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

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Running title: Hemilateral 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^{o}C or repeated immersions at 4^{o}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 pain-facilitatory bulbo-spinal pathways that mediate the spread of pain and 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 pain processing.

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^{o}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^{o}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/cm^2 (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/cm^2 to 0.6 J/cm^2 (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 ± 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 α_2-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).

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.

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Figure Legends

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.

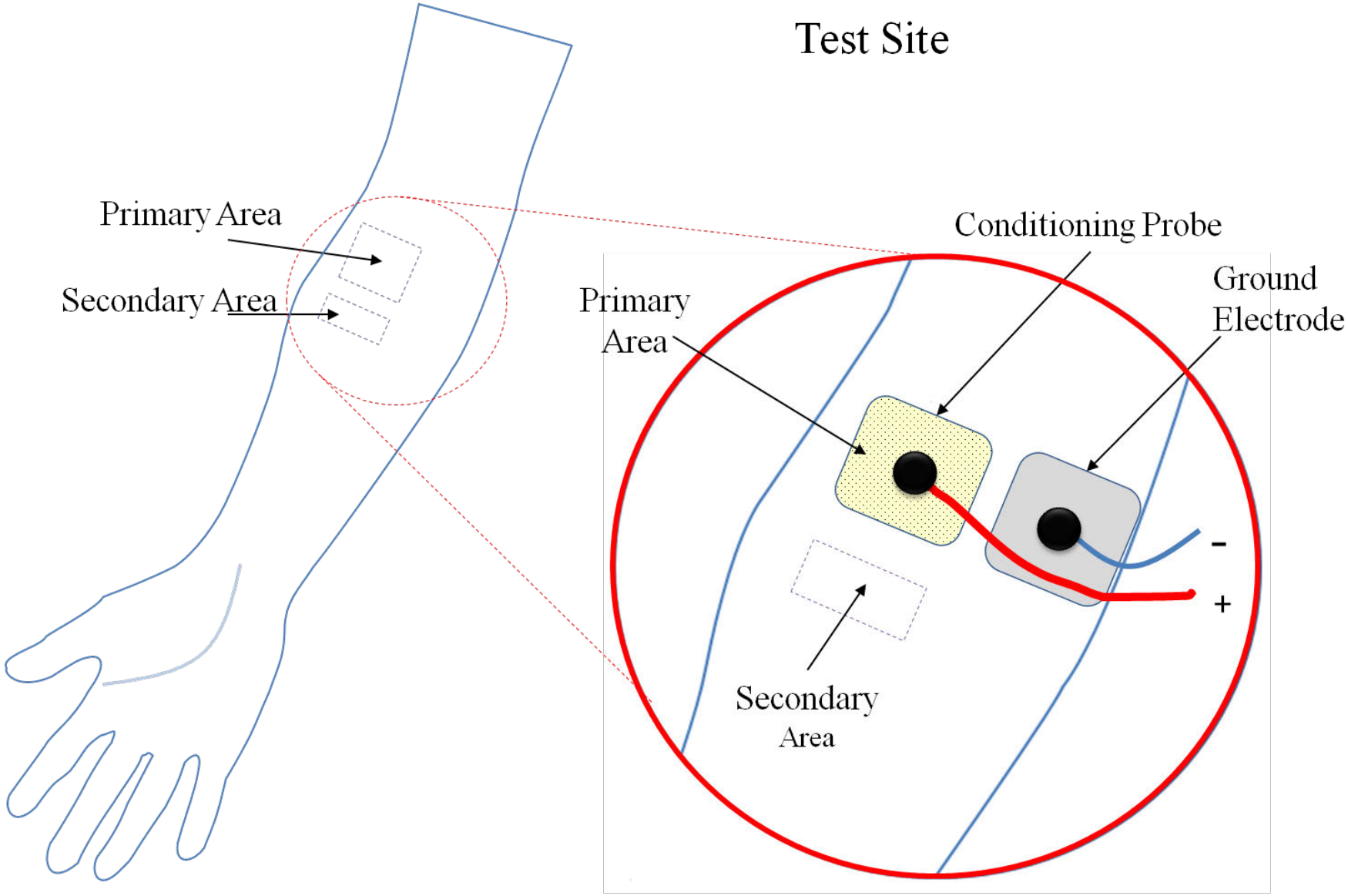
Figure 2. Mean sensitivity ± S.E. to (a) heat; (b) pinprick; (c) von Frey's monofilament; and (d) pressure-pain in the Primary and Secondary Areas before and after conditioning with HFS or UVB. Sensitivity to each stimulus increased significantly in the Primary Area after HFS- and UVB conditioning (* $p < .05$ 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.

