PHARMACOLOGICAL VALIDATION OF THE HEAT CAPSAICIN WARMTH MODEL FOR NEUROPATHIC PAIN: A DOUBLE-BLIND PLACEBO-CONTROLLED THREE-WAY CROSS-OVER DESIGN WITH GABAPENTIN AND REMIFENTANIL


(IN PREPARATION)
ABSTRACT

BACKGROUND Neuropathic pain is a chronic pain state that arises as a direct consequence of a lesion or disease affecting the somato-sensory nervous system. Neuropathic pain can be a disabling illness that often has a strong negative impact on health-related quality of life. The most common clinical signs of neuropathic pain are sensory loss, spontaneous (burning) pain, paresthesias and stimulus-evoked pain. Human experimental models for neuropathic pain can help improve the pharmacological evaluation of novel analgesics.

Recently we have described a newly developed Heat Capsaicin Warmth (HCW) model that induces allodynia, secondary hyperalgesia and continuous pain in healthy volunteers. Here we aimed to validate the HCW model with a pharmacological challenge under the hypothesis that two analgesics, from distinct pharmacological classes, would reduce painfulness, allodynia and secondary hyperalgesia induced by the model.

METHOD The HCW model uses heat (5 minutes 45 °C heat) followed by the application of 0.3% topical capsaicin cream combined with prolonged 37 °C warmth stimulation to sensitize the skin. Gabapentin (1200mg) and remifentanil (0.05μg/kg/min) were administered in a double blind, placebo-controlled three-way cross-over design.

RESULTS Our data showed that gabapentin significantly reduced the extent of secondary allodynia and hyperalgesia compared to placebo. Remifentanil also reduced secondary allodynia but not hyperalgesia and in addition alleviated continuous on-going pain. All though there were some significant changes between the first and the second visit, in general the HCW model was well repeatable and showed clear drug effects.

CONCLUSION We conclude that the HCW model can be used in a cross-over design for repeated induction of neuropathic pain symptoms in healthy volunteers and has adequate sensitivity to detect drug effects on secondary alldynia, hyperalgesia and continuous pain. Its sensitivity to the two very different classes of compounds used in this study adds to the validity of this model. Therefore, the HCW model is applicable in early clinical development of novel compounds aimed for the treatment of neuropathic pain specifically those targeting mechanisms of central sensitization as well as spontaneous pain.
6.1 INTRODUCTION

Neuropathic pain is a chronic pain state that arises as a direct consequence of a lesion or disease affecting the somato-sensory nervous system (Treede et al., 2008). Neuropathic pain can be a disabling condition that often has a strong negative impact on health-related quality of life (O’Connor, 2009; Dworkin et al., 2010; Moore et al., 2011). Two surveys in France and the UK estimated the prevalence of neuropathic pain in the general population to be as high as 7-8% (Torrance et al., 2006; Bouhassira et al., 2008).

Neuropathic pain remains difficult to manage because a patient’s response to existing medication is difficult to predict. For drugs studied in clinical trials only half of the patients reported a clinically meaningful pain relief but in most cases pain of moderate severity remains (O’Connor, 2009; Attal et al., 2010). This clearly shows there still is a great need for novel efficacious drugs for neuropathic pain. The use of valid and robust human experimental models for neuropathic pain can help to improve the pharmacological evaluation of novel drugs (Curatolo et al., 2000; Staahl & Drewes, 2004).

The most common clinical signs of neuropathic pain are sensory loss, spontaneous (burning) pain, paresthesias and stimulus-evoked pain (Walk et al., 2009). Specific aspects of neuropathic pain such as hyperalgesia (increased pain sensation to a noxious stimulus) and allodynia (a pain sensation in response to a non-noxious stimulus) can be mimicked in experimental pain models in healthy volunteers using chemical stimulants like capsaicin, the pungent ingredient in hot chilli peppers (Simone et al., 1989; LaMotte et al., 1991; Magnusson & Koskinen, 1996; Petersen & Rowbotham, 1999; Ziegler et al., 1999; Dirks et al., 2000; Schmelz et al., 2000; Petersen et al., 2001; Dirks et al., 2003). Alldynia and hyperalgesia at the site of the capsaicin application are termed ‘primary’ whereas alldynia and hyperalgesia beyond the actual application area are termed ‘secondary’. Primary alldynia and hyperalgesia are the result of sensitization in the peripheral nervous system and secondary alldynia and hyperalgesia are caused by a sensitization mechanism mediated through the central nervous system in the spinal cord (Bridges et al., 2001; Attal, 2012; von Hehn et al., 2012).

