateral to supraorbital stimuli (R2ci), and contralateral both to the HFS-treated site and to supraorbital stimuli (R2cc) in raw format before and after HFS conditioning. In general, R2 AUC at baseline (A) was greater than R2 AUC during forearm counter-irritation (B) and after HFS (C and D).
Figure 7  Mean percent change ± S.E. in R2 area under the curve (AUC) expressed in relation to R2 AUC recorded at baseline ipsilateral both to the HFS-treated site and supraorbital stimuli (R2ii), ipsilateral to the HFS-treated site but contralateral to supraorbital stimuli (R2ic), contralateral to the HFS-treated site but ipsilateral to supraorbital stimuli (R2ci), and contralateral both to the HFS-treated site and supraorbital stimuli (R2cc). R2 AUC decreased significantly (p < .01) during electrically-evoked forearm pain on both sides of the forehead both before and after HFS conditioning (Fig. 7a and 7b). However, in the absence of counter-irritation of the forearm, the R2ii AUC increased after HFS conditioning, whereas R2ic AUC, R2ci AUC, and R2cc AUC decreased (Fig. 7c). Specifically, the R2ii AUC was significantly greater than the R2ic AUC and R2ci AUC (* p ≤ .05).
Figures from Study Three

Figure 1  Pain ratings ± S.E. (a) during LFS conditioning; and (b) during HFS conditioning. Pain ratings during each subsequent 100 pulses decreased after the initial 100 pulses during LFS conditioning (* p < .05). In contrast, pain ratings during the second and third trains of HFS stimuli increased significantly compared to the first train during HFS conditioning (* p < .05)

Figure 2  Mean sensitivity ± S.E. to (a) heat; (b) pinpricks; (c) von Frey’s monofilament; and (d) pressure-pain at the primary and secondary sites before and after conditioning for HFS and LFS (* p < .05 compared with values before conditioning)

Figure 3  Pressure-pain thresholds ± S.E. in the ipsilateral and contralateral forehead before and after HFS and LFS conditioning. Ipsilateral and contralateral PPTs increased significantly after both forms of electrical conditioning (p<.05 for each side at each time point compared with baseline), with a greater increase on the ipsilateral side (* p < .05 compared to PPT on the contralateral side)
Figures from Study Four

Figure 1  Mean ± S.E. for (a) heart rate; (b) systolic blood pressure; and (c) diastolic blood pressure at baseline, after drug administration and after HFS conditioning in the yohimbine and placebo conditions. Basal heart rate was higher in the placebo than yohimbine condition, likely due to chance, but decreased after drug administration (# p < .05). Basal systolic and diastolic blood pressures were similar before yohimbine and placebo were administered. Blood pressure increased after administration of yohimbine (* p = .052 and # p < .05 compared with values at baseline and placebo). Neither blood pressure nor heart rate changed after HFS conditioning.

Figure 2  (a) Mean electrodermal activity (EDA) ± S.E. at baseline, after drug administration and after HFS conditioning in the yohimbine and placebo conditions during 2 minutes rest. (b) Increases in EDA during 5 deep breaths. At baseline, EDA at rest and during deep breathing was similar in the yohimbine and placebo conditions, but EDA at rest increased significantly after yohimbine was administered. EDA at rest increased after HFS conditioning in both conditions (* p < .05).
Figure 3  Mean forearm sensitivity ± S.E. at baseline, after drug administration and after HFS conditioning to (a) heat; (b) pinprick; and (c) pressure pain thresholds (PPT). In general, all sensations in the test and the control arms were similar at baseline, and remained stable after drug administration. After HFS conditioning, sensations differed significantly between the test and the control arms. (a) Heat sensitivity increased at HFS-conditioned site (* p < .001 compared to values before HFS), but decreased in the control arm (* p < .05). (b) Sharpness ratings to pinprick remained unchanged in the control arm but increased after HFS at primary and secondary sites in the test arm (* p < .001 compared to before HFS and in the control arm). (c) The PPT decreased after HFS at the primary site in the test arm but not in the control arm (* p < .01). In the placebo session, the PPT at the secondary site decreased in the test arm (* p < .05 compared to before HFS). In contrast, the PPT did not change at the secondary site in the yohimbine condition.

Figure 4  Mean sensitivity ± S.E. to (a) heat; (b) pinprick; and (c) pressure-pain in the ipsilateral and contralateral forehead at baseline, after drug administration, and after HFS conditioning. Sensitivity to heat and
pinprick in the ipsilateral and contralateral forehead remained stable throughout the yohimbine and placebo conditions. Ipsilateral and contralateral pressure-pain thresholds (PPT) remained unchanged after yohimbine and placebo administration but increased significantly after HFS conditioning (# p < .01 compared with their respective PPT at baseline), with a greater increase on the ipsilateral side (* p < .001 compared with PPT on the contralateral side after HFS conditioning).

Figure 5  Mean sensitivity ± S.E. to supraorbital stimuli before and after HFS conditioning in the yohimbine and placebo conditions. Before HFS conditioning, sensitivity was similar on the ipsilateral and contralateral sides of the forehead. After HFS conditioning, sensitivity remained unchanged in the yohimbine condition but decreased in the placebo condition (* p < .05 and # p < .01).

Figure 6  Mean percentage change in R2 area under the curve (AUC) after HFS conditioning expressed in relation to R2 AUC before HFS conditioning for blink reflexes ipsilateral both to the HFS-treated site and supraorbital stimulation (R2ii), ipsilateral to the HFS-treated site and contralateral to supraorbital
stimulation (R2ic), contralateral to the HFS-treated site and ipsilateral to supraorbital stimulation (R2ci), and contralateral both to the HFS-treated site and supraorbital stimulation (R2cc). After HFS conditioning, the R2 AUC for blink reflexes ipsilateral to the HFS-treated site (R2ii and R2ic) were greater in the yohimbine condition than the placebo condition. Error bars represent standard errors.

Figure 7  Mean R2 onset latency ± S.E. before and after HFS conditioning. Before HFS conditioning, the R2 onset latency was shorter on the side of supraorbital stimulation than the contralateral side, and was longer in the yohimbine than placebo condition (* p < .05). After HFS conditioning, R2 latency remained shorter on the side ipsilateral to supraorbital stimulation but decreased overall.

Figure 8  Pain ratings ± S.E. to electrical stimulation of the HFS-conditioned (test) and control sites in the forearms during ipsilateral and contralateral temple cooling. In the yohimbine condition, decreases were greater at both sites during ipsilateral than contralateral temple cooling (* p < .001 for the test site and p < .05 for the control site), and at the test
site during the recovery period after the ipsilateral temple was cooled (* p < .01). In the placebo session, decreases were greater at the test site during ipsilateral than contralateral temple cooling (* p < .05).
Chapter 1 Normal Pain Processing

1.1. Pain

Pain perception is influenced not only by noxious sensations but also by the individual’s cognitive and emotional experiences associated with that sensation [8]. Indeed, advanced brain imaging technology has shed some light into this complex phenomenon by showing that the ‘pain experience’ is associated with activation of multiple brain regions, the ‘pain matrix’, which consists of the somatosensory cortex, involved in processing the sensory-discriminant component of pain, and the amygdala, anterior cingulate cortex, insular cortex and prefrontal cortex, involved in emotional and cognitive modulation of pain [8].

Acute pain, which is short-lived and often coupled with injury and many diseases, is critical for survival as it alerts the individual to real or potential tissue injury/damage and facilitates necessary protective responses [190] (e.g., withdrawing a hand from a hot stove or escaping). The inability to detect noxious stimuli from the environment (e.g., stabbing pain from sharp objects, open-flame heat) or internal injuries (e.g., pain or discomfort from internal bleeding or a broken bone), is therefore life threatening and maladaptive [13]. Paradoxically, pain serves no benefits and is maladaptive when it develops into a chronic and debilitating state [190].
1.2. Nociception

Nociception refers to the peripheral and central physiological mechanisms involved in processing pain-related information [209, 220]. Nociception begins with the activation of primary afferents that respond to noxious stimuli, so called pain receptors or nociceptors [60]. Nociceptors are free nerve endings that are distributed throughout the skin and deep tissues [69]. The cell bodies for nociceptors are located in the dorsal root ganglia, or trigeminal ganglia for the face [27, 117]. Nociceptors are medium-diameter lightly myelinated fast conducting Aδ-fibres and small-diameter unmyelinated slow conducting C-fibres [13, 69]. The Aδ nociceptors respond to intense mechanical and extreme thermal stimuli that are below 50°C or above 45°C, and produce ‘first’ sharp, pricking, acute and localised pain which is felt within 0.1 second following the stimulus. C-fibres are polymodal which respond to noxious thermal and mechanical stimuli [13, 69]. Most C-fibres also respond to noxious chemical stimuli (e.g., acid, capsaicin) [117]. C nociceptors convey ‘second’ slow, deep aching, burning, throbbing, or dull pain which is experienced about 1 second after the stimulus [13, 69]. Another subgroup of C-fibres is called ‘sleeping’ or ‘silent’ nociceptors, which respond to heat but not mechanical stimuli under normal conditions [15]. However, they develop sensitivity to mechanical stimuli during inflammation and tissue damage and facilitate nociceptive responses in the dorsal horn and in the upper level of nociceptive pathways following tissue damage [213-215]. Not all sensory C-fibres are nociceptive as some respond to cooling, and some may mediate pleasant touch sensations as they respond to innocuous stroking in hairy skin, but not to heat or mechanical stimuli [186]. Finally, primary afferents that respond only to touch and other innocuous stimuli are large-diameter fast-conducting myelinated Aβ fibres [220].
The action potentials triggered by the activation of peripheral nociceptors travel to the dorsal horn (or trigeminal nucleus caudalis for nociceptive inputs from the face) [220]. The dorsal horn is organised into laminae (layers) [15]. The Aδ nociceptors project to lamina I as well as lamina V, C-fibres project to the superficial dorsal horn or laminae I and II, and touch-mediated Aβ fibres project to laminae III, IV and V [15]. Electrophysiological evidence further shows that lamina I neurons are mostly nociceptive-specific, whereas lamina II (substantia gelatinosa) neurons are exclusively inhibitory or facilitatory interneurons, most of which are nociceptive specific [15]. Neurons in laminae III and IV respond to innocuous stimulation (Aβ fibres) and lamina V neurons receive convergent noxious (Aδ and C fibres) and innocuous inputs (Aβ fibres), which are thus termed wide dynamic range neurons. Deeper laminae (VII and VIII) contain mostly interneurons which receive sensory inputs and form part of a reflex pathway by communicating with other interneurons and motor neurons[220]. Ascending projections from the dorsal horn mostly originate in laminae I and V [15].

In the dorsal horn (or trigeminal nucleus caudalis for the face), the primary nociceptive afferent fibres synapse into second order neurons via the release of two main excitatory neurotransmitters, the amino acid glutamate and neuropeptide substance P [59, 220]. Nociceptive inputs in the dorsal horn and trigeminal nucleus caudalis can be modulated via spinal and supraspinal mechanisms, which influence pain perception [192]. Nociception can be facilitated or inhibited depending on the mechanisms or receptors activated [192]. Mechanisms that modulate pain in the dorsal horn include gate control (segmental inhibition/facilitation), diffuse noxious inhibitory controls (DNIC), stress-induced analgesia (SIA), stress-induced
hyperalgesia, baroreflex-mediated hypoalgesia, and coeruleospinal pain modulation [192].

The axons carrying nociceptive signals then cross to the opposite of the spinal cord and reach the thalamus and brainstem via the spinothalamic and spinoreticulothalamic tracts respectively [220]. Nociceptive inputs from the face ascend from the trigeminal nuclei to the thalamus via the dorsal and ventral trigeminothalamic tracts [27, 220]. The thalamus relays the pain signals to the somatosensory cortex which enables the individual to discriminate pain (e.g., stimulus onset, intensity, quality, location, and duration) [220]. Through the spinoreticulothalamic tracts the pain signals reach the limbic structures (e.g., amygdala, anterior cingulate cortex) which are involved in processing emotional and cognitive components of pain [15, 220].

1.3. Gate Control

Melzack and Wall’s (1965) “gate theory of pain control” [167] refers to a pain modulatory mechanism that occurs in the dorsal horn of the spinal cord, which is thought to act as a gate. This theory is often known as segmental inhibition as the body area in which pain is inhibited is linked anatomically to the segments of the dorsal horn where the nociceptive and non-nociceptive afferents terminate [15]. Activation of medium-diameter lightly myelinated Aδ and small-diameter unmyelinated C nociceptors ‘opens’ this pain gate or induces pain by activating the wide dynamic range neurons in lamina V and suppressing activity of inhibitory interneurons in lamina II in the dorsal horn [15]. In contrast, activation of large-diameter myelinated non-nociceptive Aβ fibres ‘closes’ this pain gate, or inhibits
pain by suppressing activity of the wide dynamic range neurons in lamina V and activating inhibitory interneurons in lamina II [15]. The perceived sensation is the balance between nociceptive (Aδ and C-fibres) and non-nociceptive (Aβ) inputs into the dorsal horn [167, 173]. Thus, activation of the Aβ afferents responsible for touch sensation, by rubbing, applying ice or vibration to the injured area, mild transcutaneous electrical nerve stimulation (TENS) or passive movement can reduce pain perception [73, 119, 168].

1.4. Diffuse Noxious Inhibitory Controls (DNIC)

DNIC in animals, or heterotopic noxious conditioning stimulation (HNCS) and ‘pain-inhibits-pain’ counter-irritation in humans, refers to a concept in which a noxious conditioning stimulus applied in one body area reduces pain responses evoked elsewhere in the body [189]. This ‘diffuse’ or widespread inhibitory effect has been demonstrated in healthy humans through a wide range of noxious electrical, mechanical, thermal, chemical, and ischemic stimuli [6, 9, 24, 57, 72, 86, 90, 91, 118, 128, 129, 135, 141, 193, 243, 258, 268, 272]. For instance, noxious heat applied in the hand reduced pain perception to CO2 laser stimulation in the contralateral knee [118], and inhibited the RIII withdrawal reflex of the leg [268]. Infusion of hypertonic saline into the tibialis anterior muscle increased pressure-pain thresholds in the arm and ankle [90]. Tourniquet-induced arm pain enhanced pressure pain thresholds in both legs [243]. Similarly, placing a hand in ice-water [128] and rekindling of heat pain sensitised by capsaicin in the forearm [129] increased the pressure-pain threshold on both sides of the forehead.
Le Bars and colleagues [144, 145] observed that stimulation of peripheral Aδ and C nociceptors inhibited activity of wide dynamic range neurons in the spinal cord and trigeminal nuclei. The DNIC effect is mediated by spinal and supraspinal circuits as spinal cord sectioning and systemic naloxone, an opioid antagonist, abolished its effect [143-145]. It was initially thought that the periaqueductal gray-rostroventral medulla (PAG-RVM) circuitry was involved in mediating DNIC. However, lesions of the nucleus raphe magnus in RVM or in PAG, main synthesizers of serotonin and opioids within the central nervous system, did not block DNIC [147, 252]. Lesion of the subnucleus reticularis dorsalis (SRD) in the caudal medulla attenuated DNIC [25, 146, 252], indicating involvement of the medulla both in the transmission and modulation of pain signals via a spinal-supraspinal-spinal feedback loop. Thus, nociceptive inputs ascend from the spinal cord via the ventrolateral quadrant [56, 253], and excite neurons in the SRD [155, 252]. The SRD in turn recruits pain modulation circuits from the PAG, RVM, hypothalamus, amygdala and other brain structures, and projects pain modulatory influences to the spinal cord [2, 149, 154, 155, 252] via the dorsolateral funiculus [56, 253]. Indeed, serotonergic [43, 61] and opioidergic mechanisms [56, 147, 193, 268, 270] have been shown to partially mediate DNIC. The engagement of the amygdala, rostral anterior cingulate cortex, and insular cortex by the SRD suggests an involvement of emotional and cognitive factors in pain perception [189] (refer to section “Cognitive and emotional influences on pain”).

Some studies have demonstrated a positive relationship between the magnitude of DNIC and intensity of noxious conditioning stimuli [84], while others have not [9, 89, 199]. Some studies also detected DNIC with mild [89] or non-painful [142] conditioning stimuli. Interestingly, simultaneous administration of two
noxious conditioning stimuli masked DNIC, resulting in pain facilitation rather than inhibition [7]. The induction of DNIC and its time-course also depend on the conditioning stimulus [198]. For example, DNIC was only observed during experimental muscle pain but not after [229]. In contrast, depression of the RIII reflex was observed during heat conditioning, and lasted for 6-9 minutes after heat removal [268]. Moreover, DNIC was only observed 5 minutes after the removal of tourniquet-induced pain [84].

1.5. Stress-induced analgesia (SIA)

It is well-known that stress or fear suppresses pain perception, a phenomenon known as stress-induced analgesia (SIA) [162]. SIA is adaptive as it promptly triggers behavioural responses in emergency situations to expedite survival [3]. Reduced pain perception by soldiers during battle [17] and athletes during sports competitions [20] despite serious injuries are well-known examples of SIA. Experimentally-induced SIA is well-demonstrated in rats and humans. Brief electric shocks to the hindpaw induced antinociception as measured in the tail-flick test [259]. In humans, electric shocks to the hand reduced sensitivity to radiant heat [203]. SIA is also observed during other forms of painful stimulation [65, 108, 217], psychological stressors such as mental arithmetic [10, 77, 78], and memory tasks [82, 83], fear [203], startle with a loud noise [67], noxious cold stimulation [257], or electric shocks [269].

Lesion of the dorsolateral funiculus (DLF) abolished SIA [259], indicating involvement of supraspinal brain structures. Systemic or intrathecal naloxone reduced antinociception induced by electric shocks [259], and microinjection of opioid antagonists into the PAG or RVM deterred SIA [18, 79, 266], suggesting that
SIA is predominantly opioid-sensitive. Microinjection of lidocaine in the PAG or RVM blocked antinociception induced by administration of opioids into the amygdala [96]. Thus, stress or alarm signals emanating from the fear cortical network (e.g., the amygdala) attenuates pain responses in the spinal cord via the PAG-RVM descending pain-inhibitory circuitry [102]. In addition to opioids, involvement of serotonergic mechanisms [102, 173, 182, 208], noradrenergic mechanisms, and neurotransmitters and neuropeptides including GABA, glutamate and endocannabinoids [37, 173] in mediating SIA has also been reported.

The extent of SIA depends on the type of stressor, intensity, and duration of exposure [51, 273]. For instance, SIA increased when rats were forced to swim in extreme temperatures [51]. Similarly, SIA strengthened with increasing frequency and duration of electric shocks to the foot [273]. In another rat study, naltrexone, an opioid antagonist, did not affect immediate SIA induced by inescapable foot shocks (naloxone-insensitive), but effectively reversed SIA produced by exposure to the same stimuli 24 hours later (naloxone-sensitive) [66].

DNIC and SIA overlap when the stressor is a painful stimulus [279]. Although both mechanisms are mediated by opioids [1, 269], and similar neurotransmitters/neuropeptides [37], they are considered to be separate mechanisms [221]. SIA is associated with sympathetic activation (increased heart rate and skin conductance responses) triggered by stress [203], which is not observed during DNIC [146]. In addition, SIA takes longer to develop and increases over time [267, 269], whereas DNIC occurs immediately after the conditioning stimulus and decreases over time [221].
1.6. Stress-induced hyperalgesia (SIH)

Paradoxically, stress can also enhance pain, also known as stress-induced hyperalgesia (SIH) [106]. In rats, acute stress during inescapable holding, exposure to novel environments, or vibrations [115, 116, 250, 251], and prolonged stress induced by repeated exposure to a cold environment [121, 184, 185, 187, 212], restraints [53, 54, 85, 107], and forced swim [200, 201] elicits hyperalgesia. In humans, anxiety and psychological stress caused by public speaking, exams, the Stroop test, threat of electric shocks, and noxious electrical stimulation elicits hyperalgesia [41, 156, 171, 203, 232].

It is now known that the RVM can inhibit or facilitate pain via the PAG-RVM descending pain modulatory circuitry [173, 192]. Involvement of RVM in mediating both SIA [80, 175] and SIH [218] has been reported. A contribution of the RVM to pain facilitation has been demonstrated in inflammatory [228], neuropathic [35], cancer [63] and visceral pain [248]. The brain regions underlying emotion and cognition such as the amygdala, lateral hypothalamus, anterior cingulate cortex, and prefrontal cortex also modulate pain via their neuronal projections to the RVM [38, 100, 103, 162, 164, 165]. For instance, activation of the dorsomedial nucleus of the hypothalamus, which is known to regulate cardiovascular and thermogenic responses to emotional stressors, activates ON-cells, inhibits OFF-cells and increases pain responses in rats [162].

At the molecular level, noradrenaline [173, 200], serotonin [107, 162], opioids and glutamate [227] have been implicated in SIH. Rats pretreated with naloxone (an opioid antagonist), and ketamine (an NMDA antagonist), before the second swim developed thermal hyperalgesia, whereas those pretreated with
naloxone both before the first and second swims did not, suggesting that overstimulation of opioid and NMDA receptors (that occurred during the first swim in rats without naloxone or ketamine) contributes to SIH [54, 227]. Similarly, impairment of the opioid system was also reported in rats subjected to repeated cold stress [187]. Contribution of glutamate through NMDA receptors, substance P, and calcitonin gene-related peptide (CGRP) to hyperalgesia has been demonstrated following repeated cold stress in rats [136, 184, 212]. Serotonin may also contribute to hyperalgesia or analgesia depending on the types of receptors activated [173]. For example, 5-HT2, 5-HT3, and 5-HT4 enhance, whereas 5-HT1A and 5-HT1B suppress neuronal activity [173]. Although the involvement of α1-adrenoreceptors in pain-facilitation has been emphasized [188, 234], prazosin, an α1-receptor antagonist, did not affect SIH in acute restraint [191].

The same stressor can elicit SIA or SIH depending on the intensity, duration of exposure [106], and the test stimulus [191]. For example, rat restraint induced analgesia [250, 251] but elicited hyperalgesia in the paw pressure test [196] and the tail-flick test [191]. Thus, it appears that the shift of balance from pain-inhibition to pain-facilitation can be altered by the intensity of the stressor, duration of exposure [106], and the test stimulus [191, 218].

1.7. Baroreflex-mediated Hypoalgesia

Elevated blood pressure is associated with a reduced responsiveness to acute pain stimuli in healthy normotensive humans [30; 32; 45]. This hypoalgesia is believed to be mediated by a descending pain-inhibitory mechanism triggered by baroreflex receptors. Baroreceptors are mechanoreceptive sensory neurons located in
blood vessels, excited by the dilation of blood vessels during the elevation of blood 
pressure [71]. The generated action potentials are relayed to the nucleus tractus 
solitarius, the paraventricular hypothalamus, and the rostroventromedial medulla 
which sends pain-inhibitory projections to the spinal cord [71; 260]. Blockade of α2-
adrenoreceptors [31; 45] and opioid receptors (30; 32; 81] eliminated the blood 
pressure related hypoalgesia, indicating an influence of endogenous noradrenergic 
and opioidergic systems on the autonomic control of blood pressure in attenuating 
pain. Chronic pain abolishes or reverses the inverse relationship between blood 
pressure and hypoalgesia (26; 32), suggesting that chronic pain alters the 
cardiovascular and pain regulatory interaction, and this alteration could be due to the 
disruption of the endogenous opioid [33] and noradrenergic systems [31].

1.8. Coeruleospinal Pain Modulation

Most descending pain modulation mechanisms (e.g., opioidergic, 
serotonergic, dopaminergic, noradrenergic) exert broad or bilateral pain-inhibitory 
effects [192]. To our knowledge, hemilateral pain-inhibitory modulation is only 
associated with activity in noradrenergic pathways descending from the nucleus 
locus coeruleus (LC) [240, 241]. Electrical and chemical activation of the LC exerts 
direct antinociceptive effects [113, 114, 161, 264]. In addition, electrical stimulation 
of A-δ and C nociceptive afferents in the periphery [97, 244] also evokes activity in 
the LC which, in turn, suppresses nociceptive responses in the dorsal horn of the 
spinal cord [46, 113, 114] and the trigeminal subnucleus caudalis [242]. It does so 
via bilateral noradrenergic projections that act on α2-adrenoreceptors. In a rat model 
of inflammatory pain, noradrenaline increased in the dorsal horn ipsilateral to the 
infamed paw, but not contralaterally, indicating noradrenergic pain modulation was
ipsilateral rather than bilateral or diffuse [240]. Interestingly, in rats with bilateral LC lesions, shorter paw withdrawal latencies (PWLs), indicating higher pain intensity, were observed not only in the carrageenan-inflamed hindpaw but also in the ipsilateral non-inflamed forepaw [241]. As PWLs remained unchanged in the non-inflamed contralateral paws, noradrenergic pain-inhibitory influences apparently were more active ipsi- than contralaterally. In a recent study involving positron emission tomography, high-frequency electrical stimulation (HFS) of the sciatic nerve in rats enhanced metabolic activity in the amygdala and periaqueductal gray (PAG) [98]. It was suggested that activation of the PAG may also preferentially trigger ipsilateral descending pain-inhibitory influences [152].

In healthy humans, unilateral limb pain induced by immersing a limb in ice-water [128] (cold pressor test), and rekindling heat hyperalgesia in forearm skin sensitised with capsaicin (an ingredient in hot chilli peppers that sensitises heat-sensitive nociceptors) [129] increased pressure-pain thresholds on both sides of the forehead with a greater increase on the ipsilateral side. Possibly, noradrenergic mechanisms may be involved in mediating the bilateral and ipsilateral pain-inhibitory analgesia in humans.

Paradoxically, there is also substantial evidence to suggest an involvement of noradrenergic projections from the LC in pain-facilitation [28, 95, 235]. For example, LC-lesioned rats spent less time licking or lifting the inflamed hindpaw during a hotplate test following intraplantar formalin injection compared with control rats [235]. In a related study, an increase in markers of neural activity in the LC was observed following spared sural nerve injury, whereas disruption of synaptic activity or destruction of noradrenergic LC neurons with neurotoxin prevented the
development of allodynia and hyperalgesia [28]. Activation of $\alpha_1$-adrenoreceptors in the spinal cord increased responses of dorsal horn neurons to noxious stimulation [34, 74, 95, 183]. Similarly, microinjection of the $\alpha_1$-adrenoreceptor agonist phenylephrine in the RVM decreased tail flick latency, suggesting involvement of a noradrenergic mechanism in the RVM in pain-facilitation via $\alpha_1$-adrenoreceptors [76]. Furthermore, noradrenaline increased activity of RVM ON-cells, which was reversed by prazosin, an $\alpha_1$-adrenoreceptor antagonist [76]. The majority of RVM axons project to the spinal cord and trigeminal nucleus caudalis [14, 163], which implies that activation of RVM ON-cells by $\alpha_1$-adrenoreceptors facilitates nociception both in the spinal cord and the trigeminal nucleus caudalis [76, 169].