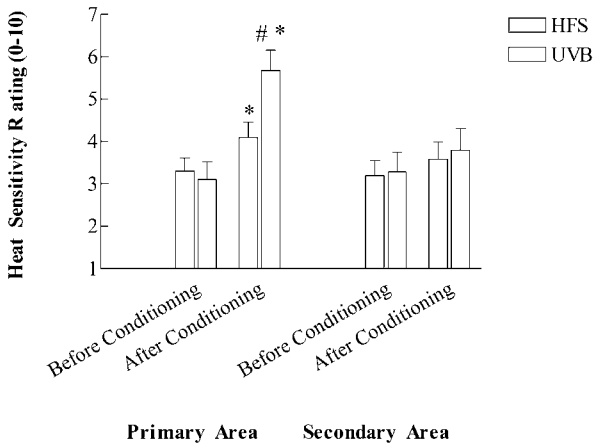
Figure 4. The effect of ipsilateral and contralateral temple cooling on electrically-evoked pain at (a) the HFS-treated site; (b) the HFS control site; (c) the UVB-treated site; and (d) the UVB control site. Ice was applied to each temple for 30 seconds. Pain ratings decreased significantly at treated and control sites during the indicated time intervals compared with a control task when ice was not applied (* $p < .05$). In addition, pain at the HFS-treated site (a) decreased significantly during ipsilateral temple cooling compared with 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.

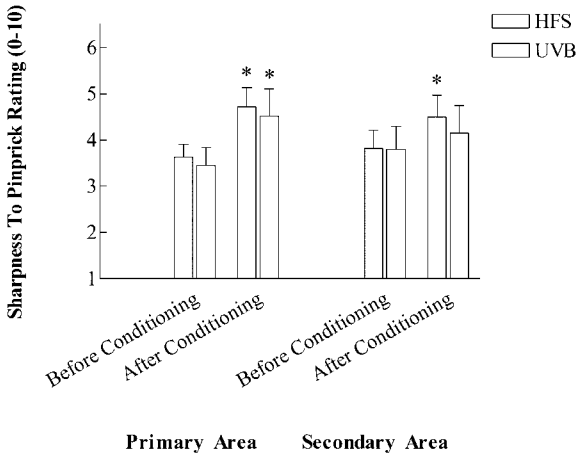
Test Site


Primary Area
Secondary Area
Conditioning Probe
Ground Electrode
Primary Area
Secondary Area
–
+

