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Title page

(i) Altered nociceptive C fibre input to primary somatosensory cortex in an animal model of hyperalgesia

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Abstract

Evaluating potentially analgesic effects of drugs and various treatments is critically dependent on valid animal models of pain. Since primary somatosensory (SI) cortex is likely to play an important role in processing sensory aspects of pain, we here assess whether monitoring SI cortex nociceptive C fibre evoked potentials can provide useful information about central changes related to hyperalgesia in rats. Recordings of tactile and CO₂-laser C fibre evoked potentials (LCEPs) in forelimb and hind limb SI cortex were made 20–24 h after UV-B irradiation of the heel at a dose that produced behavioural signs of hyperalgesia.

LCEPs from irradiated skin increased significantly in duration but showed no significant change in magnitude, measured as area under curve (AUC). By contrast, LCEPs in hind limb SI cortex from skin sites nearby the irradiated skin showed no increase in duration or onset latency but increased significantly in magnitude after UV-B irradiation. The LCEPs in forelimb or hind limb SI cortex elicited from forelimb skin did not change in magnitude, but were significantly delayed in hind limb SI cortex. Tramadol, a centrally acting analgesic known to reduce hyperalgesia, induced changes that counteracted the changes produced by UV-B irradiation on transmission to SI cortex from the hind paw, but had no significant effect on time course of LCEPs from forelimb skin. Tactile evoked potentials were not affected by UV-B irradiation or tramadol. We conclude that altered sensory processing related to hyperalgesia is reflected in altered LCEPs in SI cortex.

1. Introduction

To develop new analgesics, appropriate animal models of pain are crucial. The current models are based primarily on measuring the changes in motor responses (Sandkuhler and Gebhart, 1984; McMahon *et al.*, 1991; Koltzenburg *et al.*, 1994; Yeomans *et al.*, 1996; Valle *et al.*, 2000; Davies *et al.*, 2005; Bishop *et al.*, 2007; Munro *et al.*, 2008; Saade *et al.*, 2008). Because the nociceptive input to motor systems and to sensory systems are channelled through at least partly different central pathways, with different physiological and pharmacological properties (Weng and Schouenborg, 1996), the validity of motor responses in predicting sensory aspects of pain and analgesia is ambiguous (Kalliomaki *et al.*, 1993b; Weng and Schouenborg, 1996; Palecek *et al.*, 2002). To develop new and effective analgesics, it is therefore crucial to develop supplementary animal models that provide assessments of the activity in the brain regions involved in the sensory aspects of pain. The primary somatosensory (SI) cortex receives strong and somatotopically organized nociceptive input in humans (Apkarian *et al.*, 2005) and animals (Lamour *et al.*, 1983; Kalliomaki *et al.*, 1993a; Chang *et al.*, 2008; Qiao *et al.*, 2008). In humans, nociceptive evoked potentials in the SI cortex correlate with pain sensation in the normal (Schnitzler and Ploner, 2000) and analgesic situation (Kochs *et al.*, 1990), and sometimes after induction of hyperalgesia (Treede *et al.*, 2003). Although it is clear that other cortical areas also contribute to pain processing, SI cortex plays an important role in this aspect (Apkarian *et al.*, 2005; Lee *et al.*, 2008). Also, animal studies reveal the presence of a population of neurons, receiving nociceptive input, in the SI cortex. These show a graded response to graded nociceptive input from restricted receptive fields (Treede *et al.*, 2003), similar to many neurons in, for example, the ventral posterior lateral nucleus and the dorsal horn of the spinal cord.

Monitoring cortical potentials evoked by electrical or cutaneous CO₂ laser stimulation in animals has shown that nociceptive C fibres provide powerful input to SI cortex (Schouenborg *et al.*, 1986; Kalliomaki *et al.*, 1993b; Qiao *et al.*, 2008). This is mediated by multiple parallel spinal pathways in the rat (Schouenborg *et al.*, 1986; Kalliomaki *et al.*, 1993b). Notably, CO₂ laser C fibre evoked potentials (LCEPs) are reduced following morphine-induced spinal analgesia (Kalliomaki *et al.*, 1998) and increased in an NMDA-dependent way after spinal wind-up (Kalliomaki *et al.*, 2003). It is thus conceivable that rat LCEPs can be used to monitor pain related ascending transmission under various conditions. If this notion proves to be correct, LCEPs may provide a useful animal model for the assessment of potentially analgesic drugs.

Our aim was to evaluate whether hyperalgesia, induced by UV-B irradiation of the skin (Davies *et al.*, 2005; Bishop *et al.*, 2007; Saade *et al.*, 2008), is reflected in altered LCEPs in SI cortex. In addition, we examined whether tramadol hydrochloride, a centrally acting analgesic drug known to reduce hyperalgesia (Munro *et al.*, 2008), can counteract the changes in LCEPs following UV-B irradiation.

