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Original Article

**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 VAS) 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 et al, 1997; Inui et al., 2000). 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).

R2 onset latency

R2 onset latency was shorter ipsilateral than contralateral to supraorbital stimulation ($43.4 \pm 1.2 \text{ ms versus } 46.1 \pm 1.4 \text{ ms, } F(1, 16) = 36.52, p < .001$) 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, R2 latency decreased bilaterally during electrically-evoked forearm pain (Time x Counter-irritation interaction $F(1, 16) = 12.44, p < .01$) (Fig. 5).

R2 AUC

In general, R2 AUC decreased over the course of the experiment (Fig. 6). To investigate this statistically, changes in R2 AUC were normalised in relation to the R2 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 $\alpha_2$-adrenoreceptors at all segmental levels (Bouhassira et al., 1987; Jones and Gebhart, 1986a; b; Rahman et al., 2008; Sluka and Westlund, 1992). 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.
Author contributions

Both authors made substantial contributions to the study conception and design, statistical analysis and interpretation of data; 2) drafting and revising the article; 3) and gave final approval of the version to be published. Lechi Vo was also responsible for the acquisition of data.
References


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**Figures Legends**

**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.

**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).
Pain Ratings to Supraorbital Stimuli

(a) Without Forearm Pain  With Forearm Pain
   Baseline  After HFS Conditioning

(b) Without Forearm Pain  With Forearm Pain
   Baseline  After HFS Conditioning
R2 Latency

- Without counter-irritation
- With counter-irritation