1.9. Peripheral sensitisation and inflammation

Noxious stimulation excites and sensitises peripheral nociceptors or lowers their thresholds to normal afferent input [209]. Noxious stimuli, including mild thermal burn, UVB-irradiation, capsaicin, bradykinin, serotonin, and mustard oil trigger peripheral sensitisation and inflammation [16, 21, 52, 55, 94, 126, 130, 132, 137, 139, 205, 236, 237]. Peripheral sensitisation and inflammation manifest as enhanced pain sensitivity to thermal and mechanical stimuli at the site of stimulation [52, 126, 132, 137, 202]. Consistent with neurogenic inflammation are the developments of a red flare, heat vasodilation, swelling and sometimes loss of function at the stimulated site [101, 225].

The hyperalgesia is due to the simultaneous release of a number of inflammatory mediators by the primary afferent terminals and non-neuronal cells (e.g., mast cells and neutrophils), which is often referred to as ‘the inflammatory
soup’ [117]. These include potassium and hydrogen ions, histamine, bradykinin, prostaglandins (PGs), serotonin, adenosine triphosphate (ATP), nerve growth factor (NGF), cytokines, and TNF-α ([5, 47, 92]. Some inflammatory mediators (e.g., potassium and hydrogen ions, ATP, serotonin, and PGs) directly sensitise nociceptors by interacting with the ion channels on the nociceptor surface (ionotropic receptors), whereas others (bradykinin and NGF) bind onto metabotropic receptors, which subsequently trigger other intracellular events via other chemicals or second-messengers [275].

Some mediators also interact synergistically with others to further enhance the sensitisation and inflammation process [176]. For example, bradykinin and PGs separately sensitise polymodal nociceptors to heat [131, 159]. However, PGs also facilitate responses of bradykinin via the activation of other receptor subtypes (e.g., the EP3) [42, 170, 213]. In addition to inducing thermal and mechanical hyperalgesia [49, 62], NGF also stimulates mast cells to induce degranulation [153]. Furthermore, NGF increases the proportion of neurons in dorsal root ganglia that respond to bradykinin [120]. Adding to this ‘inflammatory soup’ are substance P and calcitonin gene-related peptide (CGRP) released from primary afferent terminals [225, 230], which cause vasodilatory effects and discharge of proteins and fluids from blood vessels (plasma extravasation), and activate mast cells and neutrophils [5, 47, 150].

The recruitment of ‘sleeping’ or ‘silent’ nociceptors during inflammation or injury also facilitates nociceptive activity in the spinal cord and in the upper level nociceptive pathways [176]. Acidosis in inflamed tissues activates polymodal nociceptors, which is enhanced in the presence of serotonin, PGs, histamine and bradykinin [224]. Finally, NMDA-receptors, which are increased in inflamed human
skin [231], are also involved in sensitisation processes [40, 68, 109]. Sensitisation and inflammation of primary afferents may also increase the excitability of spinal nociceptors [278], and consequently lead to central sensitisation [172].

1.10. Central sensitisation

Central sensitization refers to an increase in nociceptive responses of neurons in the central nervous system that elicits pain hypersensitivity [274]. Central sensitisation is robust as it can be readily evoked in healthy humans by a wide range of noxious stimuli applied either to the periphery, deep muscle tissues, or visceral organs [274]. These include capsaicin, mustard oil, acid, thermal burn, electrical stimulation, and hypertonic saline [29, 129, 132, 133, 137, 138, 210, 211, 216, 238, 246, 277]. Following noxious stimulation, signs of central sensitisation manifest as punctate hyperalgesia and dynamic allodynia not only at the conditioned site (primary) but also in surrounding skin (secondary) [127, 134, 138]. Hypersensitivity to pressure-pain [127, 138, 140, 195] and sometimes to heat [274] can also be observed both in primary and secondary zones. Thus, a C-fibre conditioning input to the superficial layers of the dorsal horn decreases the activation thresholds of central Aδ nociceptors and Aβ-fibres, enhances their responses [50], and consequently contributes to secondary hyperalgesia to pinprick and dynamic stimuli respectively [127, 134, 138, 195]. Dynamic allodynia is an important aspect of central sensitisation as heightened pain sensitivity is evoked by touch, an innocuous stimulus mediated by Aβ-fibres which does not elicit pain under normal conditions [274].
In addition to the subjective psychophysical measures, central sensitisation has also been detected using objective electrophysiological measures such as facilitation of nociceptive withdrawal reflexes [22] and amplitudes of event related potentials [246]. Furthermore, changes in neural activity in higher cortical structures have also been linked to signs of central sensitisation using brain imaging techniques (e.g., functional magnetic resonance imaging, magnetoencephalography, magnetic source imaging) [11, 12, 148, 158].

Depending on the stimulus, central sensitisation can occur immediately, outlast the conditioning stimulus by hours, and eventually return to baseline [127, 195, 276]. Central sensitisation has been detected in neurons not only in the dorsal horn, but also in the spinothalamic tract [64, 124], the rostroventral medulla (RVM), anterior cingulate cortex and amygdala [180, 197, 261].

Evidence suggests a contribution of “ON-cell” neurons in the rostroventral medulla (RVM) in the development of hyperalgesia following noxious stimulation [173, 209]. For example, bilateral RVM lesioning with a neurotoxin prevented the development of secondary heat hyperalgesia following carrageenan- and kaolin-knee inflammation or topical application of mustard oil to the rat hindleg [245]. Similarly, injection of lidocaine in the RVM reversed mechanical hyperalgesia [194] and blocked the development of tactile and heat hyperalgesia following spinal nerve ligation [35].

At a cellular level, involvement of lamina I dorsal horn neurons that express neurokinin-1 receptors is linked to central sensitisation as destruction of these neurons inhibits hyperalgesia [160]. Involvement of NMDA-receptors in central sensitisation has been reported. For example, ketamine, an NMDA-receptor
antagonist, inhibits secondary mechanical hyperalgesia evoked by electrical stimulation [134], and primary and secondary hyperalgesia following thermal burn [105], and topical [4] and intradermal capsaicin [219] in humans. Involvement of GABA receptors has also been implicated in central sensitisation. For example, oral gabapentin reduced tactile allodynia and secondary mechanical hyperalgesia following intradermal capsaicin both in healthy humans and chronic pain patients [88]. Similar inhibitory actions of pregabalin were also observed following electrical stimulation [44] and intradermal capsaicin [256]. Actions of ketamine and gabapentanoids on central sensitisation were detected with functional magnetic resonance imaging [104, 222].

1.11. Cognitive and emotional influences on pain

As discussed in the “Stress induced analgesia (SIA)” section, pain suppression during stressful situations is adaptive as it diverts attention away from pain and facilitates necessary behavioural responses to expedite survival [3, 162]. Consistent with this, research has shown that distracting or diverting participants’ attention away from the sensory aspects of the painful stimulus reduced perception of pain [36, 110, 157, 174, 206].

Attentional focus on pain following serious injury is important for survival as it triggers protective responses, which may help to prevent further injury or assist in recuperation. However, research evidence on how an attentional focus on pain influences pain perception is still diverse. Some studies suggest that focusing on painful sensations enhances pain perception in healthy [99] and chronic pain populations [70, 112, 151]. For example, patients who rated their post-surgery pain
more frequently perceived pain more intensely than those who rated pain less often [151]. Other studies argued otherwise [177, 181, 207]. For instance, both chronic pain sufferers and healthy individuals who continuously verbalised their sensation during a 7-minute cold pressor test rated their pain lower than those who did not [181]. Similarly, patients with complex regional pain syndrome perceived less pain when they were required to describe the location and the size of the tactile stimulus (a cork probe) applied to their hands [177]. It was argued that focussing on sensory-discriminant aspects of pain diverted participants’ attention away from negative affects such as anxiety, thereby reducing pain [177, 181, 207]. These findings suggest affective-motivational influences on pain. For example, intense fear caused by painful electric shocks attenuated pressure-pain [203], whereas anxiety induced by threat of electric shocks (but no shocks delivered) enhanced pressure-pain [203]. Thus, consistent with the SIA concept, in this instance salience of fear diverted attention away from painful sensations, hence reduced pain, whereas anxiety focussed attention on a more salient event, that is pain itself, thereby enhancing it [203].

In regards to the influence of pain-related anxiety on pain, Keogh et al. [125] demonstrated that healthy participants who fear pain attended towards pain-related words, whereas those with low fear of pain attended away from pain-related stimuli. Similarly, Boston and Sharpe [23] showed that healthy participants, who received no threatening information about the harmful effects of the painful stimulus (cold pressor test), attended towards sensory pain words but perceived less pain when focussing on physical sensations during the cold pressor test. In contrast, those who received threatening information attended away from sensory pain words and towards affective pain words, and reported no pain reduction [23]. It was argued that
attending to sensory aspects of the painful stimulus is helpful only if participants perceived the stimulus as not harmful, while the threatening information about the stimulus induced anxiety and worry, which might have triggered an affective schema that proved to be not beneficial when these participants were forced to attend to the sensory pain-related sensations [23]. Consistent with this, participants who have catastrophic thinking about pain tend to have attentional biases towards pain-related information, and consequently perceive more pain [39, 263], and benefit less from distraction [247, 249]. Nevertheless, one study reported that health-anxious participants reported less anxiety and pain sensitivity when they attended to sensory aspects of pain [93], suggesting that attentional biases do not always enhance pain.

However, in general, stimuli that trigger negative emotions such as viewing pictures containing negative emotional scenes [58, 122, 123, 166, 204], smelling unpleasant odours [255], listening to depressing verbal statements [19, 280], sad music [233], and non-linguistic negative emotional sounds (e.g., crying) [223] increase pain intensity. Conversely, experimentally-manipulated positive emotions (e.g., pleasant music, pleasant pictures, humorous movies, listening to non-linguistic positive sounds) reduce pain [48, 58, 87, 166, 223, 262, 280].

Evidence on effects of anger on pain is still inconclusive. Experimentally-induced anger increased sensitivity to cold pain [111] and decreased pressure-pain threshold [226], but contrasting results were reported in other studies [265]. Evidence on the influence of depressed mood on pain is sparse. One study showed that experimentally-induced feelings of helplessness and uncontrollability by painful electrical stimulation is associated with an increase in salivary cortisol (stress hormone) and ratings of helplessness and pain intensity in male participants [179].
Additionally, males rated pain intensity lower than females when attending to pain during a cold pressor test, suggesting that gender plays a role in attentional modulation of pain [125]. Past experiences, personality types and cultural backgrounds may also lead to certain expectations, beliefs or predictions about pain, which consequently influence pain [8]. For example, individuals who expect that treatment will bring pain relief perceive less pain despite the treatment being inert, a phenomenon known as ‘placebo analgesia’ [8]. Conversely, those who believe that treatment will cause or enhance pain perceive higher pain [8].

In summary, perception of pain is subjective and depends not only on the unpleasant physical sensation but also on the individual’s cognitive and emotional experiences associated with that sensation [8, 254]. The neural circuits involved in cognitive and emotional modulation of pain may involve an opiate-sensitive descending pathway from the frontal cortex to the amygdala, periaqueductal gray matter (PAG), rostroventral medulla and the spinal cord [75]. For example, distraction increased activity in the PAG, which corresponded with reduced pain sensitivity [239]. Naloxone, an opioid antagonist, inhibited activation of the PAG during placebo analgesia [8]. In addition, the cholinergic and adrenergic systems have also been implicated in the cognitive modulation of pain [178, 271].
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Chapter 2 Human Experimental Pain Models

Human pain models have been developed to further the understanding of mechanisms that underlie acute and chronic pain [74]. To date, no human pain model has yet been able to mimic all clinical aspects of chronic pain [49, 87]; however, some models have been developed to successfully produce peripheral sensitisation and/or central sensitisation [87]. In human pain research, one approach to assess somatosensory changes in the skin following a painful conditioning stimulus entails psychophysics [87]. This method involves application of various test stimuli directly onto the skin and relies upon subjective pain ratings [87]. Commonly used instruments include a pressure algometer with a contact surface to assess pain sensitivity in deeper tissues and muscles (e.g., FDX, Wagner Instruments, Greenwich, CT, USA), a contact thermode from a well-controlled Peltier system to examine thermal sensitivity, and calibrated von Frey monofilaments or metal pins with various weights to investigate sensitivity to sharpness [77]. Comprehensive standards and protocols for quantitative sensory testing are well-documented by Rolke et al. [77]. Following is a description of some commonly used human pain models and a brief review of pain modulation processes that may be involved in each model.

2.1. The Cold Pressor Test

The cold pressor test (CPT) involves the immersion of a hand or foot in cold water [100]. It was designed originally by Hines and Brown to examine cardiovascular stress reactivity as cold pain triggers “pressor” effects causing a rise
in blood pressure and heart rate [100]. The intensity of “cold pain” depends on the degree of cooling; however, immersion in water below 18 °C triggers a ‘cold’ sensation, followed by a generalised and deep aching pain approximately 60 seconds later [100]. After pain has reached its maximum intensity (60 seconds), ‘pins and needles’ are also felt in water below 12 °C, followed by an “adaptation” period in which pain gradually decreases, and finally stops [100]. Cold pain is thought to be associated with the vasoconstriction of local blood vessels in the immersed limb by cooling and is carried by small diameter, unmyelinated C-fibres [100]. In healthy humans, the CPT reduces pain sensitivity to a wide range of noxious stimuli administered elsewhere in the body of healthy individuals. These include electrical [26, 96], thermal [29, 60, 96], mechanical [28, 51, 61, 72], and chemical stimuli [1, 2, 99]. For example, the CPT increased the pressure-pain threshold in the contralateral knee [61] and on both sides of the forehead [51], and reduced R2 amplitude of blink reflexes elicited by supraorbital stimulation [26]. In addition to the central pain-inhibitory effects, the CPT may also trigger an ipsilateral pain-inhibitory mechanism, which was demonstrated by a greater increase in pressure-pain threshold on the side of the forehead ipsilateral to cold-induced limb pain [51].

In healthy individuals, a noradrenergic influence on the autonomic control of blood pressure may indirectly attenuate pain, perhaps due to activation of a pain-inhibitory mechanism through the stimulation of baroreflex receptors [9], as elevated blood pressure is associated with a reduction in pain sensitivity [8, 14, 23]. Therefore, the extent to which pain-inhibition is attributed to DNIC or a baroreflex mechanism triggered during the CPT is unclear [91].

Dysfunction in central pain-inhibitory modulation or disinhibition in numerous chronic pain disorders has also been implicated with the use of CPT as a
conditioning stimulus, including central post-stroke shoulder pain [78], headache [21], and complex regional pain syndrome (CRPS) [53]. For example, sensitivity to pressure-pain increased on both sides of the forehead of CRPS patients when the affected limb was immersed in cold water, as opposed to a bilateral decrease in sensitivity to this same stimulus in the forehead of healthy controls [53].

2.2. Capsaicin

Capsaicin, an active ingredient in hot chilli peppers, is often administered topically [54] or intradermally [57]. Topical capsaicin excites and sensitises the peripheral terminals of Aδ and unmyelinated C-fibres that express vallinoid Type I (TRPV1) receptors [11]. This triggers neurogenic inflammatory responses by releasing vasodilator agents such as substance P and calcitonin gene-related peptide (CGRP), which produce a red flare and an ongoing burning pain at the treated site [38, 65, 86]. In turn, the ongoing barrage of sensitised C nociceptors enhances nociceptive responses in the dorsal horn [90], leading to central sensitisation [33, 52, 58]. Consistent with peripheral inflammation, hyperalgesia to heat and mechanical stimuli develops at the site of application [6, 33, 53, 58]. Mechanical hyperalgesia and dynamic mechanical allodynia also develop in skin surrounding the capsaicin treatment site, consistent with central sensitisation [6, 33, 58]. In animal models of inflammatory pain, substance P and excitatory amino acids released from central terminals of C nociceptors acting at the N-methyl-D-aspartate (NMDA) receptor, contribute to central sensitisation [19, 22]. In humans, administration of the NMDA antagonist ketamine reduced capsaicin-induced hyperalgesia [70]. However, repeated or long application of capsaicin can cause a degeneration of terminals and axons of nociceptors leading to desensitisation [10, 64]. Thus, although a painful
stimulus, capsaicin can be used as an analgesic agent [3]. Indeed, capsaicin extracts reduce pain in conditions in which pain is maintained by sensitised but intact primary nociceptors, including postherpetic neuralgia [98], postmastectomy pain [97], and mixed neuropathic pain [76].

In healthy humans, capsaicin sometimes, but not always, evokes DNIC effects [18, 25, 52, 89]. Capsaicin suppressed R2 amplitude of blink reflexes elicited by supraorbital stimulation 20 minutes after treatment [18]. A bilateral forehead analgesia to sharpness developed 6 hours after topical capsaicin treatment [52]. In addition, rekindling thermal hyperalgesia by heating the capsaicin-treated site 48 hours after capsaicin treatment evoked bilateral forehead analgesia to pressure-pain with a greater analgesic effect on the ipsilateral side [52], suggesting that capsaicin may also trigger ipsilateral pain-inhibitory modulation.

2.3. Ultraviolet-B (UVB) radiation

Unlike other pain models (e.g., topical capsaicin), UVB (280 – 350 nm) produces no ongoing spontaneous pain during or after irradiation [6]. Exposing human skin at one or greater individual minimum erythema doses (MED) (the minimum amount of UVB energy required to produce a perceptible reddening of the skin) produces erythema at the site of treatment several hours after UVB irradiation [30, 31, 34, 37]. The erythema and primary hyperalgesia peak at around 24 hours and dissipates after several days [4, 5, 30, 34, 37]. Consistent with peripheral sensitisation, heat and mechanical hyperalgesia developed at the irradiated site [34]. Changes in skin blood flow at the irradiated site peaked at 18 hours and again at 36 hours, suggesting that UVB inflammation may be biphasic [4]. Indeed, a biphasic
model of UVB inflammation was observed in a rat study in which thermal hyperalgesia was detected at 3-6 hours and again at 48 hours after irradiation [81]. Mechanistically, release of inflammatory mediators such as prostaglandins [75] and tumour necrosis factor α [16] likely contribute to early inflammatory effects and heat hyperalgesia, whereas nerve growth factor may be involved in the longer lasting mechanical hyperalgesia [6].

However, under certain conditions, UVB also triggers central sensitisation [30, 31]. Human studies observed secondary mechanical hyperalgesia when a high dose of UVB radiation (3 times MED) was applied to a large area of skin (5 cm in diameter), likely induced by an increase in spontaneous activity of primary nociceptive afferents [30, 31]. Consistent with this, in a rat study, secondary mechanical hyperalgesia and allodynia developed following UVB irradiation, which was further enhanced and prolonged when heat hyperalgesia was rekindled at the UVB-treated site [17].

2.4. Electrical stimulation

Electrical stimulation can be administered peripherally or intracutaneously [74]. Different stimulation parameters (frequency, stimulus intensity, waveform, and duration) and electrode design can preferentially target different populations of nociceptors, evoke different pain sensations, and may trigger activity in different central pain pathways [74]. Two common forms of electrical stimulation currently used in human pain research are high-frequency electrical stimulation (HFS) and low-frequency electrical stimulation (LFS). Both forms of stimulation are administered peripherally via a purpose-built electrode that preferentially activates
superficial Aδ and C nociceptors [41, 68]. HFS involves five 1-second bursts of electrical stimulation at 100 Hz with a 9-s rest between bursts [50, 59], whereas LFS consists of 1000 single pulses at 1 Hz [7, 48]. A 2 ms pulse width and current intensity at 10 times the individual electrical detection threshold are used for both HFS and LFS.

LFS (1-2 Hz) has been used to examine mechanisms of hyperalgesia in humans [7, 13, 48, 55, 56, 88]. Conditioning with LFS in the periphery evokes a low to mild intensity of pin-prick like pain, which increases initially and gradually declines [7, 48]. Following LFS, pain perception to single electrical pulses also decreased, indicating that LFS induces a long-lasting decrease in synaptic strength or LTD in spinal nociceptive pathways, resulting in hypoalgesia at the site of stimulation [7, 48]. Analgesia mediated by some forms of electrical stimulation (e.g., spinal cord stimulation [69]; and cutaneous field stimulation [67]) was partially attributed to a spinal LTD mechanism, or segmental spinal inhibition via the gate control mechanism (e.g., non-painful high frequency low intensity TENS; [24]). Hypoalgesia was observed at the site of application, but not at the unstimulated control site following LFS [48], suggesting that LFS does not induce descending central pain-inhibitory mechanisms (e.g., DNIC). Secondary mechanical hyperalgesia also develops after LFS but only to a minor extent [7, 48]. However, this may depend on the intensity of stimulation as a large area of secondary pinprick hyperalgesia was observed following intracutaneous LFS (5 Hz) [55, 56, 88]. Similarly, in an electrophysiological study, a significant increase of blood flow and amplitude of the nociceptive withdrawal reflex was observed following LFS conditioning, but not HFS conditioning, suggesting that LFS also induces central sensitisation [7]. Moreover, in rodent studies, LFS (2 Hz, 2 minutes) at C-fibre
intensity (10 times the electrical detection threshold) could also induce LTP at superficial [20, 40] and deep dorsal horn neurons [35]. In particular, it was demonstrated that both HFS and LFS induced LTP at synapses between C-fibre and spinal lamina I neurons expressing neurokinin-I receptors [40].

HFS triggers a moderate to severe pin-prick like sensation which gradually increases after each train of stimulation [50, 59]. More importantly, HFS enhances sensitivity to electrical and mechanical stimuli not only at the site of conditioning, but also in adjacent skin [50, 59, 71]. In an electrophysiological study, HFS-induced secondary punctate hyperalgesia was associated with enhanced N1-P2 peak-to-peak and P300 event-related potentials [95], further supporting that HFS triggers central sensitization [50, 71]. HFS-induced hyperalgesia is long-lasting [71]. Half-lives of hyperalgesia to single electrical stimuli at the conditioned site and secondary mechanical hyperalgesia were approximately 7 hours and 5 hours, which disappeared after 48 and 24 hours respectively [71]. The absence of thermal hyperalgesia at the conditioned site, a sign of peripheral sensitisation [92], implies that HFS does not induce peripheral sensitisation [50, 59, 71]. Molecular mechanisms involved in secondary hyperalgesia are not fully understood but may involve neuropeptides such as substance P released during peripheral noxious stimulation, which diffuses to the adjacent neurons [62]. Substance P activates dorsal horn neurons that express neurokinin-1 receptors [39, 63], which are important for the induction of LTP of spinal nociceptive pathways [39] and the development of hyperalgesia [47, 66].

The persistence of secondary hyperalgesia long after the cessation of stimulation is consistent with HFS inducing long-term potentiation (LTP) of spinal
nociceptive pathways, which is in line with many rat studies (see Sandkuhler [83] and Ruscheweyh et al. (2011) [80] for reviews). However, spinal LTP induced by HFS has been challenged for several reasons. Firstly, 100Hz is significantly higher than the firing range of C-fibres (1 – 10 Hz) [42], which is essential for induction and maintenance of LTP [82]. Secondly, it is also argued that in conditions where hyperalgesia is caused by trauma and inflammation, C-fibres discharge at rather low frequencies (1-10Hz) [40]. Finally, activation of Aδ nociceptors by HFS [32], may also induce LTD, rather than LTP of spinal nociceptive pathways [12, 84].

In rat studies, HFS may also evoke activity in pain-inhibitory pathways that descend from the brainstem to all levels of the spinal cord [27, 85]. For example, disrupting descending pathways using a cold block of the thoracic spinal cord enhanced the pronociceptive effects of HFS of the sciatic nerve in rats [27]. Similarly, HFS facilitated C-fibre evoked field potentials in spinalised but not in intact rats [85]. Thus, the central sensitisation triggered by HFS may be opposed, in part, by activation of descending pain modulation pathways.

2.5. Approach

In summary, the literature reviewed above suggests that peripheral sensitisation primarily predominates in the UVB inflammatory model [6, 30, 34, 37]. LFS, on the other hand, may evoke LTD of spinal nociceptive pathways and only minor signs of secondary hyperalgesia [48], at least at low stimulus intensities. In contrast, HFS triggers more intense signs of central sensitisation [48, 50, 59, 71] compared to UVB and LFS. To my knowledge, only Biurrun Manresa et al. [7] have compared the effects of HFS and LFS on human central pain modulation processes,
and the central pain modulation processes induced by UVB are yet to be examined. It is therefore important to further characterise these pain models to expand our understanding of their effects on human central pain modulation processes.

In healthy humans, unilateral limb-pain induced by capsaicin and the cold pressor test evoked a bilateral increase in pressure-pain threshold in the forehead, with a greater increase ipsilaterally [51, 52]. This suggests that limb pain, induced by noxious stimuli can trigger central and ipsilateral descending pain-inhibitory effects [51, 52]. Thus, it is important to develop a further understanding of whether limb-pain induced by other pain models such as HFS, UVB and LFS also induce similar pain-inhibitory mechanisms. We addressed this in Study one by examining the effects of HFS [48, 50, 59, 71, 95] and UVB [6, 30, 34] on sensitivity to pressure-pain on each side of the forehead. In particular, we aimed to determine whether the ipsilateral forehead analgesia to pressure-pain was associated with signs of peripheral sensitisation evoked by UVB or central sensitisation evoked by HFS.

To further delineate pain modulation processes evoked by HFS, in Study two we examined the effects of HFS on sensitivity to heat, blunt and sharp stimuli, and nociceptive blink reflexes elicited by supraorbital stimulation. The nociceptive blink reflex is an objective assessment of human trigeminal pain pathways which involves the involuntary closure of the eyelids induced by painful stimulation of the face [26, 46]. Additionally, we also assessed the effects of electrically-evoked forearm pain (counter-irritation) on nociceptive blink reflexes before and after HFS.