2. Methods

2.1. Ethical approval

Approval for the experiments was obtained in advance from the Lund/Malmoe local ethical committee on animal experiments, regulated by the code of regulations of the Swedish Board of Agriculture. These regulations, including directives from the European Union, follow the law on animal welfare legislated by the Swedish parliament. The County Administrative Board governs the implementation of the rules. Further, the experiments were in accordance with the policies and guidelines reported by Drummond (Drummond, 2009) and IASP (Zimmermann, 1983).

2.2. Animals used

Twenty seven Sprague-Dawley (Taconic, Denmark) rats weighing 215 ± 55 g were used whereof seventeen were used in the analysis of LCEPs. All rats received food and water *ad libitum* and were kept in a 12-hour day–night cycle at a constant environmental temperature of 21°C and 65% humidity. The animals were kept in the animal facilities of the Biomedical Center at Lund University and the experiments were carried out at the Section for Neuroscience. The facilities are approved by the Swedish Board of Agriculture.

2.3. Induction of hyperalgesia using 1.3 J cm^{-2} UV-B narrowband irradiation

The right hind paw, exposing the heel, was covered with a UV-blocking film from an FR-4 clad board (ELFA, Sweden) and tin foil covered with paper to protect the rat from UV exposure. Sixteen animals were irradiated with 1.3 J cm^{-2} on the right heel (exposure area $8 \text{ mm} \times 9 \text{ mm}$), using a Philips UV-B TL/01 narrowband lamp (PL-S 9W/01, $\lambda = 300\text{--}320 \text{ nm}$). This intensity has been reported to be just below the threshold for blistering (Bishop et al., 2007). Moreover, Bishop et al (2007) show that UV-B irradiation produces a skin inflammation and a dose-dependent hyperalgesic state. Before every exposure, the lamp was left on for 3 min to allow the UV-B intensity to stabilize. UV intensity was measured before every exposure using a Varicontrol UV/PDT meter and skin tester (Herbert Waldmann GmbH & Co. KG, Germany). Recordings of laser evoked potentials were commenced 20–24 h after irradiation. At this time, discrete to moderate redness of the irradiated skin, but no skin lesions or scarring, was seen on the irradiated skin in each rat, confirming an inflammatory process as has been shown by Bishop et al (2007). However, one rat later on developed a small blister on the UV-B irradiated skin and was therefore excluded from further analysis. Animals did not exhibit any obvious signs of distress while being handled or observed and appeared to groom normally.

2.4. Nociceptive withdrawal reflex

In order to verify that an effective dose of UV-B irradiation had been given and that the UV-B irradiated rats showed similar changes as have been reported previously at a reflex level (Bishop *et al.*, 2007), behavioural hyperalgesia was assessed before surgery by measuring the threshold of the nociceptive withdrawal reflex of the irradiated and contralateral heels in awake animals ($n = 14$). A radiant heat CO₂ laser (Irradia, Sweden; model 315M Superpulse, wavelength 10.6 μm , output power 10 W, beam diameter 3.0 mm; pulse length of 18–24 ms stimulation) was used. Loosely embedded

in a towel, the rat rested calmly, with no signs of distress, in the hands of our experienced laboratory technician while the experimenter operated the laser. The duration of the CO₂ laser pulse was increased in steps of 2 ms until the threshold, defined as a response in three of five trials, was reached.

2.5. Surgery and preparation for electrophysiology

The rats were anaesthetized with isoflurane (1.8–2.0% during surgery) in a mixture of 40% oxygen and 60% nitrous oxide. The adequacy of the depth of anaesthesia was assessed throughout the surgery by applying noxious pinch to check for reflexes or by monitoring blood pressure. The trachea was cannulated and the animals were artificially ventilated. The end-expiratory PCO₂ was monitored continuously. An infusion of 2.5–4.5 ml h⁻¹ of 5% glucose (50 mg ml⁻¹) in Ringer's acetate was given through the right jugular vein. Mean arterial blood pressure was monitored continuously in the left femoral artery. The rectal temperature was kept at 36.5–38.5 °C, using a feedback-regulated heating system. The spinous process of T11 was clamped and the chest lifted to facilitate ventilation. The rat's head was fixed by ear bars after administration of local analgesia (EMLA® salve 5%; eutectic mixture of 2.5% prilocaine and 2.5% lidocaine, AstraZeneca, Södertälje, Sweden) and a nose ring. Cerebrospinal fluid was drained between the base of the skull and the first cervical vertebra to reduce the risk of cortical oedema (Kalliomaki *et al.*, 1993a). A craniotomy exposing the left parietal cortex was made. The dura mater was cut and the surface covered with paraffin oil. Local infiltration of lignocaine (Xylocaine® 20 mg ml⁻¹ + 12.5 µg ml⁻¹ adrenaline, Dentsply Ltd, Addlestone, Weybridge, England) was made during all surgery to reduce the nociceptive input. After completed surgery, the muscle relaxant pancuronium bromide 0.2 ml (Pavulon® 2 mg ml⁻¹, Organon AB, Göteborg, Sweden) was given and thereafter 0.15 ml once every hour. Also, the isoflurane level was lowered to 0.8–0.9% in the same gas mixture as before. This anaesthetic level was characterized by an EEG dominated by 4–6-Hz waves. The EEG was recorded (recording sites same as used for recording of potentials below) for periods of 90 seconds using fine silver ball-tipped electrodes (~0.3 mm diameter) and analysed with Signal 3.05 software (Cambridge Electronic Design Limited, Cambridge, England). The sampling rate for EEG recordings was 500 Hz. Experiments were terminated after any signs of deterioration such as cortical oedema, hind paw oedema or a precipitous decline in expiratory PCO₂ (five rats were discarded on these criteria). At the end of the experiment, the rats were killed with an overdose of isoflurane in a mixture of 40% oxygen and 60% nitrous oxide. When the PCO₂ and blood pressure was 0, air was injected *i.v.*