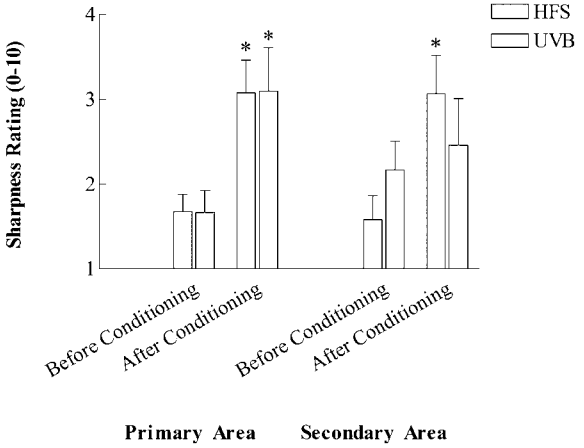
a. Heat Sensitivity



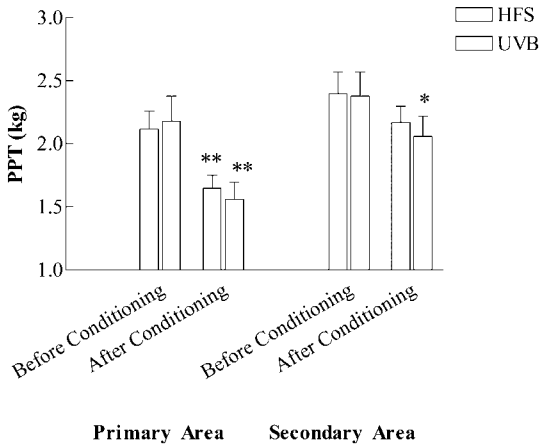
b. Sharpness Rating to Pinprick



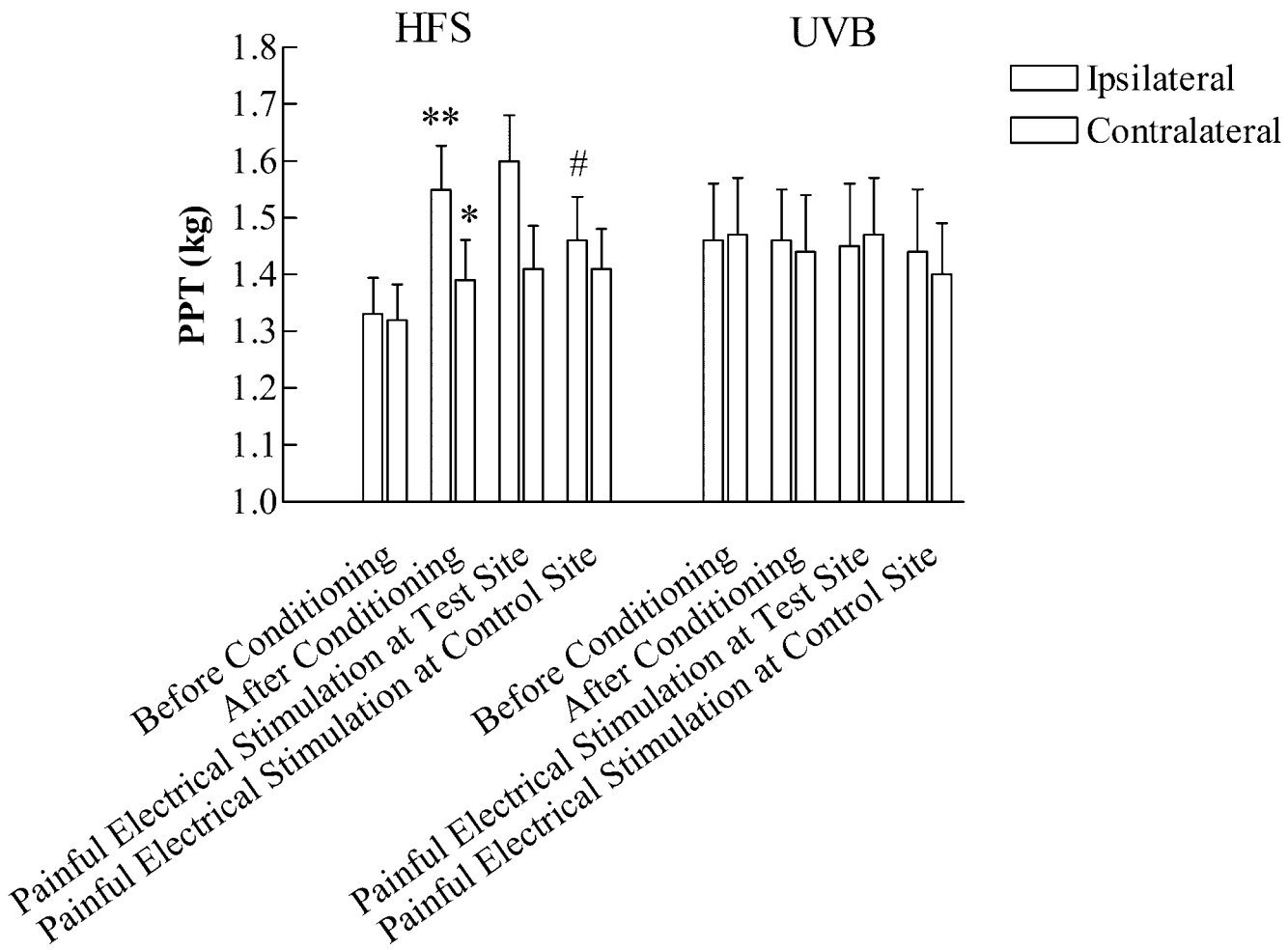
c. Sharpness Rating to Von Frey's Monofilament