Next, we wanted to determine whether central sensitisation is necessary for the development of ipsilateral analgesia in the forehead after painful stimulation of the forearm with HFS and low frequency electrically stimulation (LFS). As
discussed in the “LFS” section, in contrast to HFS, LFS (1-2 Hz) may induce LTD of spinal nociceptive pathways, causing hypoalgesia at stimulated site [45, 79] and only minor signs of secondary hyperalgesia [48]. Hence, we hypothesised that HFS would trigger more intense secondary hyperalgesia [48] and ipsilateral forehead analgesia than LFS. This hypothesis was examined in Study three.

Numerous rat studies implicate the involvement of descending noradrenergic pain-inhibitory mechanisms in mediating antinociceptive effects in the spinal cord following electrical stimulation of peripheral nociceptors [36]. These mechanisms suppress nociceptive activity in the spinal cord by acting on inhibitory $\alpha_2$-adrenoreceptors [15, 43, 44, 73]. Furthermore, noradrenaline is released in the dorsal horn ipsilateral to an inflamed hindpaw [93], and this inhibits pain not only in the inflamed hindpaw but also in the noninflamed ipsilateral forelimb [94]. We hypothesised that a similar hemilateral noradrenergic pain-inhibitory mechanism may be involved in preventing the spread of the excitability of spinal neurons to other regions on the same side of the body following HFS, thereby producing analgesia in the ipsilateral forehead. To find out whether the ipsilateral forehead analgesia is mediated by $\alpha_2$-adrenoreceptors, it would be logical to attempt to block their effects with an $\alpha_2$-adrenoreceptor antagonist. This hypothesis was tested in the final double-blind placebo-controlled crossover design using yohimbine, an $\alpha_2$-adrenoreceptor antagonist (Study four).
References


Chapter 3 Study 1

High frequency electrical stimulation concurrently induces central sensitisation and ipsilateral inhibitory pain modulation

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What's known

Nociceptive stimulation may evoke signs of central sensitisation while simultaneously suppressing pain elsewhere in the body.

High frequency electrical stimulation induces both peripheral and central sensitisation whereas ultraviolet B radiation induces mainly peripheral sensitisation.

What's new

Central sensitisation after high frequency electrical stimulation is associated with signs of hemilateral pain modulation.

Peripheral sensitisation induced by ultraviolet B radiation does not influence pain modulation elsewhere.
Abstract

**Background.** In healthy humans, analgesia to blunt pressure develops in the ipsilateral forehead during various forms of limb pain. The aim of the current study was to determine whether this analgesic response is induced by ultraviolet B radiation (UVB), which evokes signs of peripheral sensitisation, or by high-frequency electrical stimulation (HFS), which triggers signs of central sensitisation.

**Methods.** Before and after HFS- and UVB-conditioning, sensitivity to heat and to blunt and sharp stimuli was assessed at and adjacent to the treated site in the forearm. In addition, sensitivity to blunt pressure was measured bilaterally in the forehead. The effect of ipsilateral versus contralateral temple cooling on electrically-evoked pain in the forearm was then examined, to determine whether HFS- or UVB-conditioning altered inhibitory pain modulation.

**Results.** UVB-conditioning triggered signs of peripheral sensitisation, whereas HFS-conditioning triggered signs of central sensitisation. Importantly, ipsilateral forehead analgesia developed after HFS- but not UVB-conditioning. In addition, decreases in electrically-evoked pain at the HFS-treated site were greater during ipsilateral than contralateral temple cooling, whereas decreases at the UVB-treated site were similar during both procedures.

**Conclusions.** HFS-conditioning induced signs of central sensitisation in the forearm and analgesia both in the ipsilateral forehead and the HFS-treated site. This ipsilateral analgesia was not due to peripheral sensitisation or other non-specific effects, as it failed to develop after UVB conditioning. Thus, the supra-spinal mechanisms that evoke central sensitisation might also trigger a hemilateral
inhibitory pain modulation process. This inhibitory process could sharpen the boundaries of central sensitisation or limit its spread.

Key words: high frequency electrical stimulation; ultraviolet radiation; hemilateral pain control; diffuse noxious inhibitory controls; central sensitisation
Introduction

Limb pain evokes analgesia in the ipsilateral forehead of healthy men and women (Knudsen and Drummond, 2009; Knudsen and Drummond, 2011). In the first study to report this effect, sensitivity to blunt pressure was measured on each side of the forehead before and after immersion of one hand in painfully cold water (Knudsen and Drummond, 2009). Mild hand pain had no effect on forehead sensitivity. However, sensitivity to blunt pressure decreased following a single immersion of the hand in water at 2°C or repeated immersions at 4°C (intensely painful), particularly on the ipsilateral side of the forehead. In a follow-up experiment, a small patch of skin on the forearm was sensitized to heat with topical capsaicin, a substance that increases the excitability of heat-sensitive nociceptors (Knudsen and Drummond, 2011). Again, when the treated site was heated, decreases in sensitivity to blunt pressure were greater on the ipsilateral than contralateral side of the forehead.

The aim of the present study was to determine whether this ipsilateral forehead analgesia was associated with signs of central sensitisation. Central sensitisation results, at least in part, from activity in bulbo-spinal pathways that facilitate transmission of nociceptive impulses, leading to tenderness around sites of injury and inflammation (Jaggi and Singh, 2011; Millan, 1999; Millan, 2002). An association between central sensitisation and ipsilateral forehead analgesia during limb pain would imply simultaneous activation of circumscribed facilitatory and broad ipsilateral inhibitory influences on processing of nociceptive information.

Specifically, we investigated effects of limb pain induced by two different forms of sensitisation: ultraviolet B radiation (UVB) and high frequency electrical
stimulation (HFS). UVB radiation triggers signs of peripheral sensitisation at the site of inflammation, including local hypersensitivity to heat and to blunt and sharp mechanical stimulation, but has little effect on sensitivity in surrounding skin (Bishop et al., 2009; Harrison et al., 2004). In contrast, HFS evokes hypersensitivity to sharp stimulation not only at the site of electrical conditioning but also in adjacent skin (Klein et al., 2008; Lang et al., 2007; Pfau et al., 2011), consistent with central sensitisation.

We also explored the effects of cold-pain in the temple on limb pain (a form of counter-irritation; Drummond et al., 2001). In line with our hypothesis that central sensitisation after HFS would be coupled with broad ipsilateral changes in inhibitory pain modulation, we expected that decreases in electrically-evoked pain at the site conditioned by HFS would be greater when ice was applied to the ipsilateral than the contralateral temple. However, in the absence of central sensitisation (i.e., at the site conditioned by UVB and at a control site in the contralateral forearm), analgesia evoked by counter-irritation should not depend on whether the ice was applied ipsilaterally or contralaterally.

Methods

Participants

The sample consisted of 30 participants (11 males) aged between 18 and 49 years. Participants were excluded if they suffered from acute or chronic pain, diabetes, heart disease, epilepsy, or if they were pregnant or breastfeeding. Participants provided their informed consent for the procedures, which were approved by the Murdoch University Human Research Ethics Committee.
Procedures

The procedures were carried out in a laboratory maintained at around 21°C and participants sat in a comfortable armchair throughout. HFS and UVB conditioning were conducted by the same experimenter (LV), and were counterbalanced after an interval of at least seven days for the 16 participants who completed both procedures. To avoid carry-over effects, different sites on the forearm were exposed to UVB and HFS. To minimise skin electrical resistance, the ventral forearms were cleaned with pumice stone, rinsed with water and dried. One ventral forearm area was assigned as the test site and an equivalent area in the contralateral forearm as the control site. The laterality of these sites was counterbalanced across participants. The primary location for testing (Primary Area), and an area 1 cm distal to the primary location (Secondary Area), were marked as shown in Fig. 1.

Psychophysical tests.

A verbal rating scale ranging from 0 to 10 was used by participants to report pain or sharpness intensity. For pain, 0 indicated “no pain” and 10 indicated “extreme pain”. For sharpness intensity, 0 implied “not sharp” and 10 implied “extremely sharp”. To assess heat sensitivity, a 1.5 cm diameter metal probe heated to 44 ± 0.2°C was placed on the skin for 7 s. To investigate sensitivity to mild sharpness, a 10 g von Frey monofilament (Neuropen, Owen Mumford, USA) was applied at a 90° angle to the skin surface with sufficient pressure to bend the filament for 1 s. To assess sensitivity to more intense sharpness, a sharp tip with a calibrated spring mechanism exerting a force of 40 g (Neuropen, Owen Mumford, USA) was applied for 2 s. Stimuli were applied in runs alternating between the Primary and the
Secondary areas of the test site and the control site on the contralateral arm. To measure pressure-pain sensitivity, an algometer (FDX, Wagner Instruments, USA) with a modified 8 mm diameter hemispheric rubber tip was applied at each forearm site and on each side of the forehead at 100 g/sec until the participant reported pain. The side tested first alternated between each arm and between each side of the forehead in counterbalanced order across participants. Prior to baseline measurements, participants were trained until ratings and pressure-pain thresholds stabilized. To minimize effects of repeated testing, each test was performed only once in each round. The exception was during baseline when measures taken at two sites on the same forearm differed by more than 20% or 2 points on the 0-10 rating scales (approximately 20% of occasions). In such cases, the final measurement was the average of two readings.

**HFS Procedure** (N = 30). Electrical stimuli were generated by a constant current stimulator (DS7A; Digitimer, Welwyn Garden City, UK) and delivered via a purpose-built electrode that consisted of 24 copper pins with 0.2 mm diameter tips mounted on a 2 cm x 3 cm perspex block such that the tips projected 0.5 mm beyond the surface of the block. Electrodes with these properties preferentially activate superficial nociceptive A-δ and C fibers (Inui et al., 2002; Nilsson and Schouenborg, 1999). A 3.0 cm x 3.5 cm ground plate attached 1 cm from the conditioning electrode completed the electrical circuit (see Fig. 1). Initially, the electrical detection threshold (EDT) was determined using the method of limits for two ascending and two descending sets of single pulses at 2 ms pulse width. The stimulus intensity, starting at 0.1 mA, increased in steps of 0.1 mA until the participant perceived the stimulus, and then decreased in steps of 0.05 mA until the stimulus was no longer perceived. This procedure was then repeated. The EDT was defined as
the geometric mean of the 4 stimulus intensity levels. After 5 minutes rest, HFS conditioning was applied at the test site. This consisted of five 1-s bursts of electrical stimulation (100 Hz, 2 ms pulse width, at 10 times EDT up to a maximum of 8 mA) with a 9-second rest between bursts (Klein et al., 2008; Lang et al., 2007). The psychophysical tests were readministered at the test site and forehead starting 10 minutes after HFS conditioning.

**Effect of HFS on pain modulation.** Moderate pain (pain level 5 on the 0-10 VAS) was evoked via the 24-pin electrode at the test or control site in counterbalanced order across participants with electrical stimuli at 1 Hz and 0.5 ms pulse width. The current level was adjusted to maintain moderate pain for 60 s before assessing pressure-pain thresholds on each side of the forehead. In addition, the effect of HFS on electrically-evoked forearm pain during ipsilateral and contralateral temple cooling was assessed. Before each task, the current level was adjusted to bring pain ratings to 5 for at least 30 s. During temple cooling, an ice cube with an application surface area of 6 cm\(^2\) was held against the left or right temple anterior to the ear for 30 s. Electrically-evoked pain in the forearm was rated each second during the 30-s cooling period and for 30 s after the ice was removed. Participants also provided a single pain rating for the cold sensation in their temple at the end of the cooling period. In a separate control task, electrically-evoked pain in the forearm was rated each second for 60 s. Test order was counterbalanced across participants and sides, and alternated between the test and control site. The control task immediately preceded an active task whereas the other tasks were separated by at least 2 minutes to minimise carry-over effects.
UVB Procedure (N = 16). At least 24 hours before testing, the individual minimum erythema dose (MED), the minimum amount of UVB energy required to produce a perceptible reddening of the skin, was determined by exposing the forearm to different intensities of UVB through a light source with an irradiance energy level of 8.50 mW/cm² (Durham Erythema Tester Device; Hybec, UK). Psychophysical tests were conducted at the test site as described above prior to UVB conditioning. During UVB conditioning, a 2-cm diameter forearm area at the test site was exposed to the UVB radiation source at twice the predetermined MED. Six hours later, psychophysical tests were readministered. Sensitivity to pressure-pain in the forehead and effects of counter-irritation on electrically-evoked pain in the forearm were then assessed as previously described.

Statistical Analyses

Assessment of primary and secondary hyperalgesia. Changes in sensitivity to heat, sharpness, and pressure-pain at the Primary and Secondary Areas were assessed separately across time (before conditioning, after conditioning) and conditions (HFS, UVB) in repeated-measures analyses of variance.

Pressure-pain sensitivity in the forehead. Changes in sensitivity to pressure-pain between the two sides of the forehead (ipsilateral, contralateral), across time (before conditioning, after conditioning, during electrically-evoked pain at the treated site, during electrically-evoked pain at the control site) and conditions (HFS, UVB) were investigated in an analysis of variance with contrasts between consecutive time points.
Effects of temple cooling on electrically-evoked pain in the forearm.

Repeated-measures analyses of variance with simple contrasts between baseline and each subsequent 5-s interval were run separately for each site to determine whether decreases in electrically-evoked limb pain were greater during temple cooling than during a control task without temple cooling. Next, differences in electrically-evoked pain were investigated separately for each site in the HFS and UVB conditions in analyses with simple contrasts across time for ipsilateral versus contralateral temple cooling. Finally, a between-group repeated-measures analysis of variance with simple contrasts across time was conducted to compare pain rating changes at the HFS- and UVB-treated sites over consecutive 5-s intervals for ipsilateral versus contralateral temple cooling.

All analyses were conducted using SPSS for Windows Version 18. An alpha level of 0.05 was used in all analyses, and results are presented as mean ± standard error of the mean.

Results

Before conditioning, heat, sharpness and pressure-pain sensitivity were similar across the HFS and UVB conditions, and were similar at the Primary and Secondary Areas within each condition (Fig. 2a-d).

The MED for UVB ranged from 0.11 J/cm² to 0.6 J/cm² (M = 0.32 ± 0.04). No spontaneous pain was reported at the UVB-treated site during or after irradiation. However, erythema clearly developed at the treated site six hours after UVB conditioning. In addition, each participant demonstrated heat hyperalgesia at the UVB-treated site (a sign of primary hyperalgesia).
The individual EDT ranged from 0.25 mA to 1.0 mA ($M = 0.48 \pm 0.03$ mA).

After HFS conditioning at 10 times the EDT, participants described the treated site as ‘prickly’, ‘sore’ and ‘sensitive’. These sensations lasted for approximately 2.5 hours.

**Changes in forearm sensitivity after conditioning stimulation**

*Heat sensitivity in the Primary area.* After conditioning, heat sensitivity increased in the Primary Area both of the HFS-treated site ($t(29) = 3.58, p = .001$) and the UVB-treated site ($t(15) = 9.25, p < .001$) (main effect for Time $F(1, 44) = 86.39, p < .001$), with significantly greater heat sensitivity in the Primary Area of the UVB-treated site than the HFS-treated site (Time x Condition interaction $F(1, 44) = 24.78, p < .001$) (Fig. 2a).

*Heat sensitivity in the Secondary area.* When data were pooled across the HFS- and UVB-treated sites, heat sensitivity increased slightly in the Secondary Area after conditioning (main effect for Time $F(1, 39) = 6.23, p = .023$; Time x Condition interaction $F(1, 39) = .011$, not significant). However, increases did not achieve statistical significance for either site when each site was considered separately (Fig. 2a).

*Sharpness ratings to pinprick in the Primary area.* Sensitivity to pinpricks increased to a similar extent in the Primary Area after both forms of conditioning (main effect for Time $F(1, 44) = 22.67, p < .001$; Time x Condition interaction $F(1, 44) = 0.03$, not significant) (Fig. 2b).

*Sharpness ratings to pinprick in the Secondary area.* Fig. 2b also shows that after conditioning, sharpness sensitivity to pinpricks increased in the Secondary Area.
(main effect for Time F(1, 36) = 4.54, p = .040). The Time x Condition interaction was not significant (F(1, 36) = 0.48); nevertheless, when each form of conditioning was considered separately, increases in sharpness were significant only after HFS conditioning (Fig. 2b).

**Sharpness ratings to von Frey’s monofilament in the Primary area.**

Sharpness sensitivity to von Frey’s monofilament increased to a similar extent in the Primary Area after HFS and UVB conditioning (main effect for Time F(1, 44) = 29.65, p < .001; Time x Condition interaction F(1, 44) = .00, not significant) (Fig. 2c).

**Sharpness ratings to von Frey’s monofilament in the Secondary area.**

Sharpness sensitivity to stimulation with von Frey’s monofilament increased in the Secondary Area after HFS conditioning but not after UVB conditioning (main effect for Time (F(1, 36) = 11.28, p = .002; Time x Condition interaction F(1, 36) = 5.13, p =.030) (Fig. 2c).

**Forearm pressure-pain sensitivity in the Primary area.** Pressure-pain thresholds (PPT) decreased to a similar extent after HFS and UVB conditioning (main effect for Time, F(1, 44) = 58.85, p < .001; Time x Condition interaction F(1, 44) = 1.23 , not significant) (Fig. 2d).

**Forearm pressure-pain sensitivity in the Secondary area.** The PPT decreased to a similar extent in the Secondary Area after both forms of conditioning (main effect for Time, (F(1, 35) = 11.64, p =.002; Time x Condition interaction F(1, 44) = 0.34 , not significant) (Fig. 2d).
Forehead pressure-pain sensitivity

Symmetry of forehead pressure-pain sensitivity before conditioning. Before conditioning, PPTs were similar on each side of the forehead in the HFS and UVB conditions (Fig. 3). The mean PPT was slightly higher in the UVB than HFS condition, but this difference was not statistically significant.

Changes in forehead pressure-pain sensitivity after conditioning. After UVB conditioning, the PPT remained stable on both sides of the forehead (Fig. 3). However, after HFS conditioning, the PPT increased on both sides of the forehead (main effect for Time F(1, 44) = 6.06, p = .018), with a greater increase on the ipsilateral side (Time x Side interaction F(1, 44) = 8.88, p = .005; Time x Condition interaction F(1,44) = 8.75, p = .005; Time x Side x Condition interaction F(1, 44) = 5.80, p = 0.02).

Changes in forehead pressure sensitivity during electrically-evoked pain at the test site in the forearm. The PPT remained greater on the ipsilateral than contralateral side of the forehead during electrical stimulation of the HFS-treated site (Fig. 3). However, electrically-evoked pain at the UVB-treated site had no effect on the forehead PPT, either ipsilateral or contralateral to electrical stimulation.

Changes in forehead pressure-pain sensitivity during electrically-evoked pain at the control site in the forearm. The PPT in the forehead ipsilateral to the HFS-treated site decreased during electrical stimulation of the HFS control site in the forearm whereas the contralateral forehead PPT remained unchanged. In contrast, electrically-evoked pain at the UVB control site had no consistent effect on the forehead PPT (Time x Side x Condition interaction F(1, 44) = 10.16, p = 0.003) (Fig. 3).
The effect of temple cooling on forearm pain

Cold-pain ratings to ice applied to the temple for 30 s averaged around 7 (moderately to extremely painful) and were similar for each temple both after HFS- and UVB conditioning.

In the absence of temple cooling, electrically-evoked pain in the forearm decreased in a linear trend from a starting point of 5 on the 0-10 pain rating scale to around 4.5 after 60 s of stimulation. Temple cooling provoked an additional decrease in electrically-evoked pain at all sites (Fig. 4a-d). Fig. 4a demonstrates a significantly greater reduction of pain at the HFS-treated site during the final 10 seconds of cooling the ipsilateral temple compared with cooling the contralateral temple (Side x Time interaction for 21-25s after baseline F(1, 20) = 4.35, p = .05; Side x Time interaction for 26-30s after baseline F(1, 20) = 5.48, p = .03), and the ipsilateral analgesic effect continued for a further 5 seconds after the ice was removed (Side x Time interaction F(1, 20) = 4.85, p = .04). In contrast, decreases in electrically-evoked pain at the HFS control site and the UVB-treated and control sites did not depend on which temple was cooled. Thus, pain reductions were greater at the HFS- than UVB-treated site during the final 5 seconds of cooling the ipsilateral temple (Side x Time x Condition interaction F(1, 29) = 4.06, p = .053), and for 5 seconds after cooling (Side x Time x Condition interaction (F(1, 29) = 6.02, p = .02).

Discussion

Three key findings emerged from this study. First, HFS conditioning triggered signs of more intense central sensitisation than UVB conditioning. Second, ipsilateral forehead analgesia developed after HFS- but not UVB conditioning.
Third, pain inhibitory effects were greater at the HFS- than the UVB-treated site during ipsilateral temple cooling. Together, these findings suggest an association between central sensitisation and hemilateral pain modulation.

**Peripheral versus central sensitisation**

Erythema and hypersensitivity to heat and mechanical stimuli developed at the UVB-treated site six hours after UVB irradiation. UVB evokes a “sunburn-like” effect that involves primary mechanical hyperalgesia at the site of UVB treatment (Bishop et al., 2009; Harrison et al., 2004) due to release of inflammatory mediators such as prostaglandins (Rhodes et al., 2001) and tumour necrosis factor α (Cunha et al., 1992). Under certain conditions UVB may trigger central sensitisation (Davies et al., 2011; Gustorff et al., 2004). However, this seems unlikely in the present study because there was little evidence of secondary hyperalgesia in skin adjacent to UVB conditioning. Unexpectedly, the PPT decreased close to the UVB-treated site (generally a sign of primary rather than secondary hyperalgesia; Kilo et al., 1994). One possibility is that the force applied by the algometer stretched the skin, thereby encroaching on and stimulating the sensitised UVB-treated area.

Hyperalgesia to sharp stimuli developed in skin adjacent to the site of HFS conditioning, consistent with secondary hyperalgesia (Klein et al., 2008; Lang et al., 2007; Pfau et al., 2011). Punctate hyperalgesia surrounding the HFS-treated site is associated with enhanced N1-P2 peak-to-peak and P300 event-related potentials (van den Broeke et al., 2010), suggesting that HFS triggers central sensitisation (Klein et al., 2008; Pfau et al., 20011; van den Broeke et al., 2010). We also identified heat hyperalgesia at the HFS-treated site and other evidence to support peripheral sensitisation (flushed skin and sensitivity to mechanical stimulation), possibly due to
an electrically-evoked release of prostaglandins (Ferrell et al., 2002; Tartas et al., 2005; Yaksh et al., 1999) or repeated testing. Nevertheless, primary hyperalgesia was more intense after UVB- than HFS conditioning, whereas secondary hyperalgesia was greater after HFS- than UVB conditioning. Thus, we are confident that the conditioning procedures produced their intended effects.

**Bilateral forehead analgesia**

The PPT increased bilaterally in the forehead after HFS conditioning, but not after UVB conditioning. This suggests the involvement of a central pain inhibitory mechanism such as stress-induced analgesia (Bandura et al., 1988; Chesher and Chan, 1977; Gamaro et al., 1998; Janssen et al., 1998; Willer et al., 1981) or diffuse noxious inhibitory controls (DNIC) after HFS conditioning (Villanueva and Le Bars, 1995). We previously detected bilateral forehead analgesia to pressure-pain stimulation during unilateral cold-induced (Knudsen and Drummond, 2009) and capsaicin-induced limb pain (Knudsen and Drummond, 2011). The absence of spontaneous pain after UVB conditioning may explain why sensitivity to pressure-pain did not change in the forehead in this condition.

**Ipsilateral forehead analgesia and its association with central sensitisation**

Ipsilateral forehead analgesia to blunt pressure developed immediately after HFS conditioning. This finding is in harmony with previous studies that identified ipsilateral forehead analgesia to blunt pressure during unilateral cold-induced (Knudsen and Drummond, 2009) and heat-induced limb pain (Knudsen and Drummond, 2011), although analgesia was relatively short-lasting in these studies. In contrast, the ipsilateral analgesia following HFS persisted for at least two hours, consistent with the long half-life of mechanical hyperalgesia at and surrounding the
HFS-treated site (Pfau et al., 2011). As neither central sensitisation nor ipsilateral forehead analgesia developed after UVB conditioning, these observations suggest an association between signs of central sensitisation triggered by HFS and persistent ipsilateral forehead analgesia.

The locus coeruleus (LC) suppresses nociceptive activity in wide dynamic range neurons in the dorsal horn via bilateral noradrenergic projections that act on α2-adrenoreceptors at all segmental levels of the spinal cord (Bouhassira et al., 1987; Clark and Proudfit, 1992; Clark et al., 1991; Fritschy and Grzanna, 1990; Jones and Gebhart, 1986a; b; Rahman et al., 2008; Sluka and Westlund, 1992; Tsuruoka et al., 1990). This inhibitory effect can be triggered by electrical stimulation of A–δ and C nociceptors (Hitoto et al., 1998; Men and Matsui, 1994; Tyce and Yaksh, 1981; Yaksh et al., 1981). Following carrageenan-induced hindpaw inflammation, noradrenaline increased in the ipsilateral dorsal horn but not contralaterally (Tsuruoka et al., 1999), suggesting that descending modulation from the LC was active only in the dorsal horn ipsilateral to the inflamed paw (Tsuruoka et al., 2003; Tsuruoka and Willis, 1996a; 1996b). Importantly, in rats with bilateral LC lesions, heat hyperalgesia was detected not only in the carrageenan-inflamed hind-paw but also in the ipsilateral non-inflamed forepaw (Tsuruoka et al., 2004). As heat hyperalgesia did not develop in the non-inflamed contralateral hind- or forepaw, coeruleospinal pain modulation apparently inhibited nociceptive activity hemilaterally in the ipsilateral dorsal horn. This hemilateral coeruleospinal pain inhibitory mechanism might also have mediated ipsilateral forehead analgesia to pressure-pain following HFS conditioning in our study.
A substantial literature suggests that descending excitatory influences deriving from the midbrain, pons and rostral ventromedial medulla, involving noradrenergic, serotonergic, opioidergic and other mechanisms, may also facilitate pain via a spino-bulbo-spinal loop (Urban and Gebhart, 1999; Vera-Portocarrero et al., 2006; Dubner, 2004; Millan, 2002; Ossipov et al., 2000; Suzuki et al., 2004; Vera-Portocarrero et al., 2006; Roberts et al., 2009; Torsney, 2011). Evidence for involvement of noradrenergic pathways in pain facilitation is particularly intriguing. For example, LC-lesioned rats spent less time licking or lifting the inflamed hindpaw during a hotplate test following intraplantar formalin injection compared with control rats (Taylor et al., 2000). In a related study, an increase in markers of neural activity in the LC was observed following spared sural nerve injury, whereas disruption of synaptic activity or destruction of noradrenergic LC neurons with neurotoxin prevented the development of allodynia and hyperalgesia (Brightwell and Taylor, 2009). It is tempting to speculate that a similar facilitatory mechanism contributed to segmental central sensitisation following HFS in our study.