2.6. Mappings of cutaneous representation on the SI cortex

The SI representations of tactile and nociceptive input from the same skin area overlap to a large extent, with the tactile input being more (Kalliomaki *et al.*, 1993a). Moreover, it is known that neurons receiving nociceptive input in SI cortex often also receive a tactile input from the same area on the skin (Lamour *et al.*, 1983; Kalliomaki *et al.*, 1993a). To avoid input from nociceptors as much as possible, which in itself may produce excitability changes in peripheral and central pathways, tactile input was used to locate the cortical representation of the glabrous skin of the arch and heel of the right hind paw (figure 1a) and of the digit area of the right forepaw. A hand-held electromechanical stimulator with a blunt metal probe (0.8 mm diameter) attached to a coil was used for tactile stimulation. The probe was displaced 1 mm by a current pulse

(10 ms) generated by a Grass stimulator. The stimulation was adjusted to cause a light touch of the skin activating tactile A β fibres, without any visible joint movement. The recordings of the tactile evoked potentials were amplified and monitored using Signal 3.05 software. Evoked potentials were sampled from 6-18 cortical sites in hind paw and forepaw areas respectively, (coordinates: hind paw -0.6 to -3.3 mm rostral-caudal to bregma and 1.3-2.7 mm lateral to the midline; forepaw 1.0 to -2.14 mm rostral-caudal to bregma and 3.0-4.3 mm lateral to the midline). For each skin area/rat, the cortical site eliciting potentials with the highest amplitude was used for recordings. In total, three electrodes were placed on SI cortex. Figure 1b shows the distribution of the recording sites used.

2.7. Nociceptive stimulation

To elicit C fibre evoked potentials, the glabrous skin of the right hind paw/forepaw areas were stimulated using a CO₂ laser with a pulse duration of 21–33 ms. These stimulation energies have been shown to evoke late cortical field potentials reliably in the rat SI cortex through the activation of cutaneous nociceptive C fibres (Kalliomaki et al., 1993a; Kalliomaki et al., 1993b). Based on previous latency measurements (Kalliomaki et al., 1993a) we here classify evoked potentials with an onset latency exceeding 120 ms and 180 ms for forepaw and hindpaw, respectively, as C fibre evoked. Superimposed averaged recordings can be seen in figure 2. During the stimulation of a given skin area, e.g. the heel, a train of 16 CO₂ laser pulses with a frequency of 1 Hz was used. The stimulation site, within the skin area, was shifted between the pulses to avoid repeated stimulation of the same site, as this could reduce LCEPs (Kalliomaki *et al.*, 1993a). No visible damage to the skin was observed.

2.8. Electrophysiological recordings and sequence of stimulations

LCEPs elicited in the contralateral SI cortex representation area of the forepaw digits (forepaw), the heel and the arch of the hind paw were recorded simultaneously (figure 1b). Rats (n=5) that did not show clear LCEPs from forelimb stimulation, which served as a control of transmission of nociceptive input to cortex, were excluded from analysis of LCEPs. The time interval between trains of stimulations per cutaneous area and individual site was set to 10 minutes. Each train consisted of 16 stimulations. These recordings were averaged and the trains repeated five times for each cutaneous area. All data on LCEPs reported in this study are based on averaged LCEPs. From animals receiving tramadol (Tradolan® 50 mg ml⁻¹, tramadol hydrochloride, Nordic Drugs, Limhamn, Sweden), an additional five LCEPs were collected after drug administration. The first LCEPs recording per SI cortex area was not used in the analysis, as the controls showed a stable baseline after the first train. Since it is necessary to establish a stable baseline before testing the effect of a drug and the LCEP resulting from the first train tended to be larger than the subsequent LCEP, the first train of stimulation was excluded. Notably, there was no trace of potentiation of LCEP resulting from the first train of stimulation. The sequence of stimulations was at time (t in minutes) t₀ for heel, t₅ for arch of the hind paw (arch) and t₇ for forepaw. EEG was monitored 90 s every 10 minutes five times, starting at t₃.