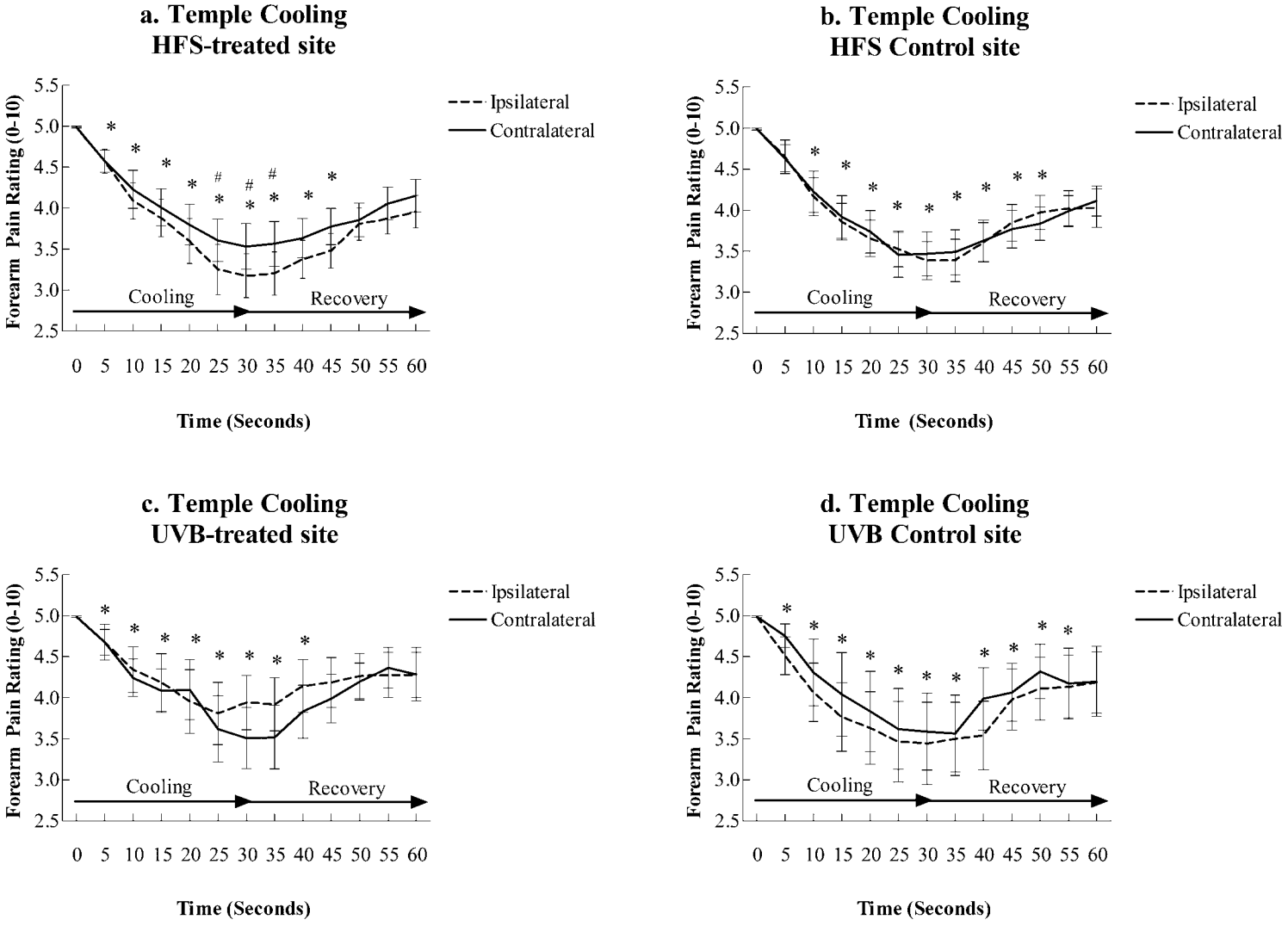


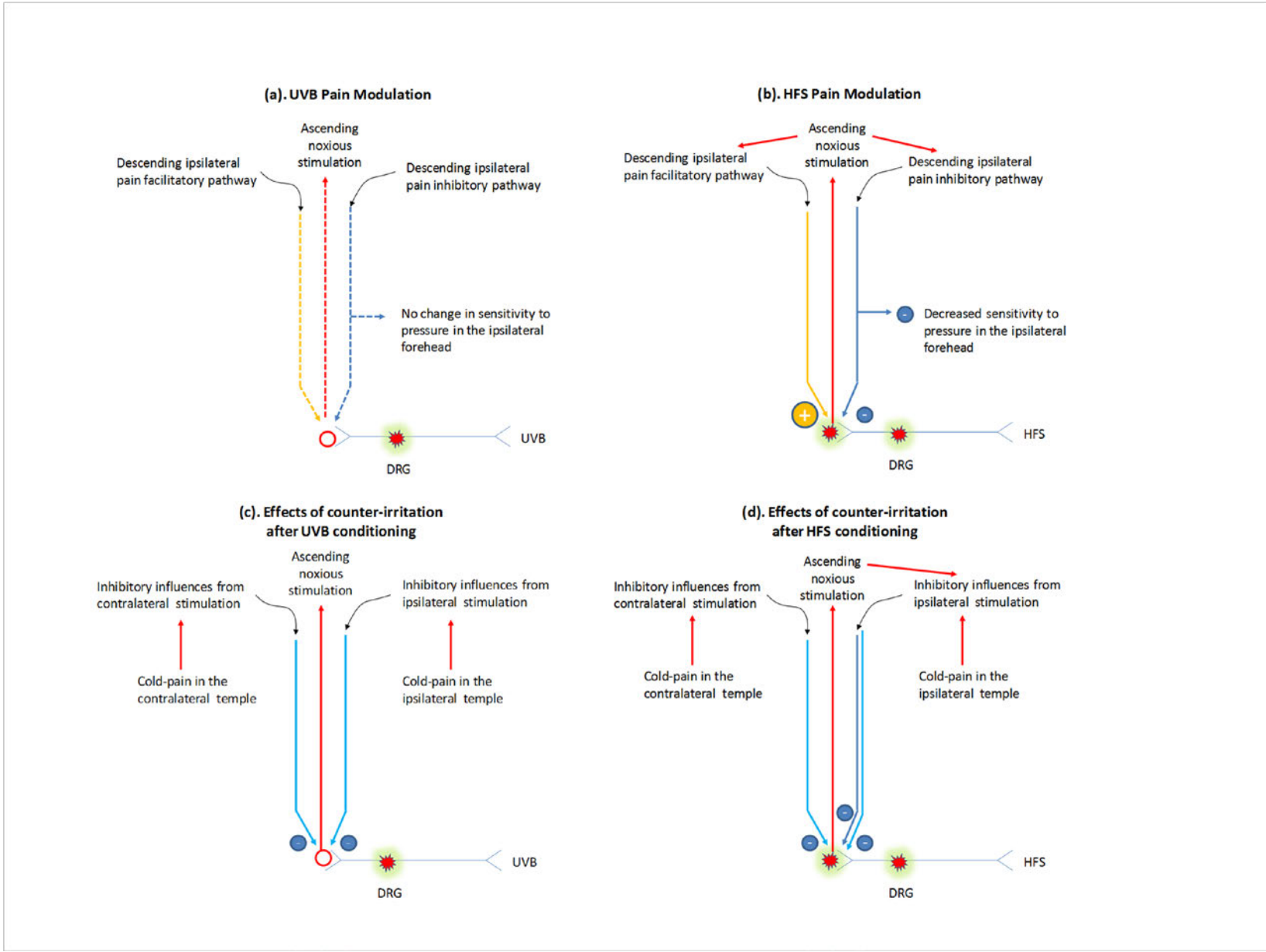
d. Forearm Pressure-Pain Thresholds



Forehead Pressure-Pain Thresholds




a. Temple Cooling
HFS-treated site
b. Temple Cooling
HFS Control site
c. Temple Cooling
UVB-treated site
d. Temple Cooling
UVB Control site
Forearm Pain Rating (0-10)
Time (Seconds)
Ipsilateral
Contralateral
Cooling
Recovery
0 5 10 15 20 25 30 35 40 45 50 55 60
2.5 3.0 3.5 4.0 4.5 5.0 5.5


(a). UVB Pain Modulation
Ascending noxious stimulation
Descending ipsilateral pain facilitatory pathway
Descending ipsilateral pain inhibitory pathway
No change in sensitivity to pressure in the ipsilateral forehead
UVB
DRG
(b). HFS Pain Modulation
Ascending noxious stimulation
Descending ipsilateral pain facilitatory pathway
Descending ipsilateral pain inhibitory pathway
Decreased sensitivity to pressure in the ipsilateral forehead
HFS
DRG
(c). Effects of counter-irritation after UVB conditioning
Ascending noxious stimulation
Inhibitory influences from contralateral stimulation
Inhibitory influences from ipsilateral stimulation
Cold-pain in the contralateral temple
Cold-pain in the ipsilateral temple
UVB
DRG
(d). Effects of counter-irritation after HFS conditioning
Ascending noxious stimulation
Inhibitory influences from contralateral stimulation
Inhibitory influences from ipsilateral stimulation
Cold-pain in the contralateral temple
Cold-pain in the ipsilateral temple
HFS
DRG