We expected that the forehead PPT would increase during electrical stimulation due to DNIC (Bouhassira et al., 1987; Villanueva and Le Bars, 1995). Surprisingly, however, the PPT ipsilateral to the HFS-treated site decreased during stimulation of the control site on the contralateral forearm. In addition to inhibitory pain modulation, noxious stimulation may also trigger hyperalgesia (Imbe et al., 2006) which, hypothetically, could oppose DNIC. This might also explain the absence of forehead analgesia during electrically-evoked forearm pain at the UVB-treated site and during electrical stimulation of control sites in the contralateral arm in both conditions.
Hemilateral pain modulation during temple cooling

Cold-pain in the temple inhibited electrically-evoked limb pain, irrespective of HFS- or UVB conditioning. This could involve spinal and supraspinal inhibitory mechanisms that result in stress-induced analgesia (Willer et al., 1981) or DNIC (Villanueva and Le Bars, 1995). Noxious stimulation elsewhere in the body might also have distracted the participant’s attention away from pain (Janssen et al., 1998). However, this seems unlikely as participants were instructed to provide pain ratings every second during these tasks.

Importantly, cooling the ipsilateral temple exerted greater analgesia at the HFS-treated site than cooling the contralateral temple, and induced greater analgesia at the HFS- than the UVB-treated site. These findings suggest that an ipsilateral inhibitory influence (e.g., coeruleospinal pain modulation) acted on pathways sensitised by HFS conditioning.

Methodological considerations

A major limitation of our study is the reliance on self-report measures of pain. To minimise potential biases, participants were blind to the hypotheses. Nevertheless, studies that incorporate more objective measures of nociceptive activity (e.g., nociceptive reflexes or evoked potentials) are required to verify and extend the present findings.

A second limitation is that most of our participants were young and well-educated. Whether the results also apply to different populations is unknown. In addition, repeated testing or electrically-evoked inflammatory responses might have contributed to sensory changes. Importantly, however, we were able to control for
the nonspecific effects of testing by comparing outcomes after HFS conditioning (which evoked signs of central sensitisation) with those following UVB conditioning (which failed to evoke clear signs of central sensitisation).

**Conclusions**

The major finding was the association between central sensitisation following HFS conditioning and ipsilateral forehead analgesia to pressure-pain. In addition, HFS conditioning was associated with heightened analgesia to ipsilateral counter-irritation. Together, these findings suggest that HFS conditioning simultaneously evoked ipsilateral segmental central sensitisation by a pain facilitatory mechanism, possibly involving the LC, and hemilateral pain inhibition also involving the LC (Figure 5). It is tempting to speculate that an ipsilateral inhibitory pain modulation process helps to limit the spread of central sensitisation or to sharpen contrasts between stronger and weaker sources of pain. Further studies are required to determine whether a shift in the balance between ipsilateral excitatory and inhibitory pain modulation processes contributes to hyperalgesia in animal and human models of inflammatory and neuropathic pain, and in chronic disorders such as complex regional pain syndrome (Drummond and Finch, 2006; Knudsen et al., 2011).
References


Sensitivity to heat, sharpness and pressure were assessed at a site in the ventral forearm conditioned by HFS or UVB radiation (the Primary Area) and in adjacent skin (the Secondary Area). During HFS, five 1-s bursts of electrical stimulation (100 Hz, 2 ms pulse width, at 10 times the electrical detection threshold up to a maximum of 8 mA) were delivered from the conditioning probe with a 9-second rest between bursts. Sensitivity to heat, sharpness and pressure were re-assessed at each site 10 minutes later.
a. Heat Sensitivity

Heat Sensitivity Rating (0-10)

Before Conditioning After Conditioning Before Conditioning After Conditioning
Primary Area Secondary Area

b. Sharpness Rating to Pinprick

Sharpness To Pinprick Rating (0-10)

Before Conditioning After Conditioning Before Conditioning After Conditioning
Primary Area Secondary Area

c. Sharpness Rating to Von Frey's Monofilament

Sharpness Rating (0-10)

Before Conditioning After Conditioning Before Conditioning After Conditioning
Primary Area Secondary Area

d. Forearm Pressure-Pain Thresholds

PPT (kg)

Before Conditioning After Conditioning Before Conditioning After Conditioning
Primary Area Secondary Area
Figure 2  Mean sensitivity ± S.E. to (a) heat; (b) pinprick; (c) von Frey’s monofilament; and (d) pressure-pain in the Primary and Secondary Areas before and after conditioning with HFS or UVB. Sensitivity to each stimulus increased significantly in the Primary Area after HFS- and UVB conditioning (* p< .05 and ** p<.01 compared with values before conditioning). However, heat sensitivity in Primary Area was greater after UVB- than HFS-conditioning (# p < .05), indicating that primary hyperalgesia was greater after UVB- than HFS conditioning. In contrast, sharpness ratings increased in the Secondary Area after HFS conditioning (consistent with central sensitisation) but not after UVB conditioning. In addition, PPT decreased in the Secondary Area after UVB (* p < .05 compared with values before conditioning).
Figure 3  Pressure-pain thresholds ± S.E. in the ipsilateral and contralateral forehead before conditioning, after conditioning, during electrically-evoked pain at the treated forearm site, and during electrically-evoked pain at the control site following HFS- and UVB conditioning. Ipsilateral and contralateral PPTs increased significantly after HFS conditioning (* p < .05 and ** p < .001 compared with values before conditioning). PPT ipsilateral to the HFS-conditioned site decreased significantly when electrical stimuli were applied to the HFS control site (# p < .05 compared with values during test-site stimulation). However, PPTs did not change significantly after UVB conditioning.
Ipsilateral
Temple Cooling
HFS-treated Site
Forearm Pain Rating
(0-10)

Contralateral
Cooling
Recovery

Time (Seconds)

Ipsilateral
b. Temple Cooling
HFS Control Site
Forearm Pain Rating
(0-10)

Contralateral
Cooling
Recovery

Time (Seconds)

Ipsilateral
c. Temple Cooling
UVB-treated Site
Forearm Pain Rating
(0-10)

Contralateral
Cooling
Recovery

Time (Seconds)

Ipsilateral
d. Temple Cooling
UVB Control Site
Forearm Pain Rating
(0-10)

Contralateral
Cooling
Recovery

Time (Seconds)
The effect of ipsilateral and contralateral temple cooling on electrically-evoked pain at (a) the HFS-treated site; (b) the HFS control site; (c) the UVB-treated site; and (d) the UVB control site. Ice was applied to each temple for 30 seconds. Pain ratings decreased significantly at treated and control sites during the indicated time intervals compared with a control task when ice was not applied (* p < .05). In addition, decreases in pain at the HFS-treated site (a) were significantly greater during ipsilateral than contralateral temple cooling during the final 10 seconds of cooling and for the following 5 seconds (# p < .05). Error bars represent standard errors.
a. HFS Pain Modulation

Descending ipsilateral pain facilitatory pathway

Descending ipsilateral pain inhibitory pathway

Ascending noxious stimulation

Decreased sensitivity to pressure in the ipsilateral forehead

DRG

HFS

b. UVB Pain Modulation

Descending ipsilateral pain facilitatory pathway

Descending ipsilateral pain inhibitory pathway

Ascending noxious stimulation

No change in sensitivity to pressure in the ipsilateral forehead

DRG

UVB

c. Effects of counter-irritation after HFS conditioning

Inhibitory influences from contralateral stimulation

Cold-pain in the contralateral temple

Inhibitory influences from ipsilateral stimulation

Cold-pain in the ipsilateral temple

DRG

HFS

d. Effects of counter-irritation after UVB conditioning

Inhibitory influences from contralateral stimulation

Cold-pain in the contralateral temple

Inhibitory influences from ipsilateral stimulation

Cold-pain in the ipsilateral temple

DRG

UVB
Figure 5  Hypothesized effects of HFS and UVB conditioning on pain modulation. (a) UVB conditioning induced signs of peripheral sensitisation (signified by the red star in the dorsal root ganglion – DRG), but did not evoke spontaneous pain, central sensitisation or activity in descending pain-facilitatory or inhibitory pathways (represented by the dashed lines). (b) HFS conditioning induced signs of peripheral and central sensitisation in ascending pain-projection pathways (shown in red). This may have triggered activity in descending ipsilateral pain facilitatory pathways (responsible for central sensitisation, shown in yellow) and hemilateral pain inhibitory pathways (responsible for ipsilateral analgesia, shown in blue). (c) Counter-irritation from noxious stimulation of either temple inhibited pain evoked by electrical stimulation of the UVB-conditioned site (inhibitory influences shown in light blue). (d) In addition to this bilateral influence, noxious stimulation of the temple ipsilateral to HFS conditioning may have evoked activity in an ipsilateral descending pain-inhibitory pathway (shown in dark blue), augmenting the analgesic effect of counter-irritation.
Chapter 4 Study 2

Coexistence of ipsilateral pain-inhibitory and facilitatory processes after high-frequency electrical stimulation

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Running head: Changes in pain modulation after HFS

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Conflicts of interest

The authors have no conflict of interest with the contents of this manuscript.

What's already known about this topic?

High-frequency electrical stimulation (HFS) triggers central sensitisation.

Ipsilateral forehead analgesia to blunt pressure develops after HFS.

What does this study add?

The excitability of the nociceptive blink reflex increases in the ipsilateral forehead after HFS.

Hemilateral inhibitory and facilitatory influences on nociceptive processing co-exist after HFS.
Abstract

Background: High-frequency electrical stimulation (HFS) of the human forearm evokes analgesia to blunt pressure in the ipsilateral forehead, consistent with descending ipsilateral inhibitory pain modulation. The aim of the current study was to further delineate pain modulation processes evoked by HFS by examining sensory changes in the arm and forehead; investigating effects of HFS on nociceptive blink reflexes elicited by supraorbital electrical stimulation; and assessing effects of counter-irritation (electrically-evoked pain at the HFS-conditioned site in the forearm) on nociceptive blink reflexes before and after HFS.

Methods: Before and after HFS conditioning, sensitivity to heat and to blunt and sharp stimuli was assessed at and adjacent to the conditioned site in the forearm, and on each side of the forehead. Nociceptive blink reflexes were also assessed before and after HFS with and without counter-irritation of the forearm.

Results: HFS triggered secondary hyperalgesia in the forearm (a sign of central sensitisation) and analgesia to blunt pressure in the ipsilateral forehead. Under most conditions, both HFS conditioning and counter-irritation of the forearm suppressed electrically-evoked pain in the forehead, and the amplitude of the blink reflex to supraorbital stimuli decreased. Importantly, however, in the absence of forearm counter-irritation, HFS conditioning facilitated ipsilateral blink reflex amplitude to supraorbital stimuli delivered ipsilateral to the HFS-conditioned site.

Conclusions: These findings suggest that HFS concurrently triggers hemilateral inhibitory and facilitatory influences on nociceptive processing over and
above more general effects of counter-irritation. The inhibitory influence may help to limit the spread of sensitisation in central nociceptive pathways.
Introduction

In healthy humans, analgesia to blunt pressure develops in the ipsilateral forehead during various forms of limb pain (Knudsen and Drummond, 2009, 2011). For example, immersing one hand in painfully cold water (Knudsen and Drummond, 2009) and heating a small patch of skin in the forearm pre-treated with topical capsaicin (an active ingredient in chilli peppers that sensitises heat nociceptors in the skin) (Knudsen and Drummond, 2011) decreases sensitivity to blunt pressure on both sides of the forehead, particularly on the ipsilateral side. Similarly, in a recent study, we identified ipsilateral forehead analgesia in healthy humans after electrically stimulating a small patch of skin in the forearm at high frequency (Vo and Drummond, 2013). In particular, we found that the high-frequency electrical stimulus (HFS) simultaneously triggered signs of central sensitisation (heightened sensitivity to sharp stimuli in skin surrounding the stimulated site) and a decrease in sensitivity to blunt pressure on the side of the forehead ipsilateral to the sensitised forearm site. Together, these findings suggest involvement of complex supra-spinal mechanisms underlying central sensitisation which might trigger an ipsilateral pain-inhibitory process.

In the present study, the nociceptive blink reflex (the involuntary closure of the eyelids induced by painful stimulation of the face) (Kaube et al., 2002; Giffin et al., 2004) was used to further delineate pain modulation processes induced by HFS. A blink reflex, elicited by electrical stimulation of the supraorbital nerve, consists of an early ipsilateral R1 component, mediated by rapidly-conducting myelinated fibres, followed by a bilateral R2 component. Specifically, the R2 component is associated with nociception and pain (Ellrich et al., 1997; Bromm et al., 1984).
Kaube et al. (2000) demonstrated that electrical stimuli which preferentially activated superficial nociceptors in the supraorbital region triggered a sharp pain in concert with R2 but not R1. This sensation, together with R2, almost disappeared after local application of topical anaesthetic agent (Kaube et al., 2000), indicating that R2 was generated by stimulation of superficial trigeminal nociceptors.

In the present study, changes in the R2 component of nociceptive blink reflexes elicited ipsilateral to a forearm site conditioned by HFS were compared with changes evoked by contralateral supraorbital stimulation. We hypothesised that R2 for blink reflexes that were ipsilateral both to supraorbital stimuli and the conditioned forearm site would be weaker than R2 to contralateral supraorbital stimuli. To determine whether inhibitory pain-modulation processes such as diffuse noxious inhibitory controls would accentuate the reduction in ipsilateral R2, we investigated the effect on blink reflexes of electrically-evoked pain at the site conditioned by HFS. We also investigated the effects of HFS on a range of sensory modalities in the forehead (pressure-pain, heat, and sharpness to pinpricks and von Frey’s monofilament), to determine whether the ipsilateral analgesia evoked by HFS was multimodal or limited to pressure-pain sensations (Knudsen and Drummond, 2009).

**Methods**

*Participants*

The participants were 10 males and 10 females aged between 17 and 51 years. Exclusion criteria included acute or chronic pain, diabetes, heart disease, epilepsy, pregnancy, or breastfeeding. Participants provided their informed consent
for the procedures, which were approved by the Murdoch University Human Research Ethics Committee.

Procedures

The experiment was carried out in a laboratory maintained at 21 ± 1°C. All procedures were conducted by the same experimenter (LV). Participants sat in a comfortable armchair throughout the experiment. To minimise skin electrical resistance, a test site on the right or left ventral forearm was gently cleaned with pumice stone, rinsed with water and dried. Sensations were investigated at the Primary Area, and also 1 cm distal to the primary location (Secondary Area).

Psychophysical tests. Participants reported pain or sharpness intensity using a verbal rating scale ranging from 0 to 10. For pain, 0 indicated “no pain” and 10 indicated “extreme pain”. For sharpness intensity, 0 implied “not sharp” and 10 implied “extremely sharp”. To assess heat sensitivity, a 1.5 cm diameter metal probe heated to 44 ± 0.2°C was placed at the site for 7 seconds. To investigate sensitivity to mild sharpness, a 10g von Frey monofilament (Neuro-pen, Owen Mumford, USA) was applied perpendicular to the skin surface with sufficient pressure to bend the monofilament for 1 second. To measure sensitivity to more intense sharpness, a sharp tip with a calibrated spring mechanism exerting a force of 40 g (Neuro-pen, Owen Mumford, USA) was applied for 2 seconds. To measure pressure-pain sensitivity, an algometer (FDX, Wagner Instruments, USA) with a modified 8 mm diameter hemispheric rubber tip was applied at each forearm site or on each side of the forehead at 100 g/second until the participant reported pain.

Prior to conducting baseline psychophysical tests, the participant was trained until ratings and pressure-pain thresholds stabilised both in the forearm and forehead.
The psychophysical tests were then conducted with each stimulus being applied in runs alternating between the Primary and the Secondary areas of the test site, and between the two sides of the forehead. The site tested first alternated between the arm and each side of the forehead in counter-balanced order across participants. As repeated testing can enhance pain sensitivity, each test was performed only once in each round. The exception was during baseline when measures taken at two sites on the forearm differed by more than 20% (or 2 points on the 0-10 rating scales) or when the participant was uncertain about their perception of the stimulus. In such cases, the final measurement was the average of two readings.

*Blink Reflex Procedure.* The stimulating electrodes were two custom-built concentric electrodes, each consisting of a copper wire cathode centred within a ring-shaped stainless steel anode with an inner diameter of 10 mm and an outer diameter of 20 mm (Kaube et al., 2000). The small cathode contact area and short anode-cathode distance enables high current density at low current intensity which preferentially activates superficial A-δ nociceptors (Kaube et al., 2000). The electrodes were attached to the supraorbital region on each side of the forehead with adhesive tape. The blink reflexes were recorded bilaterally using modified disposable Cleartrode electrodes (ConMed Corporation, NY, USA) attached over the orbicularis oculi muscle of the lower eyelid and the outer corner of each eye. A ground electrode was attached behind the right ear. Electromyograph signals were amplified with an electromyographic bio-potential amplifier (Biopac Systems, Inc., USA), digitized by an MP100 Biopac Systems Analogue/Digital Channel receptor at 2,000 Hz (Biopac Systems, Inc., USA) and displayed on a computer monitor using AcqKnowledge software (Biopac Systems, Inc., USA).
To elicit blink reflexes, two series of electrical stimuli were applied at a current intensity of 2 mA. Each series consisted of 10 monopolar square-wave electrical stimuli at 15 second intervals to minimise habituation. The stimulus was a 3-pulse train with 0.5 ms pulse duration, and an inter-pulse interval of 5 ms. Triple-pulse stimulation increases the sensation of pain and facilitates the R2 area under the curve, and is thus more suited to examining nociceptive pathways than single pulses (Giffin et al., 2004). Within each series, an equal number of stimuli were administered on each side of the forehead. Stimulus administration alternated between the sides such that no more than two stimuli were delivered sequentially on the same side. To investigate effects of counter-irritation on blink reflexes, one series was administered while moderate pain (pain level 5 on the 0-10 scale) was electrically-evoked at the test site in the forearm (1 Hz, 0.5 ms pulse width). The current level was adjusted to maintain moderate pain for 60 s before the administration of the series of supraorbital stimuli and continued at this level throughout the blink reflex series. During the administration, each supraorbital stimulus was preceded by 0.5 s by a single pulse delivered to the test site in the forearm. Participants rated pain after each supraorbital stimulus. The order of administration of the two series was counterbalanced across participants. After 5 minutes, the psychophysical tests were readministered on each side of the forehead.

*High frequency electrical stimulation (HFS).* The electrical stimuli were generated by a constant current stimulator (DS7A; Digitimer, Welwyn Garden City, UK) and delivered via a custom-built electrode that consisted of 24 copper pins with 0.2 mm diameter tips mounted on a 2 cm x 3 cm perspex block such that the tips projected 0.5 mm from the surface of the block. Electrodes with these properties preferentially activate superficial nociceptive A-δ and C fibers (Nilsson and
Schouenborg, 1999; Inui et al., 2002). The electrical detection threshold (EDT) was determined using the method of limits for 2 ascending and 2 descending sets of single pulses at 2 ms pulse width and an inter-pulse interval of 5 s. The stimulus intensity, starting at 0.1 mA, increased in steps of 0.1 mA until the participant perceived the stimulus, and then decreased in steps of 0.05 mA until the stimulus was no longer perceived. This procedure was then repeated. The EDT was defined as the geometric mean of the 4 stimulus intensity levels. After 5 minutes rest, HFS conditioning was applied at the test site. This consisted of five 1-s bursts of electrical stimulation (100 Hz, 2 ms pulse width, at 10 times EDT up to a maximum of 8 mA) with a 9-s rest between each burst (Klein et al., 2008; Lang et al., 2007). The participant rated pain after each burst of stimulation. Ten minutes later, the psychophysical tests were re-conducted at the test site and on each side of the forehead. Finally, two series of supraorbital stimuli were administered to evoke blink reflexes. One series was administered with and the other without counter-irritation at the forearm test site as previously described.

Data Filtering and Reduction

The electromyographic waveforms were filtered to remove 50 Hz electrical noise and frequencies below 20 Hz. Based on their laterality to the HFS-treated site and the supraorbital stimulus, the blink reflexes were classified into “ii” (ipsilateral to both the HFS-treated site and supraorbital stimulus), “ic” (ipsilateral to the HFS-treated site but contralateral to the supraorbital stimulus), “ci” (contralateral to the HFS-treated site but ipsilateral to the supraorbital stimulus), and “cc” (contralateral to both the HFS-treated site and supraorbital stimulus).
To identify R2 onset latency, each blink reflex was individually displayed as an unrectified waveform using the Biopac AcqKnowledge software program (Fig. 1). Latency was then measured in milliseconds from the start of the three-pulse stimulus to the point where the amplitude of the signal began to change noticeably from background noise. In addition, the R2 rectified area under the curve (AUC) was measured between 27 and 87 ms after the stimulus onset (Ellrich and Treede, 1998) (Fig. 1). The R2 AUC of blink reflexes before and after HFS conditioning was expressed as the percentage of the R2 AUC of blink reflexes administered at baseline (before HFS conditioning) without electrically-evoked forearm pain. R1 waves were not observed.

Statistical approach

Changes in sensitivity to heat, sharpness and pressure-pain were assessed in relation to site (primary and secondary areas) and time (before HFS conditioning, after HFS conditioning) in repeated-measures analyses of variance. A similar approach was used to investigate changes in sensitivity to heat, sharpness and pressure-pain between the two sides of the forehead (ipsilateral, contralateral) and across time (baseline, after the first set of blink reflexes, after HFS conditioning, after the second set of blink reflexes).

Changes in pain ratings to supraorbital electrical stimuli across time (before HFS conditioning, after HFS conditioning), and in relation to the laterality of the supraorbital stimuli (ipsilateral or contralateral to the HFS-treated site) and counter-irritation (with versus without electrically-evoked pain at the HFS-treated site), were investigated in a repeated-measures analysis of variance. Changes in R2 onset latency had an additional factor of side of supraorbital stimulation. The effect of
counter-irritation (electrically-evoked forearm pain) on the percent change in R2 AUC was investigated in relation to the laterality of HFS and the supraorbital stimuli before and after HFS conditioning. In a separate analysis, differences in R2 AUC in the presence versus absence of electrically-evoked forearm pain after HFS were assessed in relation to the laterality of HFS and the supraorbital stimuli.

**Results**

The individual EDT ranged from 0.30 mA to 1.25 mA ($M = 0.55 \pm 0.03$). After HFS conditioning, participants described the conditioned site as ‘sore’, ‘sensitive’ and ‘prickly’. These sensations lasted for approximately 2 hours.

*Changes in forearm sensitivity after HFS conditioning*

The current level required to produce moderate pain at the test site (pain level 5) was similar before and after HFS (6.7 ± 1.0 mA versus 7.1 ± 1.9 mA). As indicated in Fig. 2a, heat sensitivity did not change after HFS conditioning. However, sensitivity to pinprick (main effect for Time $F(1, 19) = 10.65, p = .004$) and von Frey’s monofilament (main effect for Time $F(1, 19) = 11.31, p = .003$), increased in the Primary and Secondary Areas, and sensitivity to pressure-pain increased mainly in the Primary Area (main effect for Time $F(1, 19) = 4.94, p = .039$) (Fig. 2b-d).

*Forehead sensitivity*

Before HFS conditioning, heat, sharpness, and pressure-pain sensitivity were similar on each side of the forehead (Fig. 3a-d). Sensitivity to heat and sharpness remained stable after HFS conditioning, and was similar on the ipsilateral and contralateral sides of the forehead (none of the effects that involved Time or Side
were statistically significant). However, PPT increased on both sides of the forehead (main effect for Time $F(1.92, 36.38) = 20.80, p < .01$), with a greater increase on the ipsilateral side (main effect for Side $F(1, 19) = 18.58, p < .001$; Time x Side interaction $F(2.56, 48.67, p < .01$) (Fig. 3d).

Pain ratings to supraorbital stimuli were greater at baseline than after HFS conditioning (main effect for Time $F(1, 19) = 9.02, p = .007$) (Fig. 4a), and were greater without than with electrically-evoked forearm pain (main effect for Forearm Stimulation $F(1, 19) = 4.54, p = .046$) (Fig. 4b). However, pain ratings did not differ between the ipsilateral and contralateral sides of the forehead before or after HFS conditioning (none of the effects that involved Forehead Side were statistically significant).

$R_2$ onset latency

$R_2$ onset latency was shorter ipsilateral than contralateral to supraorbital stimulation ($43.4 \pm 1.2$ ms versus $46.1 \pm 1.4$ ms, $F(1, 16) = 36.52, p < .001$) irrespective of HFS conditioning or the counter-irritation produced by electrical stimulation of the forearm (an example of decreased latency on the stimulated side is shown in Fig. 1). After HFS conditioning, $R_2$ latency decreased bilaterally during electrically-evoked forearm pain (Time x Counter-irritation interaction $F(1, 16) = 12.44, p < .01$) (Fig. 5).

$R_2$ AUC

In general, $R_2$ AUC decreased over the course of the experiment (Fig. 6). To investigate this statistically, changes in $R_2$ AUC were normalised in relation to the $R_2$ AUC at baseline (Fig. 6a). Under most conditions both HFS conditioning and
electrically-evoked forearm pain strongly suppressed R2 AUC (Fig 7a and 7b).

However, as shown in Fig. 7c, R2 AUC increased ipsilaterally after HFS conditioning in the absence of forearm pain (Counter-irritation x HFS Side x Side of Supraorbital Stimulation interaction $F(1, 18) = 7.29, p = .015$).