2.9. Drug administration

In the UV-B irradiated group, 2 mg kg⁻¹ of tramadol was administered *i.v.* 5–10 min after the fifth completed cycle. Tramadol at this dose is known to be analgesic (Kayser *et al.*, 1991). Twenty minutes after tramadol administration, the sequence of stimulations was repeated five times. The first recording used in the analysis with tramadol was commenced 30 min after drug injection.

2.10. Data analysis of EEG and evoked potentials

The signals (10 kHz sampling frequency), were amplified and filtered using Digitimer Neurolog system (Digitimer LTD, England) with a low cut-off frequency of 1 Hz and a high cut off frequency of 700 Hz. The epoch length was 0.7 s with a pre-stimulus interval of 10 ms for evoked potentials and 90 s for EEG recordings. Fourier analysis was used to analyze the EEG.

CO₂ laser A δ evoked potentials (onset 20–45 ms), occurred irregularly and were therefore not analysed in detail. In case of C fibre input, due to their slow conduction velocity the impulses arrive to the spinal cord during a relatively long time period. Therefore, to obtain a representative measure of the magnitude of the activity evoked by nociceptive C fibres following a laser stimulus the area under the curve (AUC) (inset in figure 2) was calculated using in-house scripts created in Scilab-4.1.1 (INRIA, France). The AUC was defined as the sum of amplitudes between the baseline level and LCEPs, with a maximum duration of 300 ms. Baseline was set to the amplitude at the onset latency of each LCEP.

As for tactile evoked potentials, the onset latency and peak amplitude of the initial positive surface potential, defined as the maximal amplitude of the averaged (n = 16) recording within an interval of 10–33 ms from the onset of the stimulus, was measured. Since the tactile input is much more synchronized and short lasting than the nociceptive input and the decay phase of the first tactile potential overlaps with subsequent more variable potentials, AUC was not used as a measure for the tactile potential.

2.11. Statistical analysis

Four averaged recordings from each cutaneous area were collected from each animal. In animals receiving tramadol an additional four CO₂ laser evoked potential trials were collected after drug administration. The amplitude of tactile evoked potentials and the AUC, duration and latency of LCEPs were used to compare the differences between the groups. The examined data was assumed to be normally distributed and Student's t test was used for statistical analysis. The unpaired two-tailed t test was used to compare the difference between naïve and irradiated rats. Paired two-tailed t test was used to analyse the thresholds of the nociceptive withdrawal reflex in irradiated rats. Furthermore, paired two-tailed t test was used in the analysis of UV-B irradiated rats before and after tramadol administration. Seven rats from the UV group were administered tramadol and used in the analysis of LCEPs. A *p* value < 0.05 was considered significant.

3. Results

3.1. General findings

Tactile evoked potentials with peak latencies between 10 and 33 ms were reliably evoked in all rats (Kalliomaki *et al.*, 1993a). Early potentials from A δ -fibres (onset latency 20-45ms) were on some occasions evoked in homotopic SI cortex (e.g. in forelimb representation on forelimb stimulation), but not in heterotopic SI cortex (e.g. hind limb representation on forelimb stimulation), and can be seen in the grand mean recordings (figures 3 and 5). It is plausible that the stimulus intensity must be higher than used here in order to reliably activate nociceptive A δ fibres (LaMotte *et al.*, 1982). Since these potentials were too variable they were not analyzed further. Similar observations have been made in previous studies using a similar stimulation paradigm (Kalliomaki *et al.*, 1993a).

In all included rats, a late surface-positive field potential was evoked with onset latencies starting around 160-200 ms on forelimb stimulation and 200-240 ms on hind limb stimulation with a duration exceeding 110 ms, corresponding to input from nociceptive C fibres (figure 3 and 5). This potential has previously been found to be due to C fibre input (Kalliomaki *et al.*, 1993a). The energy level (30-47 mJ mm⁻²) of the CO₂ laser pulse used to elicit LCEPs, corresponds to C nociceptive activation in both rats (Kalliomaki *et al.*, 1993a) and humans (Bromm *et al.*, 1984). The LCEPs exhibited the largest amplitude in the homotopic SI cortex but were also seen, albeit at lower magnitude, in heterotopic SI cortex in accordance with previous findings (Kalliomaki *et al.*, 1993b). The LCEPs from the forepaw were used to provide information on heterosegmental effects, whereas the heel and arch of the hind paw were used to test the segmental effects on transmission from the UV-B irradiated skin and adjacent non-irradiated skin. These two latter areas represent skin areas where primary hyperalgesia (Bishop *et al.*, 2007) and secondary hyperalgesia, in human (Gustorff *et al.*, 2004), may occur.

