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 $\alpha_2$-adrenoreceptors. Therefore, in the final study (Study four), to determine whether ipsilateral forehead analgesia to HFS is mediated by $\alpha_2$-adrenoreceptors, we attempted to block their effects with oral administration of yohimbine, an $\alpha_2$-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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There are many people I’d like to thank, as without them this thesis would not be possible. Firstly, I’d like to thank Professor Peter Drummond, my principle supervisor. I admire not only his exceptional expertise and knowledge in the field but also many of his personal qualities - his work ethics, his passion and dedication to research. Thank you Peter for always making time for me, for having such an open mind, for listening to my ideas and my ‘babblings’ about a topic that I knew little about, for your endless patience, and most of all for your support over the past 3 years.

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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.
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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)
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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).