**Discussion**

As in our previous study (Vo and Drummond, 2013), ipsilateral forehead analgesia to pressure-pain was detected after HFS. However, contrary to expectations, we observed an increase in R2 area under the curve for blink reflexes that were ipsilateral both to supraorbital stimulation and to the HFS-conditioned site. Thus, excitability to superficial nociceptive stimuli apparently developed in pathways subserving the ipsilateral trigeminal nociceptive blink reflex following limb pain induced by HFS. Together, these findings suggest a possible coexistence of supraspinal hemilateral inhibitory and facilitatory influences on nociceptive processing following limb pain induced by HFS.

*Central sensitisation*

Hyperalgesia to sharp stimuli (but not to blunt pressure or heat) developed in skin areas surrounding the site conditioned by HFS, consistent with the development of secondary tactile hyperalgesia. This finding is in line with previous reports of hyperalgesia to sharp stimulation in adjacent skin areas after HFS (Klein et al., 2008; Lang et al., 2007; Pfau et al., 2011; Vo and Drummond, 2013). Objective electrophysiological evidence supports an association between secondary tactile hyperalgesia and central sensitisation after HFS. This includes enhanced N1-P1 peak-to-peak and P300 event related potentials (van den Broeke et al., 2010), and an
increase in P200 amplitude of evoked potentials in response to sharp stimuli after HFS conditioning (van den Broeke et al., 2011).

**Ipsilateral forehead analgesia to pressure-pain**

Sensitivity to pressure-pain decreased on both sides of the forehead after HFS. This replicates previous findings (Vo and Drummond, 2013), and similar effects during cold- (Knudsen and Drummond, 2009) and capsaicin-induced limb pain (Knudsen and Drummond, 2011). The bilateral component of response may have been mediated by central pain inhibitory mechanisms such as diffuse noxious inhibitory controls (Villanueva and Le Bars, 1995) or stressed-induced analgesia ((Bandura et al., 1988; Chesher and Chan, 1977; Gamaro et al., 1998; Janssen et al., 1998; Willer et al., 1981).

Importantly, the analgesia to pressure-pain was greater on the ipsilateral than contralateral side of the forehead (see also Knudsen and Drummond, 2009, 2011; Vo and Drummond, 2013). This inhibitory effect persisted for at least an hour after HFS both in the present and a previous study (Vo and Drummond, 2013). Interestingly, cooling the ipsilateral temple with ice reduced electrically-evoked pain at the HFS-treated site significantly more than cooling the contralateral temple (Vo and Drummond, 2013), suggesting the involvement of a hemilateral pain inhibitory mechanism after HFS conditioning.

The nature of this mechanism is uncertain. However, electrical stimulation of A-delta and C fibres is known to trigger a pain inhibitory mechanism descending from the locus coeruleus (Hitoto et al., 1998; Men and Matsui, 1994) that suppresses nociceptive activity in wide dynamic range neurons in the dorsal horn via noradrenergic projections that act on α2-adrenoreceptors at all segmental levels.
In experiments on rats, noradrenaline increased in the dorsal horn ipsilateral to a hindpaw inflamed by carrageenan but not contralaterally (Tsuruoka et al., 1999), indicating that the adrenergic pathway was active only in the ipsilateral dorsal horn (Tsuruoka et al., 2003). Consistent with this, heat hyperalgesia developed not only in the inflamed hindpaw but also in the non-inflamed ipsilateral forepaw of rats with bilateral locus coeruleus lesions compared with sham-operated rats, but did not develop in the contralateral hind- or forepaw (Tsuruoka et al., 2004). We thus speculate that a hemilateral coeruleospinal pain inhibitory mechanism may have contributed to ipsilateral forehead analgesia to blunt pressure in the present study.

**Pain perception and nociceptive blink reflexes to supraorbital stimulation**

Pain perception and R2 AUC to supraorbital electrical stimuli decreased bilaterally during counter-irritation both before and after HFS conditioning, presumably due to pain-inhibitory processes such as stress-induced analgesia or diffuse noxious inhibitory controls (Gamaro et al., 1998; Janssen et al., 1998; Villanueva and Le Bars, 1995). In previous studies, remote noxious heat (Ellrich and Treede, 1998) and cold pain (Giffin et al., 2004) suppressed R2 amplitude and increased R2 onset latency. In contrast, in the present study, R2 onset latency to superficial nociceptive stimulation did not change during counter-irritation before HFS conditioning, and *decreased* during counter-irritation after HFS conditioning. This decrease in R2 latency is difficult to explain, but it is tempting to speculate that HFS conditioning exerted a net facilitatory influence on neurotransmission through rapidly conducting trigeminal pathways that was unmasked during counter-irritation.
due to preferential inhibition of transmission through slowly-conducting trigeminal nociceptive pathways.

In the absence of counter-irritation, a bilateral reduction in pain perception to supraorbital stimuli after HFS corresponded with a reduction in R2 AUC of blink reflexes contralateral to HFS, but was at odds with an increase in R2 AUC ipsilateral both to HFS-conditioning and supraorbital stimulation. This implies the activation of an ipsilateral facilitatory mechanism that overshadowed inhibitory influences on the nociceptive blink reflex after HFS, and that was independent of pain evoked by the supraorbital stimuli. The dissociation between R2 AUC and pain perception has been noted previously (Koh and Drummond, 2006). A substantial literature indicates that serotonergic, opioidergic and other mechanisms deriving from the midbrain, pons and rostral ventromedial medulla facilitate pain reflexes via a spino-bulbo-spinal loop (Urban and Gebhart, 1999; Vera-Portocarrero et al., 2006; Torsney, 2011; Millan, 2002; Drummond, 2012), thereby contributing to central sensitisation. Under certain conditions (e.g., inflammation and nerve injury), facilitatory adrenergic influences may overcome inhibitory ipsilateral adrenergic influences (Taylor et al., 2000; Brightwell and Taylor, 2009; Martins et al., 2010; Jeong and Holden, 2009; Makino et al., 2010; Tsuruoka et al., 2004). We speculate that a facilitatory mechanism linked with central sensitisation increased the strength of the ipsilateral trigeminal nociceptive blink reflex after HFS in our study.

**Dissociation between pressure-pain and other sensory modalities**

The dissociation between ipsilateral forehead analgesia to pressure-pain and unchanged pain in other sensory modalities is interesting, as this implies independent control of activity in deep and superficial nociceptive pathways. Similar dissociation
is sometimes seen in patients with complex regional pain syndrome (Drummond and Finch, 2006) and central post-stroke pain (Riddoch, 1938; Mailis and Bennett, 2002). Pain originating from deep tissues activates different brain structures to those activated by stimulation of superficial tissues (Henderson et al., 2006; Takahashi et al., 2011; Uematsu et al., 2011). For example, Uematsu et al. (2011) showed that cutaneous pain activated the secondary somatosensory cortex specifically, whereas deep muscle pain evoked responses from other brain structures, including the anterior mid-cingulate cortex, anterior and posterior insular cortex, dorsolateral prefrontal cortex and others. Similarly, Takahashi et al. (2011) observed that muscle stimulation evoked responses mostly from ‘emotional’ brain areas including the midbrain, bilateral amygdala, caudate, orbitofrontal cortex, hippocampus, parahippocampus and superior temporal pole compared to cutaneous stimulation. The dissociation between superficial and deep nociceptive pathways might also account for discordance between analgesia to deep pressure pain in the ipsilateral forehead after HFS and the facilitation of ipsilateral nociceptive reflexes to superficial supraorbital stimulation.

**Methodological limitations**

Although the nociceptive blink reflex provides an objective assessment of trigeminal nociception, it predominantly involves stimulation of cutaneous A-δ nociceptors. Whether methods that allow stimulation of deeply-sited nociceptors would prove to be more suitable than superficial stimulation as a neurophysiological correlate of the ipsilateral forehead analgesia to blunt pressure after HFS requires further investigation. In addition, as most of our participants were young and highly educated, the findings might not generalise to other populations. However, the
development of analgesia in the ipsilateral forehead was previously found to be unrelated to the participant’s age (Knudsen and Drummond, 2009).

Conclusions

An association between the central sensitisation triggered by HFS and ipsilateral forehead analgesia to pressure pain (Vo and Drummond, 2013) was confirmed in the present study. Also important were signs of ipsilateral facilitation to supraorbital nociceptive stimuli following HFS. Together, the present findings suggest a possible co-existence of ipsilateral facilitatory and inhibitory influences on nociceptive processing following limb pain induced by HFS in healthy humans. The ipsilateral inhibitory influence may help to limit the spread of sensitisation in central nociceptive pathways.
References


Figure 1 Unrectified and rectified waveforms of a blink reflex. Latency was measured in milliseconds from the onset of the three-pulse left-sided stimulus to the point where the amplitude of the signal began to change noticeably from background noise. In addition, the R2 rectified area under the curve (AUC) was measured between 27 and 87 ms after stimulus onset.
Figure 2 Mean sensitivity ± S.E. to (a) heat; (b) pinprick; (c) von Frey’s monofilament; and (d) pressure-pain in the Primary and Secondary Areas at baseline and after HFS conditioning. Sensitivity to pinprick and von Frey’s monofilament increased significantly in the Primary and the Secondary Areas after HFS conditioning, and sensitivity to pressure-pain increased significantly in the Primary Area (* p< .05 compared to baseline values).
Figure 3  Mean sensitivity ± S.E. to (a) heat; (b) pinprick; (c) von Frey’s monofilament; and (d) pressure-pain in the ipsilateral and contralateral forehead at baseline, after the first set of blink reflexes, after HFS conditioning, and after the second set of blink reflexes. Sensitivity to heat, pinprick and von Frey’s monofilament remained stable throughout on both sides of the forehead. In contrast, ipsilateral and contralateral pressure-pain thresholds (PPTs) increased significantly after HFS conditioning and after the second set of blink reflexes (# p < .01 compared with their respective PPT’s at baseline), with a greater increase on the ipsilateral side (* p < .001 compared with PPT on the contralateral side after HFS conditioning and after the second set of blink reflexes).
Pain ratings ± S.E. to supraorbital stimuli with and without electrically-evoked forearm pain at baseline and after HFS conditioning. a. Pain ratings decreased after HFS conditioning, both in the presence and absence of electrically-evoked forearm pain (** p = .007). b. Both before and after HFS conditioning, electrically-evoked forearm pain reduced sensitivity to supraorbital stimuli (* p = .046). However, sensitivity to supraorbital stimulation did not differ between the ipsilateral and contralateral forehead.
Before HFS conditioning, R2 onset latencies were similar irrespective of electrically-evoked forearm pain. However, after HFS conditioning, R2 onset latency decreased significantly in the presence of counter-irritation (* p < .01 compared with all other conditions).

**Figure 5** Mean R2 onset latencies ± S.E.

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<th>Without counter-irritation</th>
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Figure 6  Mean R2 area under the curve (AUC) ± S.E. for blink reflexes ipsilateral to both the HFS-treated site and supraorbital stimuli (R2ii), ipsilateral to the HFS-treated site but contralateral to supraorbital stimuli (R2ic), contralateral to the HFS-treated site but ipsilateral to supraorbital stimuli (R2ci), and contralateral both to the HFS-treated site and to supraorbital stimuli (R2cc) in raw format before and after HFS conditioning. In general, R2 AUC at baseline (A) was greater than R2 AUC during forearm counter-irritation (B) and after HFS (C and D).
Figure 7  Mean percent change ± S.E. in R2 area under the curve (AUC) expressed in relation to R2 AUC recorded at baseline ipsilateral both to the HFS-treated site and supraorbital stimuli (R2ii), ipsilateral to the HFS-treated site but contralateral to supraorbital stimuli (R2ic), contralateral to the HFS-treated site but ipsilateral to supraorbital stimuli (R2ci), and contralateral both to the HFS-treated site and supraorbital stimuli (R2cc). R2 AUC decreased significantly (p < .01) during electrically-evoked forearm pain on both sides of the forehead both before and after HFS conditioning (Fig. 7a and 7b). However, in the absence of counter-irritation of the forearm, the R2ii AUC increased after HFS conditioning, whereas R2ic AUC, R2ci AUC, and R2cc AUC decreased (Fig. 7c). Specifically, the R2ii AUC was significantly greater than the R2ic AUC and R2ci AUC (* p ≤ .05).
Chapter 5 Study 3

Analgesia to pressure-pain develops in the ipsilateral forehead after high- and low-frequency electrical stimulation of the forearm

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Running head: Changes in pain modulation after HFS and LFS

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Author Contributions: The concept and design for this study was developed in collaboration with Professor Peter Drummond. I was responsible for the acquisition of data, analysing and interpreting results, and preparing the first draft of the paper. Professor Drummond was also involved in analysing and interpreting results, reviewed and provided feedback on the first draft, and prepared the final version of the paper for publication.
Abstract

**Background.** In healthy participants, high-frequency electrical stimulation of the forearm not only evokes local hyperalgesia but also inhibits sensitivity to pressure-pain in the ipsilateral forehead, possibly due to activation of ipsilateral inhibitory pain modulation processes. The aim of this study was to compare the effects of high- and low-frequency electrical stimulation of the forearm on sensitivity to pressure-pain in the ipsilateral forehead, as inhibitory pain modulation may be stronger after low- than high-frequency electrical stimulation.

**Methods.** Before and after high- and low-frequency electrical stimulation, sensitivity to heat and to blunt and sharp stimuli was assessed at and adjacent to the electrically-conditioned site in the forearm. In addition, sensitivity to blunt pressure was measured bilaterally in the forehead.

**Results.** Pain was more intense after high- than low-frequency electrical stimulation, and was followed by primary and secondary hyperalgesia to mechanical stimulation after high- but not low-frequency electrical stimulation. Nevertheless, sensitivity to pressure-pain decreased to the same extent in the ipsilateral forehead after both forms of electrical stimulation. This decrease was associated with heightened sensitivity to pressure-pain at the electrically-conditioned forearm site, and with diminished sensitivity to heat around this site.

**Conclusions.** These findings suggest that sensitization of pressure-sensitive nociceptive afferents at the site of electrical stimulation is associated with generation of an ipsilateral pain-inhibitory process. This ipsilateral pain-inhibitory process may
decrease sensitivity to pressure-pain in the ipsilateral forehead and suppress secondary hyperalgesia to heat.

**Keywords**: HFS; LFS; diffuse noxious inhibitory controls; noradrenergic pain modulation; ipsilateral pain-inhibitory modulation; primary hyperalgesia; secondary hyperalgesia.
Introduction

In healthy humans, sensitivity to blunt pressure-pain decreases on the ipsilateral side of the forehead in the presence of upper limb pain (Knudsen and Drummond 2009, 2011). Rekindling heat hyperalgesia in capsaicin-sensitised forearm skin (Knudsen and Drummond 2011) and immersing a limb in ice-water (Knudsen and Drummond 2009) evokes this ipsilateral response, as does brief bursts of high-frequency electrical stimulation (HFS) to the forearm (Vo and Drummond 2013b, a). Specifically, HFS of the forearm enhanced pain sensitivity to mechanical punctate stimuli (pinpricks and von Frey’s monofilament) not only at the conditioned site but also in adjacent skin (Vo and Drummond 2013b, a), consistent with secondary hyperalgesia (a sign of central sensitisation) (Klein et al. 2008; Pfau et al. 2011). In addition, sensitivity to blunt pressure-pain decreased on both sides of the forehead, with a greater reduction on the ipsilateral side.

Preferential activation of nociceptors by HFS increases synaptic strength and evokes long-term potentiation in spinal nociceptive pathways (Klein et al. 2008; Lang et al. 2007; Pfau et al. 2011; van den Broeke et al. 2010). However, HFS may also evoke activity in pain-inhibitory pathways that descend from the brainstem to all levels of the spinal cord (Sandkuhler and Liu 1998). For example, disrupting descending pathways using a cold block of the thoracic spinal cord enhanced the pro-nociceptive effects of HFS of the sciatic nerve in rats (Gjerstad et al. 2001). Thus, the central sensitisation triggered by HFS may be opposed, in part, by activation of descending pain modulation pathways. Generalisation of this inhibitory effect to other regions on the ipsilateral side of the body might account for the
reduction in sensitivity to pressure-pain in the ipsilateral forehead (Vo and Drummond 2013b, a).

Recently, low frequency electrical stimulation (LFS) (1-2 Hz) has been used to examine mechanisms of hyperalgesia in humans (Seifert et al. 2009). In contrast to HFS, LFS induces a long-lasting decrease in synaptic strength in spinal nociceptive pathways, resulting in hypoalgesia at the site of stimulation (Aymanns et al. 2009; Jung et al. 2011; Rottmann et al. 2008); in addition, LFS evokes only minor signs of secondary hyperalgesia in adjacent skin (Klein et al. 2004). Together, these findings suggest that LFS triggers stronger inhibitory processes than HFS. Hence, we hypothesised that the reduction in sensitivity to pressure-pain in the ipsilateral forehead would be greater after LFS than HFS. To determine whether modality-specific changes in the forearm trigger ipsilateral forehead analgesia, we also explored the association between signs of inhibitory pain modulation in the ipsilateral forehead and changes in sensitivity to mechanical and thermal stimulation at and around the site of electrical conditioning in the forearm.

Methods

Participants

The sample consisted of 68 participants (41 females) ranging in age between 18 and 51 years. Participants were excluded if they suffered from any psychiatric or medical condition, or if they were pregnant or breast-feeding. Participants provided their informed consent for the procedures, which were approved by the Murdoch University Human Research Ethics Committee.
All procedures were carried out in a laboratory maintained at 21 ± 1°C by the same experimenter (LV). The experimental procedure is summarised in Table 1. Participants’ ventral forearms were gently exfoliated using pumice stone, rinsed and dried to minimise skin electrical resistance. One ventral forearm was assigned as the test site, and an equivalent area in the contralateral forearm (the control site) was also tested before HFS or LFS to ensure that nociceptive stimulation during the initial psychophysical assessments was equivalent in both forearms (preliminary analyses confirmed that sensitivity to heat, sharpness and pressure was similar at both sites). The laterality of the test and control sites was counter-balanced across participants. Sensitivity was assessed at the Primary Area and an area 1 cm distal to the Primary Area (Secondary Area). Participants sat in a comfortable arm-chair throughout the experiment.

Psychophysical tests. Before conducting baseline sensitivity tests, the participant was trained until ratings and pressure-pain thresholds stabilised. Participants reported pain or sharpness intensity using a verbal rating scale ranging from 0 to 10. For pain, 0 indicated “no pain” and 10 indicated “extreme pain”. For sharpness intensity, 0 implied “not sharp” and 10 implied “extremely sharp”. To assess heat sensitivity, a 1.5 cm diameter metal probe heated to 44 ± 0.2°C was placed at the site for 7 s before participants provided a pain rating. To investigate sensitivity to mild sharpness, a 10g von Frey monofilament (Neuro-pen, Owen Mumford, USA) was applied at a 90° angle to the skin surface with sufficient pressure to bend the monofilament for 1 s. To measure sensitivity to more intense sharpness, a sharp tip with a calibrated spring mechanism exerting a force of 40 g (Neuro-pen, Owen Mumford, USA) was applied for 2 s. To measure the pressure-pain threshold (PPT), an algometer (FDX, Wagner Instruments, USA) with a
modified 8 mm diameter hemispheric rubber tip was applied at each forearm site and on each side of the forehead at 100 g/s until the participant reported pain.

The psychophysical tests were then conducted with each stimulus being applied in runs alternating between the Primary and the Secondary Areas of the test and the control sites, and between the two sides of the forehead. The side tested first alternated between each arm and between each side of the forehead in counterbalanced order across participants. To minimize effects of repeated testing, each test was performed only once in each round. The exception was during baseline when measures taken at two sites on the same forearm differed by more than 20% or 2 points on the 0-10 rating scales. In such cases, the final measurement was the average of two readings.

**Conditioning Procedures (HFS: N=50; LFS: N=18).** The electrical stimuli were generated by a constant current stimulator (DS7A; Digitimer, Welwyn Garden City, UK) and delivered via a custom-built electrode that consisted of 25 copper pins with 0.2 mm diameter tips mounted on a 2 cm x 3 cm perspex block such that the tips projected 0.5 mm from the surface of the block. Electrodes with these properties preferentially activate superficial nociceptive A-δ and C fibers (Inui et al. 2002; Kaube et al. 2000; Nilsson and Schouenborg 1999). The electrical detection threshold (EDT) was determined using the method of limits for 2 ascending and 2 descending sets of single pulses at 2 ms pulse width and an inter-pulse interval of 5 s. The stimulus intensity, starting at 0.1 mA, increased in steps of 0.1 mA until the participant perceived the stimulus, and then decreased in steps of 0.05 mA until the stimulus was no longer perceived. This procedure was then repeated. The EDT was defined as the geometric mean of the 4 stimulus intensity levels. After a 5 min rest,
HFS or LFS conditioning was applied at the test site. HFS conditioning consisted of 5 1-s bursts of electrical stimuli (100 Hz, 2 ms pulse width, at 10 times EDT up to a maximum of 8 mA) with 9 s rest between bursts. The participant rated pain after each HFS burst. LFS conditioning consisted of 1,000 single electrical stimuli (1 Hz, 2 ms pulse width, at 10 times EDT up to a maximum of 8 mA). The participant rated pain after every 10 electrical stimuli. LFS lasted approximately 16.7 minutes. Ten min after conditioning, the psychophysical tests were re-conducted at the test site. Sensitivity to blunt pressure was also assessed on each side of the forehead one, ten and 60 min after conditioning.

**Statistical Approach**

A mean pain rating was derived for every 100 pulses during LFS conditioning. The change in pain ratings across the 100-pulse blocks was examined in a repeated-measures analysis of variance with simple contrasts between the first and subsequent blocks. Similarly, the change in pain ratings during 5 trains of HFS conditioning was examined in a repeated-measures analysis of variance with simple contrasts between the first and subsequent trains.

Sensory changes were assessed across sites (primary and secondary areas), time (before conditioning, after conditioning) and groups (HFS, LFS) in repeated-measures analyses of variance. Similarly, changes in sensitivity to pressure-pain in the forehead were assessed across sides (ipsilateral or contralateral to electrical stimulation), time (before conditioning, and 1, 10 and 60 min after conditioning) and groups (HFS, LFS).

To further investigate possible mechanisms of ipsilateral forehead analgesia, the association between asymmetry in the forehead PPT after electrical stimulation
(corrected for asymmetry in the forehead PPT at baseline) and the change in sensitivity to each forearm stimulus was explored in the entire group with hierarchical multiple regression analysis. Changes in sensitivity to heat, pinpricks and pressure-pain in the Primary Area (indices of peripheral sensitisation) and the Secondary Area (indices of central sensitisation) were derived by calculating the difference between values before and after conditioning. Sensitivity to pinpricks was chosen to represent sharpness because pinpricks produced a wider range of response than von Frey monofilaments. To control for bilateral changes in sensitivity to pressure-pain in the forehead after electrical stimulation, the mean change in the forehead PPT was entered in the first step of the analysis. As different physiological processes are thought to mediate primary and secondary hyperalgesia, changes in sensitivity to heat, pinpricks and pressure-pain at the site of electrical stimulation in the forearm (i.e., indices of primary hyperalgesia) were entered in the second step, and changes in sensitivity around the electrically-stimulated site (i.e., indices of secondary hyperalgesia) were entered in the final step.
Results

Pain ratings during LFS and HFS conditioning

The individual EDT’s were similar for LFS ($M \pm S.D. = 0.53 \pm 0.48 \text{ mA}$) and HFS ($M = 0.50 \pm 0.28 \text{ mA}$) ($t(66) = 0.58$, not significant). Participants perceived pain as sharp during both forms of conditioning. Mean pain ratings were greater for HFS than LFS ($7.1 \pm 1.6$ versus $2.6 \pm 1.7$ on the 0-10 verbal rating scale, $t(66) = 10.2$, $p<0.001$). Pain ratings decreased progressively across the 100-pulse blocks during LFS conditioning (Fig. 1a), but increased during the second ($p < .01$) and the third ($p < .05$) trains of HFS stimuli compared to the first (Fig. 1b).

Primary and secondary hyperalgesia in the forearm after electrical conditioning

Before conditioning, sensitivity to heat, sharpness and pressure-pain was similar in the HFS and LFS groups (Fig. 2). Heat sensitivity in the primary area increased significantly after HFS conditioning with a similar trend for LFS conditioning, but did not change in the secondary area after either form of electrical conditioning (Site x Time interaction $F(1, 61) =15.0$, $p = .000$) (Fig. 2a). However, sensitivity to pinpricks, von Frey monofilaments and pressure-pain depended on the type of electrical conditioning, with significant increases in the primary and secondary area only after HFS conditioning (Time x Group interaction: for pinpricks $F(1, 59) =5.95$, $p = .018$; for von Frey monofilaments $F(1, 59) =11.9$, $p = .001$; for pressure-pain thresholds $F(1, 58) =7.05$, $p = .010$) (Fig. 2b-d). In addition, the Site x Time x Group interaction was significant for pinpricks ($F(1, 59) =11.5$, $p = .001$) and von Frey monofilaments ($F(1, 59) =5.91$, $p = .018$) due to greater differences.
between the HFS and LFS groups at the primary than secondary site after electrical conditioning (Fig. 2b and 2c).

**Forehead sensitivity to blunt pressure-pain**

At baseline, PPTs were similar on the two sides of the forehead for both the HFS and LFS groups. Forehead PPTs increased bilaterally with a greater increase on the ipsilateral than contralateral side after both forms of electrical conditioning (main effect for Side, $F(1,66) = 28.7, p < .001$; main effect for Time, $F(1, 66) = 28.2, p < .001$; Side x Time interaction, $F(1, 66) = 11.4, p < .001$; effects involving Group not significant) (Fig. 3). The PPT increase was detected immediately after both forms of electrical conditioning, and persisted for at least 60 minutes.

**Association between primary and secondary hyperalgesia in the forearm and the development of ipsilateral forehead analgesia after electrical stimulation**

Heightened sensitivity to pressure-pain at the site of electrical stimulation in the forearm was associated with a greater reduction in sensitivity to pressure-pain in the ipsilateral than contralateral forehead [$r(66) = .283, p<.05$] (Table 2). However, neither pinprick nor heat sensitivity in the Primary Area strengthened this association (Table 3). After controlling for changes in other sensory modalities, diminished sensitivity to heat in the Secondary Area predicted the development of ipsilateral forehead analgesia [$R^2$ change for Model 3 (3, 52) = .128, $p<.05$] (Table 3).