3.2. Behavioural effects of UV-B irradiation

The effect of UV-B irradiation on the reflex threshold was evaluated by comparing the withdrawal responses from the irradiated heel on CO₂ laser stimulation with those evoked from the contralateral heel in awake animals. This comparison confirmed an increased sensitivity in the irradiated heel (Davies *et al.*, 2005; Bishop *et al.*, 2007) with reflex thresholds significantly lower (7.2%, mean of differences -15 mJ, $p < 0.01$) compared to the contralateral heel.

3.3 Effects of UV-B irradiation on cortical evoked potentials

LCEPs in homo- and heterotopic SI cortex in rats irradiated on the heel ($n=10$) were compared to naïve rats ($n=7$).

3.3.1 Responses from UV-B irradiated skin

On stimulation of the UV-B irradiated skin (heel), LCEPs in SI cortex for the corresponding area displayed a significantly longer duration (difference between means 60 ms, $p < 0.01$) (tableS1, online only) compared to naïve animals ($n = 7$) but showed no significant change in onset latency (tableS2, online only), although there was a tendency towards shorter onset latency in heel SI cortex. Similarly, the duration

(tableS1) of the LCEPs in the SI cortex area corresponding to nearby non-irradiated skin displayed a longer duration (difference between mean 91 ms, $p < 0.01$) in the UV group and showed no significant change in onset latency or magnitude (figure 4a). The LCEPs in the forepaw SI cortex of UV-B irradiated rats did not differ from naïve rats.

3.3.2. Responses from non-irradiated nearby skin

On stimulation of the arch, the magnitude (figure 4b) of the LCEPs in the arch SI cortex area was significantly higher (46%, $p < 0.05$) compared to that in naïve rats. LCEPs onset latency (tableS2) and duration (tableS1) did not differ. Furthermore, the magnitude, onset latency and duration of the LCEPs in heel SI cortex did not differ from naïve rats, although the magnitude of LCEPs in the heel SI cortex tended to increase. Likewise, the LCEPs in the forepaw SI cortex area did not differ significantly from naïve rats.

3.3.3. Responses from forepaw skin area

On forepaw stimulation, the magnitude (figure 4c) and onset latency (tableS2) of the LCEPs in the forepaw SI cortex did not differ between irradiated and naïve rats, although the duration (tableS1) was significantly longer in irradiated rats (difference between means 44 ms, $p < 0.05$). LCEPs in the heel and arch SI cortex in the irradiated rats exhibited later onset latency (difference between means 29 ms and 41 ms respectively, $p < 0.001$) compared to the naïve rats on forepaw stimulation.

3.3.4. Effect of UV-B irradiation on tactile evoked potentials

UV-B irradiation elicited no significant changes in tactile evoked potentials in homotopic cortical areas. See table S3 (online only) for details on onset latencies and peak amplitude.

3.4. Effects of tramadol on rats exposed to UV-B irradiation

LCEPs in homo- and heterotopic SI cortex in rats irradiated on the heel were compared before ($n = 7$) and after ($n = 7$) administration of 2 mg kg⁻¹ tramadol (i.v.). Detailed data on the effects are shown in figure 4d-f and tableS4 (online only) and S5 (online only). Further, the mean of the averaged recordings for all groups are shown in figure 5. Tramadol had no obvious effect on the EEG.

3.4.1. Responses from irradiated skin after tramadol administration

Tramadol did not affect the magnitude (figure 4d) of LCEPs evoked in homotopic SI cortex, but did reduce the magnitude of LCEPs in arch SI cortex (44%, $p < 0.01$). Further significant change noted was a reduction in the duration (tableS5) of LCEPs in the heel SI cortex (mean of differences 57 ms, $p < 0.05$) and in the arch SI cortex (mean of differences 100 ms, $p < 0.001$). Also, the onset latency (tableS4) of LCEPs in the arch SI cortex was delayed (mean of differences 33 ms, $p < 0.05$). These changes thus reversed the changes noted above after UV-B irradiation.

3.4.2. Responses from non-irradiated nearby skin after tramadol administration

Tramadol decreased the magnitude (figure 4e) of the LCEPs in arch (56%, $p < 0.001$) and heel (62%, $p < 0.01$) SI cortex areas on stimulation of non-irradiated nearby skin. In

these SI cortex areas, also the duration of LCEPs decreased (mean difference in arch SI cortex 69 ms and heel SI cortex 72 ms, $p < 0.05$). Furthermore, the onset latencies were unaffected. No change of LCEPs in forelimb SI cortex was seen (figure 4e, tableS4 and 4). These changes thus reversed the changes noted above after UV-B irradiation.

3.4.3. Responses from distant skin after tramadol administration

Tramadol had no significant effect on the transmission in pathways originating from the forepaw. Detailed information is shown in figure 4f and tablesS4 and tableS5.