Experimental pain models are often used to evaluate novel analgesics in early drug development and can help to understand the mechanism underlying neuropathic pain symptoms (Staahl et al., 2009b). Several experimental models have helped to show analgesic efficacy and anti-hyperalgesic effects of ketamine, alfentanil, remifentanil, fentanyl, cannabis, morphine, pregabalin, gabapentin, NSAIDS and others (for review see: (Staahl et al., 2009a; Staahl et al., 2009b). However, there are also, a number of studies that failed to find any drug effects (Wallace et al., 2002; Voller et al., 2003; Wallace et al., 2004; Kraft et al., 2008). There are many different types of models available, and no specific model is regarded as the golden standard in experimental pain research for neuropathic pain. Which model
should be used will always depend on the characteristics of the drug in combination with the characteristics of a specific patient population.

In chapter 5 I have described the new Heat-Capsaicin-Warmth (HCW) model that induces robust secondary hyperalgesia and allodynia as well as continuous pain. The HCW model uses a combination of 5 minutes 45 °C heat, 0.3% topical capsaicin and 37 °C warmth stimulation on a 3 by 3 cm area of skin to induce central sensitization and a state of continuous pain. Previously we showed that this model induced robust and stable areas of hyperalgesia and allodynia compared to other topical capsaicin models (Harbers and Konopka, 2013 submitted). The HCW model is the first and to our knowledge only capsaicin model that generates a stable period of continuous pain combined with allodynia and secondary hyperalgesia. Because many neuropathic pain patients have continuous pain as a symptom we believe that this adds to the validity of the capsaicin model. Intradermal capsaicin (IDC) models also induce on-going pain; however the spontaneous pain is not stable and decreases over time. Within 30 to 60 minutes after the injection, reported pain rating are below 10 out of 100 (Gustafsson et al., 2009). Only the electrical sensitization (ES) model described by Koppert et al. (Koppert et al., 2001) also induces continuous pain in combination with allodynia and hyperalgesia.

The next step in the development of the HCW model is to validate the model using well- known analgesic compounds. Gabapentin is regarded as a reasonably effective first line medication for treatment across different neuropathic pain conditions (Attal et al., 2010; Dworkin et al., 2010; Moore et al., 2011). Gabapentin is thought to bind to the α2-δ subunit of voltaged-gated calcium channels and inhibits neurotransmitter release by blocking new synapse formation (Eroglu et al., 2009; Dworkin et al., 2010; Moore et al., 2011). Typical side effects of gabapentin are sedation, dizziness, somnolence and headache. Several studies using human experimental capsaicin models report that gabapentin reduces the area of mechanical hyperalgesia and allodynia (Dirks et al., 2002; Gottrup et al., 2004; Mathiesen et al., 2006). However, other capsaicin studies failed to find an effect of gabapentin on hyperalgesia (Gustorff et al., 2004; Wallace & Schulteis, 2008).

Although opioids are not regarded as the first drug of choice for the treatment of neuropathic pain, the weak mu-opioid receptor binding Tramadol and other strong opioids are regarded as 2nd line medication for neuropathic pain patients (Dworkin et al., 2007; Moore et al., 2007; Attal et al., 2010). Representing strong opioids, remifentanil is a potent ultra-short acting, selective mu-opioid receptor agonist with a typical opioid pharmacodynamic profile (Scott & Perry, 2005). To achieve analgesia without anesthesia, remifentanil hydrochloride is administered as an intravenous infusion ranging from 0.025 μg/kg/min to 0.15 μg/kg/min (Staahl et al., 2009a). Typical side-effects at this dose range are nausea, vomiting, dizziness and cardio-respiratory effects (Servin & Billard, 2008).
Remifentanil has a rapid onset of analgesic action (one minute) and a fast offset of action (3 – 10 minutes) irrespective of the duration of the infusion. Therefore remifentanil is considered a useful pharmacological agent in experimental pain models and clinical trials (Scott & Perry, 2005). Remifentanil has been studied in several human experimental capsaicin models and was found to reduce hyperalgesia, alldynia and continuous pain (Petersen et al., 2001; Hood et al., 2003; Petersen et al., 2003).