**Discussion**

HFS was very painful and evoked primary and secondary hyperalgesia in the forearm whereas LFS was only mildly painful and did not generate primary or secondary hyperalgesia. Furthermore, pain rose during HFS but decreased gradually
during LFS conditioning (Biurrun Manresa et al. 2010; Klein et al. 2004), possibly due to habituation or the development of long term depression in spinal nociceptive pathways (Rankin et al. 2009; Rottmann et al. 2008). Despite these differences, decreases in sensitivity to pressure-pain in the forehead were similar after both forms of electrical stimulation, suggesting that HFS and LFS triggered similar pain inhibitory processes.

*Primary and secondary hyperalgesia after electrical stimulation of the forearm*

Heat sensitivity at the conditioned site increased after HFS with a similar trend after LFS, possibly due to local release of prostaglandins (Ferrell et al. 2002; Tartas et al. 2005; Ohishi et al. 1999; Yaksh et al. 1999). The PPT decreased at the primary site after HFS, indicating sensitisation of pressure-sensitive nociceptors. The PPT also decreased nearby, possibly due to central sensitisation or to inadvertent stimulation of the primary site by stretching the sensitised skin. In addition, hyperalgesia to pinpricks and von Frey’s monofilament developed at and around the HFS-conditioned site, consistent with the development of central sensitisation (Klein et al. 2008; Pfau et al. 2011; Vo and Drummond 2013b, a; van den Broeke et al. 2011; van den Broeke et al. 2010). A substantial literature suggests that central sensitisation is mediated by facilitatory influences on spinal nociception that emanate from the rostroventral medulla (Millan 2002; Sandkuhler 2009; Urban et al. 1999; Pertovaara et al. 1996; Burgess et al. 2002).

In contrast to HFS, sensitivity to pressure, pinprick and von Frey’s monofilament remained unchanged at and adjacent to the LFS-conditioned site. In addition, electrically-evoked pain decreased at the LFS-conditioned site (Klein et al. 2004; Rottmann et al. 2008), consistent with activation of central inhibitory pain
control mechanisms (Jung et al. 2012; Rottmann et al. 2010) and/or long-term depression in central pain processing pathways (Klein et al. 2004). Thus, the primary response to electrical stimulation differed markedly between the HFS and LFS conditions.

Analgesia to blunt pressure in the forehead

Despite these differences, sensitivity to blunt pressure decreased across the forehead for at least an hour after both forms of conditioning. Similar effects after HFS (Vo and Drummond 2013b, a) and cold-induced limb pain (Knudsen and Drummond 2009) were attributed to activation of descending pain-inhibitory mechanisms such as diffuse noxious inhibitory controls (Villanueva and Le Bars 1995) and/or stress-induced analgesia (Bandura et al. 1988; Janssen et al. 1998). Importantly, however, analgesia to pressure-pain was greater on the ipsilateral than contralateral side of the forehead not only after HFS (Vo and Drummond 2013b, a) but also after LFS, implying the involvement of an inhibitory influence that extended hemilaterally.

Pain can be modulated spinally via noradrenergic, opioidergic, serotonergic, dopaminergic, and other mechanisms (Millan 2002). Although most of these mechanisms exert broad bilateral pain-inhibitory effects (Pertovaara and Almeida 2006), noradrenergic pathways inhibit nociceptive activity hemilaterally. Electrical stimulation of A-δ and C nociceptors evokes activity in the locus coeruleus (LC) which, in turn, suppresses nociceptive responses via bilateral noradrenergic projections that act on spinal α2-adrenoreceptors (Clark and Proudfit 1992; Jones and Gebhart 1986b, a). Following injection of carrageen in the rat hindpaw, noradrenaline increased in the dorsal horn ipsilateral to the inflamed paw, but not
contralaterally (Tsuruoka et al. 1999), indicating that noradrenergic pain modulation was ipsilateral rather than diffuse (Tsuruoka et al. 2003; Tsuruoka and Willis 1996a, b). Interestingly, in rats with bilateral LC lesions, paw withdrawal latencies were shorter not only in the carrageenan-inflamed hindpaw but also in the ipsilateral non-inflamed forepaw (Tsuruoka et al. 2004). In a recent study involving positron emission tomography, an increase in metabolic activity was detected in the amygdala and periaqueductal gray (PAG) following HFS of the sciatic nerve in rats (Hjornevik et al. 2008). In turn, PAG activation may trigger ipsilateral descending pain-inhibitory influences (Levine et al. 1991). Thus, in the present study, a hemilateral noradrenergic pain-inhibitory mechanism involving the brainstem, midbrain and higher cortical centres might have mediated ipsilateral forehead analgesia to pressure-pain following LFS and HFS.

Analgesia to pressure-pain in the ipsilateral forehead was associated with primary hyperalgesia to pressure-pain in the forearm, likely mediated by sensitised pressure-sensitive cutaneous nociceptors. Thus, an association may exist between an ipsilateral pain-inhibitory mechanism and the spinal and supraspinal processes that sensitise nociceptors. In the skin, this appears to preferentially involve pressure-sensitive nociceptors whereas other mechanisms may be involved in muscles (Valeriani et al. 2005). However, the association may be complex because the analgesic response to pressure-pain in the ipsilateral forehead developed after LFS in the absence of primary hyperalgesia to pressure-pain in the forearm.

In our previous study, cold-evoked pain in the ipsilateral temple reduced electrically-evoked pain at the HFS-conditioned forearm site to a greater extent than cold-evoked pain in the contralateral temple, implying an ipsilateral pain-inhibitory
influence on nociception in the forearm (Vo and Drummond 2013b). In the present study, analgesia to pressure-pain in the ipsilateral forehead after electrical stimulation was associated with decreases in sensitivity to heat around the site of electrical stimulation, after changes in other sensory modalities had been taken into account. Further studies are required to confirm this observation; nevertheless, the present findings suggest that the ipsilateral pain-inhibitory influence evoked by electrical stimulation of the forearm acted more strongly on thermal than mechanical nociceptors. This might explain why secondary hyperalgesia developed more readily to mechanical than thermal stimuli after electrical stimulation in this and previous studies (Klein et al. 2008; Lang et al. 2007; Vo and Drummond 2013b, a).

Conclusions

Ipsilateral forehead analgesia to blunt pressure was detected following both HFS and LFS, irrespective of pain intensity or the development of secondary hyperalgesia around the site of stimulation. Together, these findings suggest that the descending facilitatory processes that maintain central sensitisation are independent from the descending inhibitory processes that modulate pain. Nevertheless, inhibitory mechanisms such as those identified in this study may limit the spread of secondary hyperalgesia. Moreover, stronger inhibitory influences on thermal than mechanical nociceptive activity could explain why secondary hyperalgesia develops more readily in mechanical than thermal modalities after certain forms of injury (Ali et al. 1996; Gustorff et al. 2013; Pedersen 2000). Objective measures of activity in central pain pathways are now required to further validate these findings.

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Conflict of interest statement

The authors have no conflict of interest with the contents of this manuscript.
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<td>10</td>
<td>Perform psychophysical test training</td>
</tr>
<tr>
<td>10</td>
<td>Assess sensitivity to heat, sharpness and pressure-pain at the primary and the secondary sites in the forearm</td>
</tr>
<tr>
<td>5</td>
<td>Assess sensitivity to pressure-pain on each side of the forehead</td>
</tr>
<tr>
<td>10</td>
<td>Determine the electrical detection threshold</td>
</tr>
<tr>
<td>5</td>
<td>Rest</td>
</tr>
<tr>
<td>17</td>
<td>Administer LFS conditioning or</td>
</tr>
<tr>
<td>1</td>
<td>Administer HFS conditioning</td>
</tr>
<tr>
<td>10</td>
<td>Rest</td>
</tr>
<tr>
<td>10</td>
<td>Reassess sensitivity to heat, sharpness and pressure-pain at the primary and the secondary sites in the forearm</td>
</tr>
<tr>
<td>5</td>
<td>Reassess sensitivity to pressure-pain on each side of the forehead</td>
</tr>
</tbody>
</table>
Table 2  Association between hyperalgesia in the forearm after electrical stimulation and a greater reduction in sensitivity to pressure-pain on the ipsilateral than contralateral side of the forehead

<table>
<thead>
<tr>
<th></th>
<th>Pearson’s correlation</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Reduction in sensitivity to pressure-pain greater on the ipsilateral than contralateral side of the forehead</td>
</tr>
<tr>
<td>Mean pain rating during electrical stimulation</td>
<td>- .120</td>
</tr>
<tr>
<td>Mean reduction in sensitivity to pressure-pain in the forehead</td>
<td>.049</td>
</tr>
<tr>
<td><strong>Forearm sensitivity at the electrically stimulated site to:</strong></td>
<td></td>
</tr>
<tr>
<td>heat</td>
<td>.027</td>
</tr>
<tr>
<td>pinprick</td>
<td>-.045</td>
</tr>
<tr>
<td>pressure</td>
<td>.283*</td>
</tr>
<tr>
<td><strong>Forearm sensitivity around the electrically stimulated site to:</strong></td>
<td></td>
</tr>
<tr>
<td>heat</td>
<td>.188</td>
</tr>
<tr>
<td>pinprick</td>
<td>-.043</td>
</tr>
<tr>
<td>pressure</td>
<td>.270*</td>
</tr>
</tbody>
</table>

* p<.05
Table 3  Regression analysis predicting greater analgesia to pressure-pain in the ipsilateral than contralateral forehead after electrical stimulation

<table>
<thead>
<tr>
<th>Predictor</th>
<th>$R^2$</th>
<th>$R^2$ change</th>
<th>$\beta$ weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean increase in the forehead pressure-pain threshold</td>
<td></td>
<td></td>
<td>-.082</td>
</tr>
<tr>
<td>Model 2: including indices of primary hyperalgesia</td>
<td>.104</td>
<td>.097</td>
<td></td>
</tr>
<tr>
<td>Mean increase in the forehead pressure-pain threshold</td>
<td></td>
<td></td>
<td>-.081</td>
</tr>
<tr>
<td>Sensitivity to heat at the HFS site in the forearm</td>
<td></td>
<td></td>
<td>-.159</td>
</tr>
<tr>
<td>Sensitivity to pinprick at the HFS site in the forearm</td>
<td></td>
<td></td>
<td>.042</td>
</tr>
<tr>
<td>Sensitivity to pressure-pain at the HFS site in the forearm</td>
<td></td>
<td></td>
<td>.284*</td>
</tr>
<tr>
<td>Model 3: including indices of secondary hyperalgesia</td>
<td>.233</td>
<td>.128*</td>
<td></td>
</tr>
<tr>
<td>Mean increase in the forehead pressure-pain threshold</td>
<td></td>
<td></td>
<td>-.140</td>
</tr>
<tr>
<td>Sensitivity to heat at the HFS site in the forearm</td>
<td></td>
<td></td>
<td>.109</td>
</tr>
<tr>
<td>Sensitivity to pinprick at the HFS site in the forearm</td>
<td></td>
<td></td>
<td>.164</td>
</tr>
<tr>
<td>Sensitivity to pressure-pain at the HFS site in the forearm</td>
<td></td>
<td></td>
<td>.250</td>
</tr>
<tr>
<td>Sensitivity to heat around the HFS site in the forearm</td>
<td></td>
<td></td>
<td>-.532*</td>
</tr>
<tr>
<td>Sensitivity to pinprick around the HFS site in the forearm</td>
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<td>.042</td>
</tr>
<tr>
<td>Sensitivity to pressure-pain around the HFS site in the forearm</td>
<td></td>
<td></td>
<td>.166</td>
</tr>
</tbody>
</table>

*p<.05
Figure 1  Pain ratings ± S.E. (a) during LFS conditioning; and (b) during HFS conditioning. Pain ratings during each subsequent 100 pulses decreased after the initial 100 pulses during LFS conditioning (* p < .05). In contrast, pain ratings during the second and third trains of HFS stimuli increased significantly compared to the first train during HFS conditioning (* p < .05).
Figure 2  Mean sensitivity ± S.E. to (a) heat; (b) pinpricks; (c) von Frey’s monofilament; and (d) pressure-pain at the primary and secondary sites before and after conditioning for HFS and LFS (* p < .05 compared with values before conditioning)
Ipsilateral and contralateral PPTs increased significantly after both forms of electrical conditioning (p<.05 for each side at each time point compared with baseline), with a greater increase on the ipsilateral side (* p < .05 compared to PPT on the contralateral side).
Chapter 6 Study 4

Involvement of $\alpha_2$-adrenoceptors in inhibitory and facilitatory pain modulation processes

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Author Contributions: The concept and design for this study was developed in collaboration with Professor Peter Drummond. I was responsible for the acquisition of data, analysing and interpreting results, and preparing the first draft of the paper. Professor Drummond was also involved in analysing and interpreting results, reviewed and provided feedback on the first draft, and prepared the final version of the paper for publication.
Number of text pages (including tables and figures): 40
Number of figures: 8
Number of tables: 2 + 1 supplementary table
Number of words in the Abstract: 229
Number of words in the Introduction: 444
Number of words in the Discussion: 1,343
Abstract

In healthy humans, high-frequency electrical stimulation (HFS) of the forearm reduces sensitivity to pressure-pain on the ipsilateral side of the forehead, consistent with activation of an ipsilateral inhibitory pain-modulation process. The aim of this study was to determine whether this inhibitory pain-modulation process could be blocked by yohimbine, an $\alpha_2$-adrenoceptor antagonist, in a double-blind placebo-controlled crossover study involving 22 healthy participants. Sensitivity to heat and to blunt and sharp stimuli at and adjacent to the treated site in the forearm, and on both sides of the forehead, was assessed at baseline, after yohimbine or placebo administration, and after HFS conditioning. In addition, the nociceptive blink reflex elicited by supraorbital stimulation was assessed before and after HFS conditioning, and the effect of ipsilateral versus contralateral temple cooling on electrically evoked pain at test and control sites in the forearms was explored. Blood pressure and electrodermal activity increased after yohimbine administration, indicating blockade of central $\alpha_2$-adrenoceptors. Yohimbine facilitated the nociceptive blink reflex in the ipsilateral forehead after HFS but, paradoxically, also augmented the analgesic effect of ipsilateral temple cooling on electrically-evoked pain in the forearm. Yohimbine had no consistent effect on primary or secondary hyperalgesia in the forearm or analgesia to pressure-pain in the ipsilateral forehead. These findings imply involvement of $\alpha_2$-adrenoceptors both in anti-nociceptive and pro-nociceptive pain modulation processes. However, non-adrenergic mechanism(s) appear to mediate ipsilateral forehead analgesia following HFS.
Key words: α2-adrenoceptors; high-frequency electrical stimulation; nociceptive blink reflex; ipsilateral pain modulation
**Introduction**

In healthy humans, various forms of upper limb pain decrease sensitivity to blunt pressure in the forehead, particularly on the ipsilateral side [45, 46, 89, 90]. This implies activation of a pain modulation process, possibly mediated by $\alpha_2$-adrenoceptors in the ipsilateral dorsal horn of the spinal cord [84, 86]. Remarkably, after a bilateral locus coeruleus lesion, rats developed thermal hyperalgesia not only in the inflamed hindpaw but also in the ipsilateral non-inflamed forepaw [85]. However, thermal hyperalgesia did not develop in contralateral paws. Together, these findings suggest that coeruleospinal pain modulation inhibits nociceptive activity not only in the inflamed dermatome but also at more distant ipsilateral sites.

The main aim of this study was to determine whether a similar ipsilateral noradrenergic mechanism contributed to analgesia in the ipsilateral forehead after conditioning the forearm of healthy human participants with high-frequency electrical stimulation (HFS). This procedure not only induces primary and secondary hyperalgesia at the site of stimulation in the forearm [43, 44, 70] but also triggers analgesia to pressure-pain in the ipsilateral forehead [89, 90]. To investigate the role of noradrenaline in these processes, the $\alpha_2$-adrenoceptor antagonist yohimbine was administered before HFS conditioning. Yohimbine facilitates pain by opposing the anti-nociceptive effects of endogenous and exogenous spinal $\alpha_2$-adrenoceptor agonists [7, 41, 53, 54, 56, 66, 67, 83, 87]. For example, in rat studies, intravenous injection of delta-9-tetrahydrocannabinol increased tail flick latencies by activating a spinal $\alpha_2$-adrenoceptor mechanism that, in turn, was blocked by intrathecal administration of yohimbine [53]. Similar effects were detected in the carrageenan model of inflammation [83]. In humans, extradural administration of clonidine, an
α2-adrenoceptor agonist, induced post-operative sedation and analgesia [54]. However, oral yohimbine partly reversed these effects. In additional studies, yohimbine induced rectal contraction and increased the perception of rectal balloon distension and pain sensitivity [3, 59, 60]. Thus, we hypothesised that yohimbine would enhance primary and secondary hyperalgesia at and around the site conditioned by HFS, and would oppose analgesia to blunt pressure in the ipsilateral forehead.

We previously reported that cold-pain in the temple ipsilateral to HFS-induced limb pain induced a greater reduction of electrically-evoked pain at the HFS-conditioned site than cold-pain in the contralateral temple [90]. This supports the view that HFS triggers an ipsilateral pain modulation process, possibly involving noradrenergic projections from the locus coeruleus to the medullary and spinal dorsal horn [82, 85]. Hence, we hypothesized that yohimbine would oppose the analgesic effect of ipsilateral temple cooling in this study.

We also investigated the effect of yohimbine on nociceptive blink reflexes before and after HFS conditioning. As HFS conditioning facilitates the R2 component of the ipsilateral waveform [89], we expected that yohimbine would further augment this response.

**Method**

**Participants**

The sample consisted of 13 males and 9 females aged between 18 and 52 years. Participants were excluded if they were pregnant or breast-feeding, if they suffered from any chronic pain condition or psychiatric disorder, if they had a
medical condition that affected the heart, blood vessels, skin, liver or kidneys that required regular treatment with medication, or they had a known sensitivity to yohimbine. Participants provided their informed consent for the procedures, which were approved by the Murdoch University Human Research Ethics Committee.

**Study design**

This study followed a double-blind, placebo-controlled crossover design. Yohimbine was administered in one session and placebo in the other session. The order of the two sessions was randomised and counterbalanced across participants. We employed an oral dose of 16 mg of yohimbine (Pfizer Limited, Tadworth, Surrey, UK) as this dose reversed sedation and anti-nociceptive effects induced by clonidine in humans [54]. Yohimbine and placebo tablets were physically indistinguishable, and were contained in separate bottles labelled as A or B under the supervision of personnel who were not involved in the data collection process. On the day of the experiment, participants had a normal breakfast and abstained from alcohol and caffeine. Effects of circadian rhythms were controlled by conducting the procedures at the same time of day in both sessions. To minimise carry-over effects, the two sessions were separated by at least 7 days for male participants. To control for menstrual cycle effects on pain, the two sessions were separated by 28 days for female participants; however, the cycle stage varied across participants.

**Procedures**

All procedures were performed by one experimenter (LV) in a laboratory maintained at approximately 22°C. To minimise skin electrical resistance, the ventral forearms were gently cleaned with pumice stone, rinsed with water and dried. One
ventral forearm area was allocated as the test site, and the equivalent ventral forearm area in the contralateral forearm as the control site. The laterality of these sites was counterbalanced across participants. In the test arm, an area 1 cm from the Primary Site was assigned as the Secondary Site. To prepare for measurement of electrodermal activity, both hands were washed with soap, rinsed, and dried. Participants sat in a comfortable armchair throughout the experiment.

The experimental procedures are summarised in Table 1. Each session consisted of three stages (pre-drug, post-drug and post-HFS). The first stage began with administration of baseline psychophysical tests in the test and control arms, and on each side of the forehead. Blood pressure and heart rate were measured, followed by measurement of electrodermal activity. The second stage began with yohimbine or placebo administration. Sixty minutes later, the psychophysical tests were repeated, followed by assessment of blood pressure, heart rate, and electrodermal activity. The blink reflex procedure was then administered, followed by HFS five minutes later. Ten minutes after HFS, the third stage began with psychophysical tests, followed by blood pressure, heart rate and electrodermal activity measurement, and then the blink reflex procedure. The session concluded with temple cooling. Each session lasted approximately 3.5 hours.

Psychophysical tests. Participants reported pain or sharpness intensity using a verbal rating scale ranging from 0 to 10. For pain, 0 indicated “no pain” and 10 indicated “extreme pain”. For sharpness intensity, 0 implied “not sharp” and 10 implied “extremely sharp”. To assess heat sensitivity, a 1.5 cm diameter metal probe heated to 44 ± 0.2°C was placed at the site for 7 seconds. To investigate sensitivity to sharpness, a sharp tip with a calibrated spring mechanism exerting a force of 40 g
(Neuro-pen, Owen Mumford, USA) was applied for 2 seconds. To measure pressure-pain sensitivity, an algometer (FDX, Wagner Instruments, USA) with a modified 8 mm diameter hemispheric rubber tip was applied at each forearm site or on each side of the forehead at 100 g/second until the participant reported pain.

The psychophysical tests were conducted with each stimulus being applied in runs alternating between the test and the control sites, and between the two sides of the forehead. The side tested first alternated between each arm and between each side of the forehead in counter-balanced order across participants. To reduce variability in ratings, the participant was trained until ratings and pressure-pain thresholds stabilised both in the forearm and forehead prior to conducting baseline psychophysical tests. Repeated testing can enhance pain sensitivity. To minimize this effect, each test was performed only once in each round. The exception was during baseline when measures taken at two sites on the same forearm differed by more than 20% (or 2 points on the 0-10 rating scales) or when the participant was uncertain about their perception of the initial stimulus. In such cases, the final measurement was the average of two readings.

**Blink Reflex Procedure.** The stimulating electrodes were two custom-built concentric electrodes, each consisting of a copper wire cathode centred within a ring-shaped stainless steel anode with an inner diameter of 10 mm and an outer diameter of 20 mm [42]. The small cathode contact area and short anode-cathode distance enables high current density at low current intensity, which preferentially activates superficial A-δ nociceptors. The electrodes were attached to the supraorbital region on each side of the forehead with adhesive tape. The blink reflexes were recorded bilaterally using modified disposable Cleartrode electrodes (ConMed Corporation,
NY, USA) attached over the orbicularis oculi muscle of the lower eyelid and the outer corner of each eye. A ground electrode was attached behind the right ear. Electromyograph signals were amplified with an electromyographic bio-potential amplifier (Biopac Systems, Inc., USA), digitized by an MP100 Biopac Systems Analogue/Digital Channel receptor at 2,000 Hz and displayed on a computer monitor using AcqKnowledge software (Biopac Systems, Inc., USA).

To elicit blink reflexes, 20 monopolar square-wave electrical stimuli were applied at a current intensity of 2 mA. An equal number of stimuli were administered on each side of the forehead. Stimulus administration alternated between the sides such that no more than two stimuli were delivered sequentially on the same side. The stimuli were separated by 15 s intervals to minimise habituation. The stimulus was a 3-pulse train with 0.5 ms pulse duration, and an inter-pulse interval of 5 ms. Triple-pulse stimulation increases the sensation of pain and facilitates the R2 area under the curve, and is thus more suited to examining nociceptive pathways than single pulses [29].

*High-frequency electrical stimulation (HFS).* The electrical stimuli were generated by a constant current stimulator (DS7A; Digitimer, Welwyn Garden City, UK) and delivered via a custom-built electrode that consisted of 25 copper pins (5 rows of pins separated by approximately 3.5 mm) with 0.2 mm diameter tips mounted on a 2 cm x 2 cm perspex block such that the tips projected 0.5 mm from the surface of the block. Electrodes with these properties preferentially activate superficial nociceptive A-δ and C fibers [39, 65]. A 3.0 cm x 3.5 cm ground plate attached 1 cm from the conditioning electrode completed the electrical circuit. The electrical detection threshold (EDT) was determined using the method of limits for 2
ascending and 2 descending sets of single pulses at 2 ms pulse width and an inter-pulse interval of 5 s. The stimulus intensity, starting at 0.1 mA, increased in steps of 0.1 mA until the participant perceived the stimulus, and then decreased in steps of 0.05 mA until the stimulus was no longer perceived. This procedure was then repeated. The EDT was defined as the geometric mean of the 4 stimulus intensity levels. After 5 minutes rest, HFS conditioning was applied at the test site. This consisted of five 1-s bursts of electrical stimulation (100 Hz, 2 ms pulse width, at 10 times EDT up to a maximum of 8 mA) with a 9-s rest between each burst [44, 49]. The participant rated pain after each burst of stimulation. Ten minutes later, the psychophysical tests were re-conducted at the test site and on each side of the forehead. Finally, the blink reflex procedure was repeated.

Electrodermal activity. Four Ag/AgCl electrodes (0.8 cm internal diameter) were filled with conducting gel (Johnson and Johnson KY lubricating Jelly, Sydney Australia). The electrodes were attached to the distal phalanges of the middle and the index fingers of each hand with Micropore tape. During each stage of the experiment, electrodermal activity was recorded for two minutes while participants rested quietly, and changes in electrodermal activity were measured while the participant took 5 deep breaths at 1 minute intervals. During deep breathing, participants were instructed to breathe in slowly and deeply through their nose for 3 s, hold their breath for 2 s, and slowly exhale through their mouth for 4 s.

Blood pressure and heart rate. Systolic blood pressure, diastolic blood pressure and heart rate were measured twice two minutes apart from the upper arm at heart level using an Omron M4 digital sphygmomanometer that detected blood
pressure using the oscillometric method. The final reading was the average of the two measurements.

*The effect of temple cooling on electrically-evoked forearm pain.* Moderate pain (pain level of 5 on the 0-10 verbal rating scale) was evoked at the test site or the control site with electrical stimuli (1 Hz and 0.5 ms pulse width) delivered via the conditioning electrodes used to administer HFS. The current was adjusted to maintain moderate pain for at least 32 s before assessing the effects of ipsilateral and contralateral temple cooling. During temple cooling, an ice cube with an application surface area of 6 cm² was held against the left or right temple anterior to the ear for 32 s. Participants rated electrically-evoked forearm pain every 2 s for 32 s prior to the ice being applied, during the 32-s cooling period, and for 32 s after the ice was removed. At the end of each cooling period, participants rated pain induced in the temple by the cold stimulus. In a separate control task, participants rated electrically-evoked forearm pain every 2 s for 96 s without temple cooling. Test order was counterbalanced across participants and forehead sides, and alternated between the test and control site. To minimise carry-over effects, 2 minutes rest was allowed between tasks except when the task was preceded by the control task.