3.4.4. The effect of tramadol on tactile evoked potentials

Tramadol administration had no effect on tactile evoked potentials in UV-B-irradiated rats. See tableS3 for details on onset latencies and peak amplitude.

4. Discussion

In the present study, we recorded nociceptive C fibre evoked potentials from the SI cortex. These potentials arise from depolarization of the deeper layers of the cortex on synchronous input from several ascending pathways, mediating input from nociceptive C fibres (Kalliomaki *et al.*, 1993b). For this reason, they may provide information on the overall ascending activity, as they reflect the summed activity in many ascending pathways. From previous studies it is known that spinal opioidergic analgesia or potentiation can be monitored by recording these potentials (Kalliomaki *et al.*, 1998). The present data indicate, in addition, that it is possible to monitor changes in central nociceptive transmission after UV-B induced hyperalgesia. By recording the representations of the skin area of inflammation, an adjacent skin area not exposed directly to UV-B and a skin area distant from the affected regions, we were able to monitor changes that are reminiscent of primary hyperalgesia and secondary hyperalgesia in the same animal. An analogous method to produce hyperalgesia and record transmission to the cortex is possible in humans, making translational research conceivable. The present study also indicates that tramadol, a centrally acting opiate, reverses hyperalgesia. While nociceptive A δ fibre evoked potentials were too irregular to allow detailed statistical analysis, it may be worth commenting that these potentials only occurred in the area exhibiting maximal tactile potentials in the control animals, whereas the nociceptive C fibre evoked potentials were much more widespread. It is tempting to speculate that this difference in cortical activation between A δ and C nociceptive input underlies the well known difference in spatial characteristics of first and second pain in humans (Lewis and Pochin, 1937). This remains to be tested using evoked potentials in humans.

4.1. Hyperalgesia — features and mechanisms

Transmission from the UV-B irradiated skin area to the target area in the SI cortex showed signs of primary hyperalgesia such as longer duration and a tendency towards shorter latency of the response. Similar observations have been made in dorsal horn neurons (wide dynamic-range (WDR) neurons) after UV-B exposure (Urban *et al.*, 1993). In particular, after UV irradiation of their receptive fields, WDR neurons responded to both tactile and nociceptive input with increased duration in response to heat stimulation as well as exhibiting expanded receptive fields. Nociceptive-specific neurons also exhibit a lower threshold and increased response on cutaneous stimulation

in the hyperalgesic situation (Hedo *et al.*, 1999; Sandkuhler, 2009). These changes are known to involve both central and peripheral mechanisms, although in the case of primary hyperalgesia, the peripheral sensitization appears to play a major role (LaMotte *et al.*, 1982; LaMotte *et al.*, 1992). The reason why the magnitude, i.e. area under curve, of the LCEPs did not increase in the pathway from the irradiated skin area to its primary projection area in the SI cortex is not clear. Because evoked potentials are dependent on relatively synchronous input, one conceivable explanation is that the nociceptive C fibre input from the primary skin area and consequent activity in the dorsal horn are desynchronized somewhat by the ongoing spontaneous activity, thereby partly masking the stronger nociceptive input from the primary hyperalgesic skin area. An alternative, but not mutually exclusive, possibility is that the response frequency in nociceptive C fibres on CO₂ laser stimulation of the irradiated skin area, despite being sensitized (Andrew and Greenspan, 1999), decreases in the hyperalgesic situation. Nociceptive C fibres fatigue easily (Torebjork *et al.*, 1984), and there is evidence that, after skin inflammation, induced by injection of complete Freund's adjuvant, the response frequency on moderate to strong noxious heat stimulation decreases in C fibres (Andrew and Greenspan, 1999). A third possibility is that the transmission pathways from nociceptors from UV-B irradiated and adjacent skin areas (secondary hyperalgesic) to the SI cortex are under different control. There is evidence for a differential descending inhibitory control of transmission from primary hyperalgesic skin areas and excitatory control of secondary hyperalgesic areas (Vanegas and Schaible, 2004). Further studies will be necessary to resolve this issue. Notably, the potentiation of the LCEPs elicited from the arch, assumed to be related to mechanisms of secondary hyperalgesia, was surprisingly stronger than the changes in the transmission from the primary hyperalgesic skin area. The mechanisms underlying secondary hyperalgesia have for a long time been assumed to arise mainly from central mechanisms (Torebjork *et al.*, 1984; Sandkuhler, 2009). It is conceivable, but remains to be tested, that NMDA dependent mechanisms triggered by ongoing spontaneous input from sensitized nociceptors are involved, since it is known that MK-801 (an NMDA antagonist) blocks frequency dependent potentiation of LCEP (Kalliomaki *et al.*, 2003). We stimulated a skin area on the arch located about 5–15 mm away from the border of the UV-B irradiated skin area. This distance is greater than the known expansion of receptive fields caused by inflammation (Andrew and Greenspan, 1999). Nevertheless, if the spread of hypothetical algogenic chemicals, caused by inflammation, to nearby skin areas sensitize mechano-insensitive C nociceptors at a distance, then such changes may contribute to the enhanced evoked potentials from the arch. As mentioned above, there is evidence for an excitatory supraspinal control of transmission from the secondary hyperalgesic skin area, which, if operative, might have contributed in the present situation. An additional central mechanism underlying the increased transmission from the adjacent skin area is the expansion of receptive fields of dorsal horn neurons (Hylden *et al.*, 1989; Urban *et al.*, 1993). Also, thalamic neurons exhibit enhanced responses to heat and mechanical stimuli in parts of their receptive field remote from the injury site (Guilbaud *et al.*, 1986).