In the present study we applied a double-blind, placebo-controlled, three-way cross-over design with the aim to pharmacologically validate the HCW model. The selected pharmacological interventions consisted of a single oral dose of 1200 mg gabapentin given 90 min prior to the capsaicin challenge and remifentanil (0.05 μg/kg/min for 45 min as infusion) 30 minutes after the start of the capsaicin model. Efficacy of the compounds was assessed on VAS ratings for continuous pain, the extent (in cm) of secondary alldynia and secondary hyperalgesia. To investigate the effects of repeated application of the HCW model we measured the mechanical detection and pain threshold before and after each session.

6.2 METHOD

6.2.1 SUBJECTS
The study adhered to the declaration of Helsinki and was approved by the medical ethics committee ‘Stichting Beoordeling Ethiek BioMedisch Onderzoek’, P.O. Box 1004, 9400 BA Assen, The Netherlands. This committee is acknowledged by the Central Committee on Research Involving Human Subjects (known by its Dutch initials, CCMO). All subjects were recruited through the recruitment department of PRA International (Zuidlaren, The Netherlands), were pain-free and refrained from any medication at least two weeks prior to the study. Other exclusion criteria were: actual pain, history of neurological or psychiatric disorders, history of cardiovascular disease or bronchospastic respiratory disease and recent drug or alcohol abuse. Subjects signed an informed consent form prior to participating in the study and received a financial compensation for their time and travel expenses. A total of 24 male subjects participated in this study, mean age was 24.9 (range 19-37). The study was performed at the site of PRA (Zuidlaren, The Netherlands). All measurements were performed by well-trained medical research assistants.
6.2.2 EXPERIMENTAL PROCEDURES

6.2.2.1 SCREENING
After the standard medical health screening all subjects were familiarized with all experimental procedures and exposed to 0.3% capsaicin cream for 30 minutes to check for possible allergic reactions to capsaicin and to confirm secondary hyperalgesia was present extending at least 3 cm beyond the area of the capsaicin application. For the familiarization subjects were tested on the non-dominant inner forearm, while during the actual study period the dominant arm was used.

6.2.2.2 THERMAL STIMULATION
All heat and warmth stimulations were applied using a computer-controlled thermode (Pathway-ATS, Medoc, Ramat Yishai, Israel) with a surface area of 3 by 3 cm.

6.2.2.3 CAPSAICIN APPLICATION
Capsaicin cream (0.3%) was supplied by the pharmacy of PRA (Zuidlaren, The Netherlands). One gram of this cream was applied on a 3 by 3 cm area of the skin of the inner fore arm demarcated by an adhesive non-woven fabric (Fixomull stretch, BSN Medical, Hamburg, Germany). The upper border of the capsaicin area was located 2 cm from the inner elbow. The area of capsaicin was covered by a single 5 by 5 cm non-woven swab (Medicomp, Hartman, Heidelberg, Germany). Finally, an adhesive non-woven fabric (Fixomull stretch, BSN Medical, Hamburg, Germany) was applied for fixation.

6.2.2.4 ASSESSMENT OF CONTINUOUS PAIN
Subjects were asked to rate the continuous pain intensity on a Visual Analogue Scale (VAS). The 100 mm VAS was anchored by ‘no pain’ on the left end and ‘the worst pain imaginable’ on the right. VAS scores are expressed on a score between 0 and 100 (mm).

6.2.2.5 ASSESSMENT OF SECONDARY HYPERALGESIA AND ALLODYNIA
The assessment of the secondary hyperalgesia was performed by stimulating the skin with a 64 mN von Frey filament (Optihair 2, Marstock, Schriesheim, Germany). The filament was applied perpendicular to the skin until it was bent, ensuring that a uniform force was applied. The assessment of the secondary allodynia took place by stimulating the skin with a standardized brush (SenseLab™, Somedic, Höby, Sweden). The brush was applied across the width of the arm over a length of approximately 2 cm.

For the assessment of hyperalgesia and allodynia both the surface area and the extension along the midline of the arm were measured. To measure the midline extent,
the first stimulus was presented at the midline of forearm just above the wrist and well outside the anticipated area of secondary hyperalgesia and alldynia. Stimulation with the filament or the brush was continued along the midline of the arm towards the area of capsaicin application in steps of 0.5 cm. Stimulation would stop as soon as the subjects reported a clear change in the sensation of the stimulus (e.g. burning, pricking, painfulness, or a higher intensity of touch sensation). The location of the stimulation where the change in perception occurred was marked and the distance to the border of the area of capsaicin application was measured.