*Data filtering and reduction*

The electromyographic waveforms were filtered to remove 50 Hz electrical noise and frequencies below 20 Hz. Based on their laterality to the HFS-treated site and then to the stimulus, the blink reflexes were classified as “ii” (ipsilateral to both the HFS-treated site and supraorbital stimulus), “cc” (contralateral to both the HFS-treated site and supraorbital stimulus), “ci” (contralateral to the HFS-treated site and
ipsilateral to the supraorbital stimulus), and “ic” (ipsilateral to the HFS-treated site and contralateral to the supraorbital stimulus). R2 onset latency was individually extracted for each blink reflex from the unrectified waveform from the start of the first of the three electrical pulses to the point where the amplitude of the signal began to change noticeably from background noise. For each blink reflex, the R2 rectified area under the curve (AUC) was measured between 27 and 87 ms after the stimulus onset [23]. The change in R2 AUC of all blink reflexes after HFS conditioning was expressed as the percentage of the R2 AUC of blink reflexes administered before HFS conditioning.

Statistical approach

Changes to all dependent variables across time (from before to after drug administration, and after HFS conditioning) and sessions (yohimbine, placebo) were investigated using repeated-measures analyses of variance with planned contrasts between consecutive time points. Sensitivity to heat, sharpness and pressure-pain at the primary and secondary sites, EDA at rest, and EDA during 5 deep breaths were also compared between the two arms (test, control). Additionally, differences in pain ratings to supraorbital stimulation, R2 latency, R2 AUC and forehead sensitivity to heat, sharpness and pressure-pain were examined between forehead sides (ipsilateral versus contralateral to HFS), and sides of supraorbital stimulation (ipsilateral versus contralateral to the supraorbital stimulus).

Results

Yohimbine side effects
All but one participant correctly guessed when they took yohimbine. Approximately 40 minutes after taking yohimbine, four participants reported a vague feeling of sexual arousal which lasted for approximately 10 to 15 minutes. Five participants felt their heart racing. Nine participants reported feeling anxious, and most participants appeared restless and agitated around 40 minutes after yohimbine was administered. Mild hand tremor was also experienced by two participants. Cold and sweaty hands were reported by half of the participants, as was fatigue and sleepiness. One participant who experienced nausea and headache was excluded from the study.

**Haemodynamic effects**

Basal heart rate before the administration of placebo was higher than before the administration of yohimbine ($t(21) = 2.46, p = .023$) (Fig. 1a), which was likely a chance effect. However, elevated heart rate in the placebo session was associated with a significant decrease in heart rate during the second stage of the experiment compared to yohimbine (Drug x Time interaction $F(1, 20) = 7.65, p < .05$). Heart rate remained unchanged after HFS conditioning in both sessions.

At baseline, systolic and diastolic blood pressures were similar in the yohimbine and the placebo sessions (Fig. 1b-c). Blood pressure increased after the administration of yohimbine but did not change after the administration of placebo (for systolic blood pressure, Drug x Time interaction $F(1, 21) = 7.69, p < .01$; for diastolic blood pressure, Drug x Time interaction $F(1, 21) = 9.90, p < .01$). Both systolic and diastolic blood pressure remained greater in the yohimbine than placebo session after HFS conditioning.


Electrodermal activity (EDA)

Before drugs were administered, EDA at rest and during deep breathing was similar in the yohimbine and placebo sessions (Fig. 2). After drug administration, increases in resting EDA were greater in the yohimbine than placebo session (Drug x Time interaction $F(1, 19) = 8.97, p = .007$). Resting EDA increased after HFS conditioning in both sessions (main effect for Time $F(1, 21) = 7.98, p = .01$). Increases in EDA during deep breathing were similar in the yohimbine and placebo sessions, and remained unchanged after HFS conditioning.

Forearm Sensitivity

Both before and after drug administration, forearm sensitivity to heat, pinpricks and pressure-pain was similar at the test and the control sites. However, HFS conditioning triggered primary hyperalgesia to heat, pinpricks and blunt pressure, and secondary hyperalgesia to pinpricks (Fig. 3). In general, this heightened sensitivity was comparable in the yohimbine and placebo sessions.

Heat. After HFS conditioning, heat sensitivity at the primary site was greater for the test than control arm in both sessions (Time x Arm interaction $F(1, 21) = 66.6, p < .001$). Further exploration of this interaction showed that heat sensitivity increased after HFS conditioning at the primary site in the test arm (p < .001), but decreased slightly in the control arm (p < .05) (Fig. 3a). Sensitivity to heat did not change at the secondary site in the test arm after HFS conditioning.

Pinpricks. After HFS conditioning, sharpness ratings to pinprick was greater for the test than control arm at the primary and secondary sites in both sessions (Primary site: Time x Arm interaction $F(1, 21) = 28.0, p < .001$; Secondary site:}
Time x Arm interaction $F(1, 21) = 21.05, p < .001$). Further exploration of the Time x Arm interactions showed that sensitivity to pinpricks remained unchanged in the control arm, but increased significantly after HFS conditioning at primary and secondary sites in the test arm (Fig. 3b).

Blunt pressure. After HFS conditioning, sensitivity to pressure-pain at the primary site was greater for the test than control arm in both sessions (Time x Arm interaction $F(1, 21) = 15.5, p < .001$). Further exploration of the Time x Arm interaction showed that the PPT decreased in the test arm ($p < .001$) but not in the control arm (Fig. 3c). In contrast, in the secondary area, changes in the PPT after HFS conditioning differed between the yohimbine and placebo sessions (Drug x Time x Arm interaction $F(1, 21) = 9.77, p = .005$). In particular, the PPT decreased after HFS conditioning at the secondary site in the test arm in the placebo session ($p < .05$), but did not change in the yohimbine session.

**Forehead Sensitivity**

At baseline, sensitivity to heat, pinprick and pressure-pain was similar on the ipsilateral and contralateral sides of the forehead in both sessions (Fig. 4). Forehead sensitivity did not change in any modality after yohimbine or placebo administration. However, after HFS conditioning, the PPT increased on both sides of the forehead with a greater increase on the ipsilateral side both in the yohimbine and placebo session (Time x Side interaction $F(1, 21) = 40.44, p < .001$).

**Pain ratings to supraorbital stimulation**

After HFS conditioning, differences between the yohimbine and placebo sessions in pain perception to supraorbital stimulation depended on laterality to the
HFS-conditioned site and side of supraorbital stimulation (Drug x Time x Side interaction $F(1, 21) = 4.84, p = .039$) (Fig. 5). Investigation of this interaction showed that pain perception to supraorbital stimulation remained stable in the yohimbine session but decreased on both sides of the forehead after HFS conditioning in the placebo session ($p < .001$) with a trend for decreases to be greater on the contralateral side ($p = .08$).

**R2 AUC**

After HFS conditioning, the percentage increase for R2 AUC for blink reflexes ipsilateral to the HFS-conditioned site (R2ii and R2ic) was greater in the yohimbine than placebo session, irrespective of the side of supraorbital stimulation (Drug x HFS Side $F(1, 21) = 4.60, p = .044$) (Fig. 6).

**R2 onset latency**

Before HFS conditioning, the R2 onset latency could be measured in 18 of the 22 participants. R2 latency was shorter on the side of supraorbital stimulation ($M = 44.70 \pm 1.08$ msec) than the contralateral side ($M = 46.00 \pm 0.91$ msec) ($F(1, 17) = 4.72, p = .044$), and was greater in the yohimbine session ($M = 46.67 \pm 1.18$ msec) than the placebo session ($M = 43.73 \pm 1.17$ msec) (main effect for Drug $F(1, 17) = 4.87, p = .041$) (Fig. 7).

After HFS conditioning, the R2 onset latency could be measured in 19 of the 22 participants. R2 latency remained shorter on the side of supraorbital stimulation ($M = 43.41 \pm 0.95$ msec) than the contralateral side ($M = 45.16 \pm 0.95$ msec) ($F(1, 18) = 17.51, p < .001$). In addition, the R2 latency was greater on the HFS-conditioned side than contralaterally ($F(1, 18) = 6.13, p = .023$) (Fig. 7).
In the 17 participants with complete data, the R2 onset latency decreased from 45.22 ± 1.03 msec before HFS to 44.27 ± 1.02 msec after HFS (main effect for Time $F(1, 16) = 4.51, p = .050$).

**Temple cooling**

Ice applied to the temple for 32 s induced moderate pain in each temple both in the yohimbine and placebo session (Table S1). Electrically-evoked forearm pain decreased from a starting point of 5 (on the 0 – 10 verbal rating scale) to around 4.2 after 96 s during control trials without temple cooling. To partial out these changes, decreases in electrically-evoked forearm pain during the control trial were first subtracted from that of each temple cooling trial. Changes in electrically-evoked forearm pain during temple cooling were then investigated in relation to Drug (yohimbine versus placebo), HFS-conditioning (HFS-treated versus control site) and Side Cooled (ipsilateral versus contralateral temple cooling) in a repeated-measures analysis of variance with simple contrasts across Time (baseline versus cooling and recovery).

Temple cooling further decreased electrically-evoked pain both during the cooling period ($F(1, 18) = 40.24, p < .001$) and recovery ($F(1, 18) = 35.10, p < .001$) (Fig. 8). However, the decrease during recovery was greater in the yohimbine than placebo session (Drug x Time interaction, $F(1, 18) = 5.36, p = .033$). In general, decreases in electrically-evoked pain were greater when the ipsilateral than contralateral temple was cooled (during cooling, Time x Side Cooled interaction, $F(1, 18) = 4.96, p = .039$; during recovery, Time x Side Cooled interaction, $F(1, 18) = 5.18, p = .035$). However, this ipsilateral inhibitory effect was greater in the
yohimbine than placebo session (Drug x Side Cooled interaction, $F(1, 18) = 5.99, p = .025$). In addition, decreases in electrically-evoked pain were greater during the recovery period at the HFS-conditioned test site than the control site (HFS-conditioning x Time interaction, $F(1, 18) = 9.61, p = .006$), particularly when the ipsilateral temple was cooled (HFS-conditioning x Time x Side Cooled interaction, $F(1, 18) = 4.69, p = .044$).

**Association between change in systolic blood pressure and pain indices**

Elevated systolic blood pressure is associated with hypoalgesia [5, 14, 27]; however, results from Pearson’s $r$ indicated no correlation between systolic blood pressure after drug administration and any of the pain indices either in the placebo or yohimbine session (all Pearson’s $r$’s not significant).

**Discussion**

We used placebo-controlled $\alpha_2$-adrenoceptor blockade to determine whether $\alpha_2$-adrenoceptors were involved in pain modulation mechanisms triggered by HFS. However, our findings were mixed (Table 2). After HFS conditioning, yohimbine facilitated ipsilateral nociceptive blink reflexes and blocked anti-nociceptive influences on pain to supraorbital stimulation. Nevertheless, yohimbine administration did not alter primary or secondary hyperalgesia in the forearm, or block analgesia to pressure-pain in the ipsilateral forehead. Moreover, yohimbine facilitated the analgesic effect of ipsilateral temple cooling on electrically-evoked forearm pain indicating, paradoxically, that yohimbine exerted anti-nociceptive effects in addition to pro-nociceptive effects.
Changes in blood pressure and heart rate

Yohimbine increased systolic and diastolic blood pressure in seated participants. Some past studies that used a similar or higher single dose of oral yohimbine than ours reported no increase in blood pressure [33], whereas others produced comparable results [13, 79]. The extent to which yohimbine becomes available to the target tissue after administration ranges from 7% to 87% [35]. Such great variability in bioavailability between individuals may explain these different findings.

Our results also showed that yohimbine had no effect on heart rate. This finding is consistent with most studies that used oral yohimbine at similar or higher doses [13, 35, 79]. Intravenous yohimbine increased heart rate at a dose of 0.125 mg/kg (i.e., 8.75 mg for a 70 kg participant) [34], likely due to higher systemic concentrations after intravenous than oral administration. This suggests that the yohimbine dosage required to increase heart rate differs to that of blood pressure. Alternatively, yohimbine might have increased heart rate, which was masked by a baroreflex mechanism, a process by which increases in blood pressure decrease heart rate through the activation of a parasympathetic vagal reflex [31]. Activation of brainstem α2-adrenoreceptors with clonidine inhibits sympathetic tone, resulting in a decrease in blood pressure and heart rate [79]. Conversely, blocking presynaptic α2-adrenoreceptors with yohimbine stimulates noradrenergic neurons to enhance noradrenaline release, thereby elevating sympathetic tone [78]. Thus, elevated blood pressure in this study suggests that yohimbine was somewhat effective in blocking central α2-adrenoreceptors [34].
Changes in electrodermal activity

Resting electrodermal activity or psychogenic sweating also increased after yohimbine in comparison to placebo. Psychogenic sweating through eccrine sweat glands in the palm and sole is induced by neurophysiological arousal, anticipation, anxiety, stress and other negative emotions [16]. In this study, the most frequently reported and observed symptoms of anxiety after yohimbine included restlessness, agitation and reports of anxiety, which may have contributed to an increase in electrodermal activity. Empirical evidence also suggests that yohimbine facilitates electrodermal activity by opposing the inhibitory effects of α2-adrenoreceptors in the spinal cord. In anaesthetised cats, intrathecal administration of clonidine at the C6 to T2 spinal levels inhibited electrodermal activity evoked by electrical stimulation of the hypothalamus [47]. Similarly, intravenous clonidine attenuated electrodermal activity following electrical stimulation of pre- and post-ganglionic sympathetic nerves [47]. Moreover, yohimbine reversed clonidine-induced inhibitory effects irrespective of the site of electrical stimulation [47]. These results indicate that yohimbine may facilitate centrally-evoked EDA by opposing inhibitory influences of α2-adrenoreceptor activation in the spinal cord.

Painful stimuli elicit a greater increase in electrodermal activity than painless stimuli, and this has been observed with thermal pain [20, 55, 71], electrical stimulation [11], post-operative pain [51] and heel pricks in children [24]. This association may account for the increase in electrodermal activity following painful HFS conditioning stimuli both after yohimbine and placebo. A functional magnetic
resonance imaging study involving healthy humans demonstrated that pain-related electrodermal activity is associated with the activation of some higher brain structures that underlie pain, particularly those involved in both pain and emotional responses to pain [20]. These include the anterior cingulate cortex, the thalamus, the hypothalamus and the amygdala [20]. Consistent with this, electrical stimulation of human limbic brain structures including the amygdala, the anterior and posterior hippocampi, and the anterior cingulate gyri elicited electrodermal activity [62]. Thus, it is possible that yohimbine may elicit electrodermal activity in humans by acting also at supraspinal brain structures. However, deep breathing increased EDA in both yohimbine and placebo conditions, possibly suggesting that the activation of sympathetic nervous system during deep breathing was not mediated by a2-adrenoreceptors as it was not inhibited by yohimbine.

**Changes in forearm sensitivity**

Similar to previous studies [44, 49, 70, 89, 90], hypersensitivity to heat, sharpness and pressure-pain developed at the HFS-conditioned site (primary hyperalgesia), and hypersensitivity to sharpness also developed nearby (secondary hyperalgesia). Contrary to expectations, yohimbine did not enhance primary or secondary hyperalgesia, and even appeared to inhibit secondary hyperalgesia to pressure-pain. In rats, various routes of yohimbine administration (e.g., topical, intradermal injection and iontophoresis) blocked the peripheral antinociceptive effects elicited by various types of α2-adrenoreceptor agonists [17;18;63;73;75]. Theoretically, yohimbine blocking α2-adrenoreceptors triggers greater release of noradrenaline, which may exacerbate pain by an action on peripheral α1-adrenoreceptors in inflamed skin [52]. However, in our study, sensitivity to heat, to
sharpness, and blunt pressure remained unchanged both at the HFS-treated site and the control site following yohimbine, indicating that this mechanism had little effect on nociception under our experimental conditions.

In the absence of sustained pain or injury, the influence of noradrenergic mechanisms on sensitivity to brief noxious stimulation could be minimal [65]. This was demonstrated by the lack of consistent increase in pain sensitivity following blockade of α2-adrenoreceptors with antagonists [68], knockout of some subtypes of α2-adrenoreceptors [61], and knockout of the dopamine β-hydroxylase gene required for the production of noradrenaline [40].

In healthy individuals, an adrenergic influence on the autonomic control of blood pressure may indirectly attenuate pain, perhaps due to activation of a pain-inhibitory mechanism through the stimulation of baroreflex receptors [6], as elevated blood pressure is associated with a reduction in pain sensitivity [5, 15, 27]. In this study, there was no association between elevated systolic blood pressure and any of the pain responses. This could due to HFS conditioning being brief and less intense compared to severe noxious stimuli being applied for a longer duration as used in studies that identified relationships between blood pressure and pain sensitivity, such as applying 2000g of pressure on the index finger for 1 minute [5, 15] or immersing a limb in 20 C water (cold pressor test) up to 4 minutes [27]. Nevertheless, we cannot rule out a possible influence of blood pressure-related hypoalgesia to pain responses as blood pressure increased following yohimbine in this study. Possibly, this blood-pressure related hypoalgesia could have masked hyperalgesia elicited by yohimbine, resulting in no net change in pain sensitivity. Alternatively, brief and only mildly noxious psychophysical stimuli (heat, sharpness, and blunt pressure) in
our study might have failed to evoke hyperalgesia in the presence of yohimbine. Importantly, yohimbine is known to enhance anxiety [31], consistent with the restlessness, anxiety and agitation reported by participants and observed by the experimenter in this study. Stressful or anxiety-provoking situations enhance the release of noradrenaline and endogenous opioids [12], which might have opposed some pronociceptive effects elicited by yohimbine (in blocking α2-adrenoreceptors).

Furthermore, the supraorbital stimuli eliciting blink reflexes, followed by highly painful HFS conditioning stimuli (pain rating of 7 on the 0-10 VAS), may have also evoked diffuse noxious inhibitory controls (DNIC) and further strengthened stress-induced analgesia (SIA) elicited by yohimbine itself, and together might have compensated for any hyperalgesia associated with yohimbine in this study.

**Analgesia to blunt pressure in the forehead**

In support of previous findings, sensitivity to blunt pressure decreased on both sides of the forehead after HFS conditioning, presumably due to inhibitory processes such as diffuse noxious inhibitory controls (DNIC) or stress induced analgesia [45, 46, 89, 90]. As noted previously [71,72], these decreases were greater on the ipsilateral side. We hypothesized that noradrenergic projections from the locus coeruleus to the ipsilateral medullary and dorsal horn [84, 85] would mediate analgesia in the ipsilateral forehead. However, pre-treatment with yohimbine did not affect the pressure-pain threshold on either side of the forehead. One interpretation of these findings is that noradrenergic mechanism(s) are not involved in analgesia to pressure-pain in the forehead following unilateral upper limb pain. However, this is
at odds with a substantial literature that demonstrates involvement of noradrenergic mechanisms in spinal analgesia [3, 30, 37, 41, 54, 59, 60, 82, 85]. A second explanation could be that yohimbine was ineffective in blocking central $\alpha_2$-adrenoceptors. However, yohimbine enhanced blood pressure and electrodermal activity, most likely by occupying presynaptic $\alpha_2$-adrenergic autoreceptors within the central nervous system, thereby increasing synaptic concentrations of noradrenaline and augmenting autonomic activity [13, 32, 34, 47, 78, 79]. A third possibility is that the hyperalgesic effect of yohimbine was masked by inhibitory mechanisms such as DNIC, stress-induced analgesia, or other non-adrenergic mechanisms.

**Nociceptive responses to supraorbital stimulation**

In the placebo session, pain perception to supraorbital stimulation decreased on both sides of the forehead after HFS conditioning, possibly due to pain modulation mechanisms such as stress-induced analgesia or DNIC. In contrast, pain perception to supraorbital stimuli did not change after HFS conditioning in the yohimbine session. Together, these findings suggest that yohimbine blocked an analgesic mechanism. Similarly, yohimbine potentiated the R2 AUC ipsilateral to the HFS-conditioned site. The mechanism of this ipsilateral facilitation is unknown, but one possibility is that HFS, together with yohimbine, increased synaptic concentrations of noradrenaline in central ipsilateral nociceptive pathways. Since $\alpha_2$-adrenoceptors were blocked by yohimbine, these high synaptic concentrations of noradrenaline might instead have stimulated excitatory $\alpha_1$-adrenoceptors on nociceptive afferents, thus disrupting inhibitory pain modulation. In line with this possibility, stimulation of brainstem $\alpha_1$-adrenoceptors with phenylephrine blocked the inhibitory effect of DNIC on the C-fibre reflex to electrical stimulation of the rat
hindpaw [58]. Interestingly, destruction of noradrenergic neurons attenuates signs of hyperalgesia in neuropathic pain models. For example, spared sural nerve injury triggered an increase in markers of neural activity in the locus coeruleus, whereas disruption of synaptic activity or destruction of adrenergic locus coeruleus neurons with neurotoxin prevented the development of allodynia and hyperalgesia [4]. Similarly, following intraplantar formalin injection, locus coeruleus-lesioned rats spent less time licking or lifting the inflamed hindpaw during a hotplate test compared with control rats [80]. Hence, we speculate that a facilitatory adrenergic mechanism contributed to an increase in the excitability of the ipsilateral trigeminal nociceptive blink reflex following HFS in our study.

R2 onset latency was greater after yohimbine than placebo administration, consistent with an excitatory influence of α2-adrenoceptors on transmission of the nociceptive blink reflex. This result was unexpected as α2-adrenoceptors appear to inhibit rather than potentiate the facial motor neuron response to stimulation [64]. One possibility might be that α2-adrenoceptors facilitate neurotransmission primarily in the afferent limb of the reflex. R2 latency decreased after HFS conditioning, perhaps due to a facilitatory influence of heightened arousal. This decrease was greater contralateral to HFS, suggesting that HFS disrupted normal modulation of the nociceptive blink reflex.

**Dissociation between pressure-pain sensitivity and nociceptive blink reflexes**

Dissociation between ipsilateral forehead analgesia and heightened excitability of the ipsilateral nociceptive blink reflex was detected both in this and a previous study [89]. Pain originating from different tissues appears to activate
different brain circuits [36, 76, 88]. For example, stimulation of nociceptive afferents in muscle evokes greater activity in limbic circuits than stimulation of cutaneous nociceptive afferents [36, 76, 88]. Interestingly, dissociation between sharp and blunt pressure-pain is sometimes seen in patients with complex regional pain syndrome [19] and central post-stroke pain [57]. It would be interesting to determine whether adrenergic modulation of nociception is greater for superficial than deep sensations, or sharp than blunt sensations, both under normal conditions and in patients with chronic pain.

**Temple cooling**

Electrically-evoked forearm pain decreased substantially during and after noxious temple cooling, presumably due to generalised pain-inhibitory mechanisms such as stress-induced analgesia or DNIC. Decreases were greater during ipsilateral than contralateral temple cooling [90], suggesting that cold pain in the ipsilateral temple activated an ipsilateral pain-modulation mechanism. We hypothesised that yohimbine would oppose the reduction of forearm pain during ipsilateral temple cooling by blocking an inhibitory mechanism mediated by α2-adrenoceptors [84,85]. Instead, the yohimbine pre-treatment facilitated analgesia.

Under certain conditions, yohimbine may enhance rather than oppose antinociceptive effects. For example, Chung et al. [15] noted that yohimbine reduced the intensity and unpleasantness of chronic back pain whereas placebo treatment did not. Numerous animal and human studies have demonstrated that opioid receptor antagonists increase pain behaviours by opposing stress-induced analgesia and DNIC [8, 25, 26, 50, 72, 77]. Furthermore, opioid and adrenergic pain modulation
processes appear to interact synergistically [12, 30]. In particular, blocking one of these mechanisms may up-regulate anti-nociceptive effects elicited by the other, further contributing to hypoalgesia. For example, Gear et al. [28] reported that yohimbine alone did not elicit hyperalgesic effects but reduced pain when combined with morphine. These synergistic interactions result in cross-tolerance and cross-dependence between the anti-nociceptive actions of µ-opioid and α₂-adrenoceptor agonists [1], and may be linked with reciprocal connectivity between noradrenergic nuclei and opioidergic projections from the periaqueductal gray [2, 48]. Thus, it is tempting to speculate that the analgesic effects of yohimbine pre-treatment were due to compensatory µ-opioid effects following blockade of α₂-adrenoceptors.

Methodological Considerations

Care must be taken in comparing our findings with those of other studies involving different yohimbine administration schedules and dosage [5, 28, 38]. For example, differential yohimbine administration schedule and dosage would imply that yohimbine bioavailability would differ significantly between our study and others [28]. According to Sturgill et al. [74], after an average of approximately 24 minutes, maximum plasma concentration would be reached for both single doses of 5.4 mg and 16.2 mg oral yohimbine in healthy participants. However, maximum plasma concentration for the 16.2 mg dose was significantly greater compared to that of the 5.4 mg dose (400 ± 314 nanogram/millilitre versus 50.9 ± 46.1 nanogram/millitre). In addition, repeated 5.4 mg doses administered three consecutive days preoperatively and every six hours on the surgery day [28] would alter plasma concentration significantly more than a single dose. Similarly, the influence of yohimbine on pain sensitivity elicited by HFS conditioning could also
differ from real life post-operative pain examined by Gear et al. [28]. Another consideration could involve the yohimbine dose. Bruehl et al. [5] and Holmberg and Gershon [38] respectively administered 0.4 mg and 0.5 mg of yohimbine per kilogram of body weight intravenously, which equates to 28 mg and 35 mg respectively for a 70-kg person. Based on their studies, 16 mg of yohimbine appeared insufficient for our sample with an average of 70 kg. Nevertheless, this relatively low dose of yohimbine was administered in the present study to minimise nonspecific effects mediated by blockade of α₁-adrenoceptors [32] and serotonin receptors [81]. Nevertheless, we are confident that the 16 mg oral dose was sufficient to block α₂-adrenoceptors as it provoked increases in autonomic activity and enhanced the excitability of the ipsilateral nociceptive blink reflex.

In healthy individuals, an adrenergic influence on the autonomic control of blood pressure may attenuate pain due to activation of a pain-inhibitory baroreflex mechanism [5, 15, 27]. However, there was no association between elevated systolic blood pressure and pain in the present study, possibly because most of the noxious stimuli were mild and brief. Nonetheless, it remains possible that blood pressure-related hypoalgesia and/or stress-induced analgesia associated with the anxiogenic effects of yohimbine masked the pro-nociceptive effects of yohimbine. Our sample consisted of 22 participants. Stronger yohimbine effects may have been detected in a larger sample size.