4.2. Effects of tramadol

Our data are consistent with a previous report showing that UV light-induced hyperalgesia can be reduced with opioids and non steroidal anti-inflammatory drugs (NSAIDs) (Bishop *et al.*, 2007). Our present data suggest that tramadol (2 mg kg⁻¹) to a

large extent reverses the changes noted after UV-B irradiation, but had no effect on transmission from forepaw. Notably, tramadol administration lessened both the increase in transmission from the arch (nearly non-irradiated skin) to the arch and heel SI cortex, and the prolonged duration of the input from the heel (irradiated skin) input to the heel and arch representation. Interestingly, tramadol has been reported to affect hyperalgesia as measured using reflex responses (Munro *et al.*, 2008). Interestingly, a significantly increased duration of forelimb LCEP evoked by forelimb stimulation after UV-B irradiation and increased latencies for the arch and heel LCEP evoked on forelimb stimulation was found, supporting a report (Kayser and Guilbaud, 1987) showing that hyperalgesia in one body part may affect nociceptive transmission from distant body parts. That tramadol did not affect this distant effect may indicate that it is produced by mechanisms other than those related to primary and secondary hyperalgesia.

We emphasize that our aim was not to characterize the effects of tramadol *per se*, but rather to evaluate the hypothesis that the LCEPs can be used to monitor the changes occurring after induction of hyperalgesia. That it was possible to reduce the changes in the LCEPs occurring after UV-B exposure adds strength to this hypothesis.

4.3. Animal models for hyperalgesia

Several animal models are used to study hyperalgesia, and different agents can be used to induce inflammation. In both humans and rats, cutaneous UV exposure has emerged as an important inflammatory pain model because it causes hyperalgesia (Urban *et al.*, 1993; Davies *et al.*, 2005; Bishop *et al.*, 2007; Saade *et al.*, 2008) by releasing endogenous substances, which evoke inflammation and sensitize the peripheral nociceptive terminals (Treede *et al.*, 1992). This model has several advantages, and most importantly, the model can be used in analogous human studies safely to permit translational studies. UV-B irradiation elicits both primary hyperalgesia and secondary hyperalgesia in humans (Gustorff *et al.*, 2004).

At present, most animal studies of nociceptive transmission and analgesia are based on measuring different types of behavioural changes (Sandkuhler and Gebhart, 1984; McMahon *et al.*, 1991; Koltzenburg *et al.*, 1994; Yeomans *et al.*, 1996; Valle *et al.*, 2000; Davies *et al.*, 2005; Bishop *et al.*, 2007; Munro *et al.*, 2008; Saade *et al.*, 2008); in particular, reflex responses. Because the reflex pathways and the ascending pathways to SI cortex differ at least partly, it is not always clear to what extent information derived from reflex tests is a valid predictor of the sensory aspects of pain related activity. LCEPs thus have the potential to be a useful complement to behavioural and reflex tests when screening potential analgesic drugs.

4.4. Conclusion

In summary, changes in transmission in nociceptive pathways to primary somatosensory cortex induced by UV-B irradiation of the skin can be monitored by recording CO₂ laser C fibre evoked potentials in SI cortex. Therefore, this way of monitoring the pain related pathways appears to be a useful supplement to animal behavioural tests of mechanisms related to pain and analgesia.

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Figures

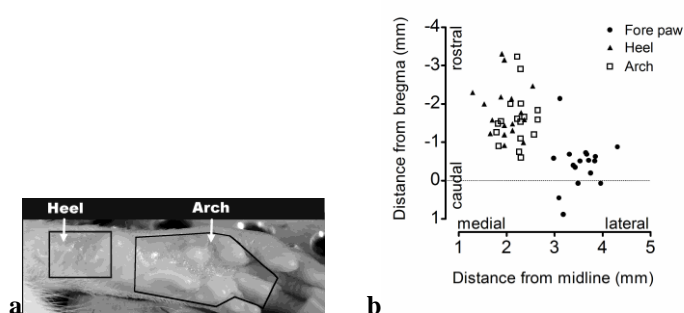


Figure 1. a) Hind paw stimulation areas. The heel and arch areas of the rat hind paw that were subjected to stimulation are indicated. b) Recording sites on SI cortex. All animals ($n = 17$) used in the LCEPs analysis are included. Coordinates in mm are given with respect to bregma and midline for forepaw digits, heel (heel of the hind paw), arch (arch of the hind paw)