Conclusions

The current findings suggest that α₂-adrenoceptors are involved both in anti-nociceptive and pro-nociceptive processes. Although further research is required to
clarify the site and mode of action of these opposing effects, the existence of a supraspinal pro-nociceptive influence might explain why systemic administration of $\alpha_2$-adrenoceptor agents is less successful than local or spinal administration for treating post-operative and neuropathic pain [9, 10, 21, 22]. Our findings also suggest that non-adrenergic (possibly $\mu$-opioid) mechanisms mediate ipsilateral forehead analgesia following HFS. Understanding this ipsilateral pain modulation process is important, as it may fail in complex regional pain syndrome [19].
Acknowledgements

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References


<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td></td>
<td>Start Time (minutes)</td>
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<tr>
<td>Pre-drug</td>
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<tr>
<td></td>
<td>10</td>
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<tr>
<td></td>
<td>20</td>
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<tr>
<td></td>
<td>25</td>
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<tr>
<td>Post-drug</td>
<td>35</td>
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<tr>
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<td>95</td>
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<tr>
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Table 2 Differences between the yohimbine (Y) and placebo (P) conditions

<table>
<thead>
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<th>Variable</th>
<th>Expected result</th>
<th>Actual result</th>
<th>Interpretation</th>
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<tr>
<td>Systolic and diastolic blood pressure after drug administration</td>
<td>$Y &gt; P$</td>
<td>$Y &gt; P$</td>
<td>$\alpha_2$-adrenoceptor blockade facilitated autonomic activity</td>
</tr>
<tr>
<td>Electrodermal activity after drug administration</td>
<td>$Y &gt; P$</td>
<td>$Y &gt; P$</td>
<td>$\alpha_2$-adrenoceptor blockade facilitated autonomic activity</td>
</tr>
<tr>
<td>Development of primary and secondary hyperalgesia after HFS</td>
<td>$Y &gt; P$</td>
<td>$Y \leq P$</td>
<td>$\alpha_2$-adrenoceptor blockade had no effect on primary hyperalgesia evoked by HFS, and inhibited secondary hyperalgesia to pressure-pain</td>
</tr>
<tr>
<td>Development of ipsilateral forehead analgesia to pressure-pain after HFS</td>
<td>$Y &lt; P$</td>
<td>$Y = P$</td>
<td>$\alpha_2$-adrenoceptor blockade had no effect on ipsilateral forehead analgesia to pressure-pain</td>
</tr>
<tr>
<td>Pain ratings to supraorbital stimulation after drug administration and HFS</td>
<td>$Y &gt; P$</td>
<td>$Y &gt; P$</td>
<td>$\alpha_2$-adrenoceptor blockade mediated pro-nociceptive effects</td>
</tr>
<tr>
<td>Ipsilateral nociceptive blink reflex amplitude after HFS</td>
<td>$Y &gt; P$</td>
<td>$Y &gt; P$</td>
<td>$\alpha_2$-adrenoceptor blockade mediated pro-nociceptive effects</td>
</tr>
<tr>
<td>Reduction of electrically-evoked forearm pain during ipsilateral temple cooling</td>
<td>$Y &lt; P$</td>
<td>$Y &gt; P$</td>
<td>$\alpha_2$-adrenoceptor blockade facilitated the anti-nociceptive effect of ipsilateral temple cooling on electrically-evoked forearm pain</td>
</tr>
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</table>
# Table S1

Cold pain rating ± S.E. in the temple during temple cooling

<table>
<thead>
<tr>
<th>Electrically-evoked pain</th>
<th>Temple Cooling</th>
<th>Yohimbine</th>
<th>Placebo</th>
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<tr>
<td>Test Site</td>
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<tr>
<td>Ipsilateral</td>
<td>6.56 ± 0.39</td>
<td>6.26 ± 0.47</td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>6.08 ± 0.33</td>
<td>5.97 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>Control Site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>6.14 ± 0.37</td>
<td>5.88 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>6.28 ± 0.41</td>
<td>6.19 ± 0.46</td>
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</table>
Figure 1  
Mean ± S.E. for (a) heart rate; (b) systolic blood pressure; and (c) diastolic blood pressure at baseline, after drug administration and after HFS conditioning in the yohimbine and placebo conditions. Basal heart rate was higher in the placebo than yohimbine condition, likely due to chance, but decreased after drug administration (# p < .05). Basal systolic and diastolic blood pressures were similar before yohimbine and placebo were administered. Blood pressure increased after administration of yohimbine (* p = .052 and # p < .05 compared with values at baseline and placebo). Neither blood pressure nor heart rate changed after HFS conditioning.
Figure 2   (a) Mean electrodermal activity (EDA) ± S.E. at baseline, after drug administration and after HFS conditioning in the yohimbine and placebo conditions during 2 minutes rest. (b) Increases in EDA during 5 deep breaths. At baseline, EDA at rest and during deep breathing was similar in the yohimbine and placebo conditions, but EDA at rest increased significantly after yohimbine was administered. EDA at rest increased after HFS conditioning in both conditions (* p < .05).
a. Sensitivity to heat

b. Sensitivity to pinprick

c. Pressure-pain thresholds
Figure 3  Mean forearm sensitivity ± S.E. at baseline, after drug administration and after HFS conditioning to (a) heat; (b) pinprick; and (c) pressure pain thresholds (PPT). In general, all sensations in the test and the control arms were similar at baseline, and remained stable after drug administration. After HFS conditioning, sensations differed significantly between the test and the control arms. (a) Heat sensitivity increased at HFS-conditioned site (* p < .001 compared to values before HFS), but decreased in the control arm (* p < .05). (b) Sharpness ratings to pinprick remained unchanged in the control arm but increased after HFS at primary and secondary sites in the test arm (* p < .001 compared to before HFS and in the control arm). (c) The PPT decreased after HFS at the primary site in the test arm but not in the control arm (* p < .01). In the placebo session, the PPT at the secondary site decreased in the test arm (* p < .05 compared to before HFS). In contrast, the PPT did not change at the secondary site in the yohimbine condition.
Figure 4  Mean sensitivity ± S.E. to (a) heat; (b) pinprick; and (c) pressure-pain in the ipsilateral and contralateral forehead at baseline, after drug administration, and after HFS conditioning. Sensitivity to heat and pinprick in the ipsilateral and contralateral forehead remained stable throughout the yohimbine and placebo conditions. Ipsilateral and contralateral pressure-pain thresholds (PPT) remained unchanged after yohimbine and placebo administration but increased significantly after HFS conditioning (# p < .01 compared with their respective PPT at baseline), with a greater increase on the ipsilateral side (* p < .001 compared with PPT on the contralateral side after HFS conditioning).
Figure 5  Mean sensitivity ± S.E. to supraorbital stimuli before and after HFS conditioning in the yohimbine and placebo conditions. Before HFS conditioning, sensitivity was similar on the ipsilateral and contralateral sides of the forehead. After HFS conditioning, sensitivity remained unchanged in the yohimbine condition but decreased in the placebo condition (* p < .05 and # p < .01).
Figure 6  Mean percentage change in R2 area under the curve (AUC) after HFS conditioning expressed in relation to R2 AUC before HFS conditioning for blink reflexes ipsilateral both to the HFS-treated site and supraorbital stimulation (R2ii), ipsilateral to the HFS-treated site and contralateral to supraorbital stimulation (R2ic), contralateral to the HFS-treated site and ipsilateral to supraorbital stimulation (R2ci), and contralateral both to the HFS-treated site and supraorbital stimulation (R2cc). After HFS conditioning, the R2 AUC for blink reflexes ipsilateral to the HFS-treated site (R2ii and R2ic) were greater in the yohimbine condition than the placebo condition. Error bars represent standard errors.
Figure 7  Mean R2 onset latency ± S.E. before and after HFS conditioning. Before HFS conditioning, the R2 onset latency was shorter on the side of supraorbital stimulation than the contralateral side, and was longer in the yohimbine than placebo condition (* p < .05). After HFS conditioning, R2 latency remained shorter on the side ipsilateral to supraorbital stimulation but decreased overall.
Figure 8  Pain ratings ± S.E. to electrical stimulation of the HFS-conditioned (test) and control sites in the forearms during ipsilateral and contralateral temple cooling. In the yohimbine condition, decreases were greater at both sites during ipsilateral than contralateral temple cooling (* p < .001 for the test site and p < .05 for the control site), and at the test site during the recovery period after the ipsilateral temple was cooled (* p < .01). In the placebo session, decreases were greater at the test site during ipsilateral than contralateral temple cooling (* p < .05).
Chapter 7 Conclusions

7.1. Summary of all studies

The purpose of this thesis was to examine the remote effects of unilateral experimentally-induced limb pain, in order to develop a better understanding of pain modulation processes, specifically hemilateral pain processing in healthy humans. In Study one [51], the effects of HFS and UVB conditioning on sensitivity to heat, sharpness, and pressure-pain in the conditioned forearm site and the control site in the contralateral forearm, and on each side of the forehead were compared in samples of 30 (HFS) and 16 (UVB) healthy participants, who were randomly selected from the HFS sample. In addition, the effects of ipsilateral versus contralateral temple cooling on electrically-evoked forearm pain following HFS and UVB conditioning were assessed. In line with past studies, UVB conditioning triggered signs of peripheral sensitization [4], whereas HFS conditioning triggered signs of central sensitization [32; 43]. Importantly, ipsilateral forehead analgesia developed after HFS but not UVB conditioning. Furthermore, reductions in electrically-evoked pain at the HFS-treated site were greater during ipsilateral than contralateral temple cooling, whereas pain reductions at the UVB-treated site were similar irrespective of the side that was cooled. Thus, HFS conditioning induced signs of central sensitization in the forearm and analgesia both in the ipsilateral forehead and the HFS-treated site. This ipsilateral analgesia was not due to peripheral sensitization or other non-specific effects, as it failed to develop after UVB conditioning. Thus, the supra-spinal mechanisms that evoke central
sensitization might also trigger a hemilateral inhibitory pain modulation process, which could sharpen the boundaries of central sensitization or limit its spread.

One limitation of Study one is that repeated testing or electrically evoked inflammatory responses might have contributed to sensory changes. However, the nonspecific effects of testing were controlled by comparing outcomes after HFS conditioning with those following UVB conditioning. Nevertheless, it would be interesting in future studies to re-investigate the effects of UVB irradiation on ipsilateral forehead analgesia and nociceptive blink reflex as intense signs of central sensitisation following UVB irradiation have been reported in recent studies [13; 25].

Although we minimised potential biases by keeping participants blind to the hypotheses, reliance on self-reported measures of pain was also another limitation of Study one. This limitation was addressed in Study two [52] in which we examined the effects of HFS on the nociceptive blink reflex to supraorbital stimulation, which is an objective assessment of activity in human trigeminal pain pathways [22; 30]. The nociceptive blink reflex was assessed on each side of the forehead with and without counter-irritation using electrically-evoked forearm pain. In addition, sensory changes in the forearm and forehead before and after HFS conditioning were assessed. In line with Study one, secondary hyperalgesia in the forearm and analgesia to pressure-pain in the ipsilateral forehead developed after HFS. Pain perception and the amplitude of the blink reflex to supraorbital stimuli were suppressed both by forearm counter-irritation and HFS conditioning under most conditions. Importantly, however, in the absence of forearm counter-irritation, the amplitude of the nociceptive blink reflex ipsilateral to supraorbital stimuli delivered ipsilateral to the HFS-conditioned site increased after HFS conditioning. These
findings suggest a coexistence of hemilateral inhibitory and facilitatory influences on nociceptive processing following HFS conditioning. The inhibitory influence may help to limit the spread of sensitisation in central nociceptive pathways. However, the heightened excitability of the nociceptive blink reflex ipsilateral to HFS-conditioned site was unexpected and is worthy of further exploration in healthy as well as in chronic pain populations such as complex regional pain syndrome (CRPS).

Although the nociceptive blink reflex provides an objective assessment of trigeminal nociception, it predominantly involves stimulation of cutaneous A-δ nociceptors. Whether methods that allow stimulation of deeply-sited nociceptors would prove to be more suitable than superficial stimulation as a neurophysiological correlate of the ipsilateral forehead analgesia to blunt pressure after HFS requires further investigation.

In Study Three [53], we wanted to ascertain whether central sensitisation is necessary for the development of ipsilateral forehead analgesia by assessing the effects of HFS versus LFS conditioning on sensory changes at the conditioned site and the contralateral control site, and on each side of the forehead in samples of 50 (HFS), being pooled from Study One [51] and Study Two [52], and 18 (LFS) participants. Pain was more intense after HFS than LFS, and signs of central sensitisation developed locally after HFS but not LFS. However, sensitivity to pressure-pain decreased in the ipsilateral forehead not only after HFS but also LFS. This reduction was associated with reduced heat sensitivity around the site of HFS, and a heightened sensitivity to pressure-pain at the electrically-conditioned forearm site. These findings suggest that an ipsilateral pain-inhibitory process triggered by electrical stimulation of the forearm might have suppressed ipsilateral forehead
sensitivity to pressure-pain and secondary hyperalgesia to heat. It would be worthwhile in future studies to determine whether intradermal LFS elicits similar effects on human pain modulation to transcutaneous LFS and HFS, as it is known to produce secondary hyperalgesia [9; 36; 37].

The failure to induce ipsilateral forehead analgesia to pressure-pain following UVB irradiation suggests that peripheral sensitisation caused by UVB was not sufficient to evoke central descending pain-inhibitory mechanisms. Interestingly, an unpublished study in our laboratory detected ipsilateral forehead hyperalgesia 2 days following UVB-irradiation of human forearm skin, possibly suggesting that UVB might have triggered descending pain-facilitatory mechanism(s). LFS did not induce peripheral sensitisation in our study. Instead, hypoalgesia developed at the site of stimulation possibly due to long-term depression in spinal nociceptive pathways [31]. The hypoalgesia at the site of stimulation, the lack of secondary hyperalgesia and the development of ipsilateral forehead analgesia to pressure-pain following LFS suggests that LFS triggered stronger pain-inhibitory than pain-facilitatory effects. In addition, UVB is known to activate primarily heat-sensitive nociceptors [4], whereas LFS might have stimulated a broad range of peripheral nerve fibres, further supporting the speculation that LFS and UVB triggered different central pain-modulatory mechanisms.

Reports of an increase in noradrenaline in the ipsilateral dorsal horn [48], and the ipsilateral spread of pain in rats with bilateral locus coeruleus lesions following inflammation in rats [49] but not contralaterally, suggest noradrenergic influences on ipsilateral pain-inhibitory modulation. We speculated that a similar hemilateral noradrenergic pain-inhibitory mechanism may contribute to the ipsilateral forehead
analgesia to pressure-pain in healthy humans following HFS [51; 52; 53]. To investigate this, in Study four, we administered oral yohimbine, an $\alpha_2$-adrenoreceptor antagonist, in a double-blind placebo-controlled crossover design to 22 male and female participants. Sensory changes to heat, sharpness and pressure-pain at the conditioned site and the contralateral control site were assessed at baseline, after yohimbine, and after HFS conditioning. Changes in blood pressure, heart rate and electrodermal activity were also assessed across these three stages. Nociceptive blink reflexes to supraorbital stimulation were also examined on each side of the forehead following yohimbine and after HFS conditioning. The effects of cold pain on each temple on electrically-evoked forearm pain were also assessed following HFS.

In line with our previous studies, in the placebo condition, HFS evoked primary and secondary hyperalgesia in the forearm, ipsilateral forehead analgesia to pressure-pain [51; 52; 53] and a greater reduction of electrically-evoked forearm pain during ipsilateral than contralateral temple cooling [51]. However, the findings were mixed in the yohimbine condition. Yohimbine increased blood pressure and electrodermal activity, consistent with yohimbine increasing sympathetic activity [24]. Yohimbine also significantly enhanced the excitability of the ipsilateral nociceptive blink reflex in comparison to placebo, consistent with a pro-nociceptive effect of yohimbine [41]. Interestingly, however, yohimbine may also enhance analgesia [5; 11; 21] as decreases in electrically-evoked forearm pain evoked by ipsilateral temple cooling were greater in the yohimbine than placebo condition at the HFS-treated site. This analgesia could be due to the compensatory effects of endogenous opioid activity following $\alpha_2$-adrenoreceptor blockade [7; 23]. Thus, to
elucidate ipsilateral forehead analgesia to pressure-pain following HFS, future research should consider blocking both noradrenergic and opioidergic mechanisms.

Simultaneous development of ipsilateral forehead analgesia to pressure-pain and an enhanced excitability of the nociceptive blink reflex following HFS suggest possible involvement of descending noradrenergic mechanisms both in pain-inhibitory and pain-facilitatory controls, mediated by α2-adrenoreceptors [12; 28; 29] and α1-adrenoreceptors [16] respectively. Additionally, this may implicate some interacting and/or competing effects between central α2-adrenoreceptors and α1-adrenoreceptors on normal pain modulation following HFS. Our recommendation of combined opioid and α2-adrenoreceptor blockade would shed more light into the role of α2-adrenoreceptors and endogenous opioids in normal pain modulation. Inhibition of the ipsilateral forehead analgesia by α2-adrenoreceptors and endogenous opioids blockade following HFS implicates the involvement of α2-adrenoreceptors and endogenous opioids in inhibiting pain. Thus, the next logical step would be to simultaneously block α2-adrenoreceptors and endogenous opioids. Finally, a subsequent step would be to determine whether α1-adrenoreceptor blockade would inhibit pain in this model and in chronic pain disorders such as CRPS [1], post-herpetic neuralgia [10], post-amputation pain [40] and fibromyalgia [42], in which α1-adrenoreceptors appear to play a part in facilitating pain [14; 15; 16] (refer to Coeruleopsinal Pain Modulation in Chapter 1 for further discussion on the adrenergic influence in pain-facilitation in CRPS).

Some methodological considerations apply to Study four. First, administration route, dosage, and frequency of administration of yohimbine influence the plasma concentration of yohimbine [47]. Thus, care must be taken
when comparing our results with other studies involving yohimbine. Additionally, pain induced by HFS differs to real life post-operative pain [21] and pain induction methods used in other studies involving yohimbine. Furthermore, the considerable length of the Study four (3.5 hours) could elicit anxiety symptoms, which potentially could influence autonomic activity. However, this seems unlikely because neither blood pressure nor heart rate increased significantly over the course of the experiment in the placebo condition.

Finally, one limitation that applies to all studies in this thesis is that as most of our participants were young and well educated, the findings might not generalise to other populations. However, the development of analgesia in the ipsilateral forehead was previously found to be unrelated to the participant’s age [33].

7.2. Implications for non-CRPS chronic pain and CRPS

The coexistence of central sensitisation and ipsilateral forehead analgesia to pressure-pain following HFS led us to hypothesise that there was an association between these two processes [51]. However, detection of this ipsilateral forehead analgesia despite LFS failing to produce signs of central sensitisation (Study Three [53]) indicated that these two processes are independent. In other words, descending pain-facilitatory mechanisms seem to be independent from mechanisms that modulate pain intensity and inhibit pain. Simultaneous development of analgesia to pressure-pain and enhanced nociceptive blink reflex in the ipsilateral forehead following HFS in Study Two, which was replicated in Study Four even after attempting to block the effects of $\alpha_2$-adrenoreceptors with yohimbine, further supports this hypothesis. A recent study reported that healthy humans adapted to
pain evoked by noxious electrical stimulation but simultaneously developed secondary hyperalgesia in nearby skin [46]. On the other hand, CRPS patients were less adaptive to pain evoked by this same method of stimulation and developed a larger area of secondary hyperalgesia, indicating a shift from pain-inhibition to pain-facilitation of nociceptive inputs [46]. Nevertheless, these findings indicate that descending pain-inhibitory and pain-facilitatory mechanisms appear to exist independently both in pain-free humans and in CRPS patients. Further clarification of their relationship is important as it may open additional avenues for treating chronic pain.

Continual activation of pain-inhibitory mechanisms during chronic pain exhausts endogenous opioid reserves and ironically further disrupts pain-inhibitory modulation [6], which has been observed in many types of chronic pain [55, 56]. Hypothetically, if central sensitisation, a dominant characteristic observed in many types of chronic pain [2], is not necessary for the activation of descending pain-inhibitory modulation then treatment for chronic pain should primarily focus on boosting pain-inhibitory mechanisms. Conversely, if they coexist independently, then chronic pain treatment should focus both on enhancing pain-inhibitory mechanisms and reducing signs of central sensitisation (desensitising the peripheral and central nervous systems to nociceptive input [2]). However, shifting from pain-inhibition to pain-facilitation in chronic pain is well-documented in pain research [2, 44, 46]. Thus, an ideal treatment for chronic pain needs to be a multimodel approach which should focus firstly on reducing central pain-facilitatory effects and signs of central sensitisation, and secondly on improving the functioning of central pain-inhibitory mechanisms.
Knudsen et al. (2011) [35] identified ipsilateral forehead hyperalgesia to pressure-pain in 14% of patients with other chronic pain (neuropathic pain, postherpetic neuralgia) compared to 59% of CRPS patients. Similar to CRPS patients with ipsilateral forehead hyperalgesia, ipsilateral forehead hyperalgesia in non-CRPS patients is associated with hypersensitivity to sharpness and swelling at the pain site [35], suggesting that constant peripheral nociceptive input at the affected site may contribute to the ipsilateral spread of pain in some chronic pain patients [35]. This finding suggests that hemilateral pain-inhibitory modulation may also be disrupted in a small proportion of chronic pain patients. Speculatively, weak or disrupted hemilateral pain-inhibitory modulation would suggest that chronic pain patients with ipsilateral forehead hyperalgesia could be more susceptible to develop CRPS. Whether any of these chronic pain patients would develop CRPS symptoms is worth investigating in future studies. Hemilateral pain modulation may also be important in migraine headache as two-thirds of migraine headache sufferers develop a migraine headache unilaterally [39]. The mechanism(s) that triggers episodes of unilateral migraine headache is unclear but may possibly involve intermittent failure of a hemilateral pain-inhibitory mechanism. Further examination of the involvement of hemilateral pain modulatory processes in chronic pain is recommended for future research.

Despite methodological issues, this thesis revealed some findings in a healthy population that differed significantly from those observed in CRPS. Firstly, in healthy humans, HFS conditioning [51; 52; 53; Study four] and LFS (Study three [53]) reduced sensitivity to pressure-pain on both sides of the forehead. Bilateral forehead analgesia to pressure-pain was also observed following noxious cold
stimulation and topical capsaicin in healthy participants [33; 34]. Furthermore, cold pain in the temple inhibited electrically evoked limb pain, irrespective of HFS [51; Study four] or UVB conditioning [51]. These widespread inhibitory effects following noxious stimulation elsewhere in the body may be mediated by stress-induced analgesia [54] or DNIC [50]. However, in CRPS patients, noxious cold stimulation of the unaffected limb only modestly reduced CRPS pain and forehead pressure-pain sensitivity compared to healthy humans, whereas noxious cold stimulation of the CRPS-affected limb increased rather than decreased forehead sensitivity to pressure-pain [35]. Furthermore, cold pain in the forehead increased CRPS pain [18]. Interestingly, Seifert et al. [46] observed that CRPS patients adapted to pain more slowly than healthy controls in relation to slower decreases in pain sensitivity to noxious electrical stimulation in the affected and unaffected limbs compared to healthy controls. In contrast, the area of hyperalgesia to tactile stimuli in the CRPS-affected limb was larger than in the unaffected limb and those in healthy controls [46]. These findings together indicate a decrease in function of descending inhibitory pain-control processes in CRPS.

Secondly, one of the dominant features of CRPS is the hypersensitivity to pressure-pain in the forehead side ipsilateral to the affected limb [17; 35]. In pain-free healthy humans, sensitivity to pressure-pain did not differ between the ipsilateral and contralateral sides of the forehead [33; 34; 51; 52; 53; Study four]. Furthermore, following HFS [51; 52; 53; Study four] and LFS conditioning (Study three [53]), sensitivity to pressure-pain decreased on both sides of the forehead, with a greater reduction on the ipsilateral side. Similar effects were also observed following noxious cold stimulation [33] and topical capsaicin treatment [34]. In addition, cold
pain in the ipsilateral temple reduced electrically-evoked pain at the HFS-treated site more effectively than cold pain in the contralateral temple following HFS [51; Study four]. In contrast, increases in pain were greater in CRPS-affected limb when cooling the ipsilateral than contralateral temple [18].

As previously discussed, the ipsilateral forehead analgesic response observed in healthy volunteers following peripheral electrical stimulation [51; 52; 53; Study four], noxious cold stimulation, and topical capsaicin [33; 34] may be mediated by ipsilateral noradrenergic pain-inhibitory mechanisms descending from the locus coeruleus. This mechanism could be disrupted in CRPS.

There is further evidence to suggest that descending noradrenergic pain-inhibitory control could be compromised in CRPS. Knudsen et al. [35] observed an increase in CRPS-affected limb pain and greater auditory discomfort when patients were startled with a loud noise in the ipsilateral ear compared to when the loud noise was presented in the contralateral ear, consistent with a facilitatory response in the cochlear nucleus and/or the auditory cortex. Activation of the sympathetic nervous system by the ipsilateral startle stimulus potentially could trigger a shift towards facilitation from the LC, which could potentiate nociceptive responses in the dorsal horn [26; 27] and cochlear nucleus [8; 20; 38; 45] by acting on excitatory $\alpha_1$-adrenoreceptors.

Finally, yohimbine failed to inhibit the ipsilateral forehead analgesia and enhanced analgesia as demonstrated by a greater reduction of electrically-evoked forearm pain during ipsilateral temple cooling following HFS (Study four). This indicates possible involvement of other non-noradrenergic pain modulation systems,
predominantly the endogenous opioids, which are known for their significant contribution to descending pain-inhibitory control in healthy humans [3]. In chronic pain patients, continual activation of descending pain-inhibitory control by persistent pain may cause opioid tolerance and exhaust endogenous opioid sources [6]. It is tempting to speculate that endogenous adrenergic and/or opioid systems are compromised in CRPS, which in turn could be a key factor that underlies dysfunction in descending inhibitory pain-control mechanisms.
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