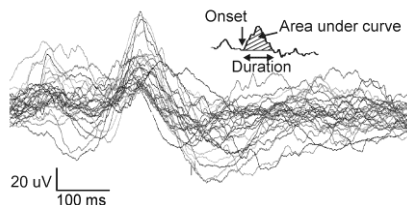


Figure 2. Superimposed recordings of LCEPs. The raw data recordings from naïve ($n = 7$) animals (28 averaged recordings) after nociceptive stimulation illustrates the variation of the onset, duration and AUC of the LCEPs. The inset shows a single averaged ($n = 16$) recording.

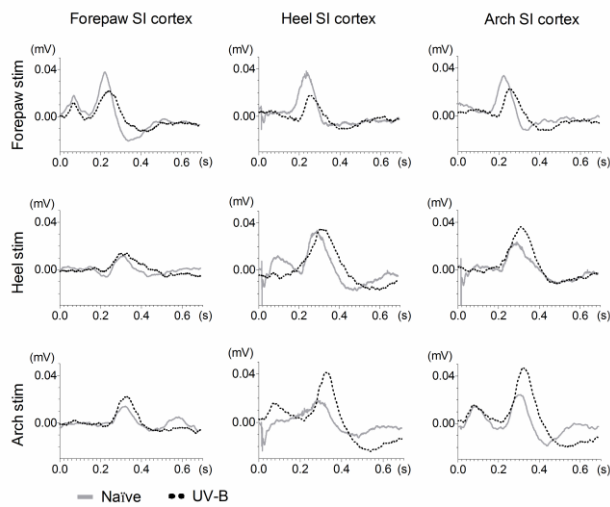


Figure 3. Grand mean of LCEPs recordings from three cortical areas. The grand mean of recordings from naïve ($n = 7$) and UV-B-irradiated rats ($n = 10$) are plotted. CO₂ laser stimulation (stim) evoked potentials starting at ~200 ms for the hind paw areas and at ~160 ms for the forepaw digits (forepaw). Arch denotes the arch of the hind paw.

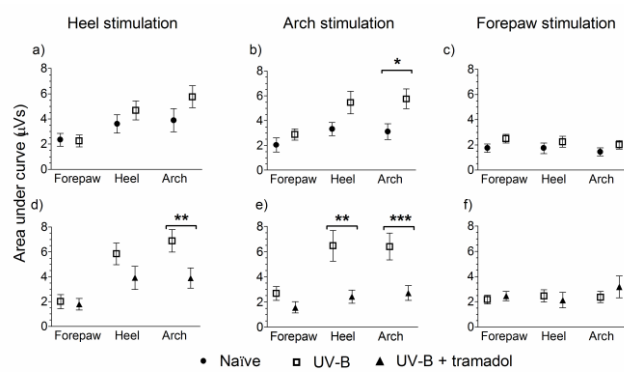


Figure 4. Magnitude of LCEPs recordings from three cortical areas. Top: The difference between means and S.E.M of area under the curve (AUC) for the naïve (black circles) and the UV-B exposed (white boxes) groups are shown.

Bottom: The mean of differences and S.E.M of AUC in UV-B exposed rats ($n = 7$) before (white boxes) and after ($n = 7$) (black triangles) tramadol administration are shown. The x-axis depicts the recording areas on SI cortex. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

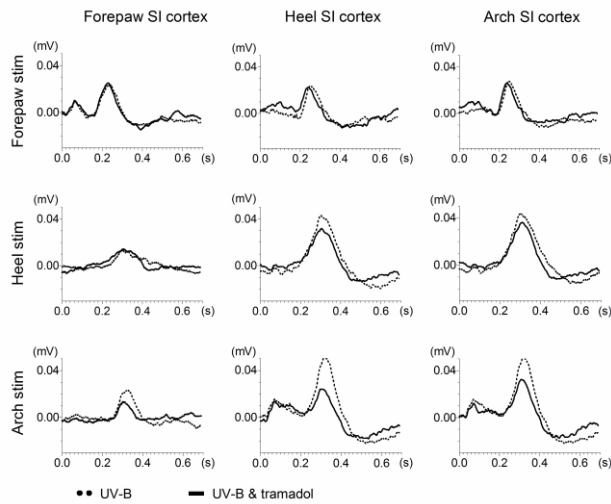


Figure 5. Grand mean of LCEPs recordings from three cortical areas. The grand mean of recordings from UV-B irradiated rats before ($n = 7$) and after ($n = 7$) tramadol administration are shown. CO₂ laser stimulation (stim) evoked potentials starting at ~200 ms for the hind paw areas and at ~170 for forepaw digit (Forepaw) stimulation. Arch denotes the arch of the hind paw.

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