To calculate the surface areas of hyperalgesia and allodynia, filament and brush applications were measured in five different directions. The first line was the midline of the arm, two horizontal measurements on the side of the capsaicin patch and two diagonal lines running from the corners of the plaster covering the capsaicin with an angle of 45 degrees towards the wrist. The area was calculated as the polygon of the 5 points on the lines minus the 9 cm2 of the area where the capsaicin cream was applied.

6.2.2.6 MECHANICAL DETECTION THRESHOLD (MDT)

Subjects were presented with a series of ascending and descending Von Frey filament intensities. To measure the MDT, subjects were asked to indicate if they felt the stimulation with the filament or not. Filament forces varied between 2 and 512 mN on a 2-log scale. The filaments were applied to the skin on the midline of the inner forearm at the area of the capsaicin application and 2 cm outside of the capsaicin area with 2 seconds in between stimulations. Ascending stimulus forces were presented until the subject reported that the stimulus was felt. Next, descending filament forces were presented until the subject would no longer report they felt a stimulus. This procedure was repeated 5 times. The MDT was calculated as the geometrical mean of the ten registered filament forces.

6.2.2.7 MECHANICAL PAIN THRESHOLD (MPT)

Subjects were presented with a series of ascending and descending pinprick intensities. During the MPT procedure subjects were asked to indicate if a pinprick stimulus felt ‘sharp’ or ‘blunt’. Pinpricks forces varied between 8 and 512 mN on a 2-log scale. The pinprick stimuli were applied to the midline of skin of the inner forearm at the area of capsaicin application and 2 cm outside of the application site with 2 seconds in between two pinprick stimulations. Ascending pinprick forces were presented until the subjects reported the pinprick as ‘sharp’. Next, descending pinprick forces were presented until the subject would report the pinprick to feel ‘blunt’. This procedure was repeated five times. The MPT was calculated as the geometric mean of the ten registered pinprick forces.
6.2.3 Pharmacological Study Design

A double-blind placebo-controlled three-way crossover design was used with gabapentin/placebo, placebo/remifentanil and placebo/placebo as treatment combinations. Treatment consisted of either 1200 mg gabapentin orally or 0.05 μg/kg/min intravenous infusion of remifentanil hydrochloride (Ultiva®, Glaxo SmithKline). Gabapentin capsules were administered at T= 0 min, 90 minutes before the start of the capsaicin application while the remifentanil infusion started at T=130 min and lasted for 45 minutes until T=175 min (see Figure 1). Gabapentin placebo were identical capsules, remifentanil placebo was a saline infusion. Treatment order was randomized across subjects.

![Figure 1](image.png)

**Figure 6.1** Schedule of assessments applying the HCW model. The timing of the pharmacological interventions is represented by the grey arrows. Gabapentin was administered orally at t=0 min. Remifentanil (0.05 μg/kg/min) was administered as an infusion starting at t=130 and finished at t=175. M0 to M6 reflect the different time points at which the different outcome measures were taken. M0 (t= -15); M1 (t= -10); M2 (t=120); M3 (t=135); M4 (t=150); M5 (t=165); M6 (t=185). The application of the HCW model starts at t=85 min with a 5 min 45 °C heat stimulation followed by 0.3% topical capsaicin with concomitant 37 °C warmth application starting at t=90 min for 90 minutes until t=180 min.

6.2.4 The HCW Model Design

The HCW model started with a heat sensitization stimulus of 45 °C presented for 5 min applied with the thermode of the Medoc Pathway system. Subsequently the capsaicin cream (0.3% capsaicin) was applied on exactly the same skin area as the heat stimulation took place. In addition, a concomitant warmth stimulus of 37 °C was applied by placing the thermode on top of the covered capsaicin. The capsaicin cream in combination with the warmth stimulation was administered for a period of 90 min. In chapter 5 a continuous warmth stimulus of 33 °C was used. Data from later experiments showed that an increased temperature of 37 °C resulted in a more stable continuous pain sensation and improved the induction of alldynia (unpublished data). Therefore in this study a continuous warmth stimulus of 37 °C was used.
The intensity of continuous pain, secondary allodynia and secondary hyperalgesia were measured at baseline, i.e. 10 min before gabapentin administration (M1, t= -10). Subsequent measures took place once the HCW model was applied at t=120 (M2), t=135 (M3), t=150 (M4) and t=165 (M5) min. The MDT and the MPT were measured within the area of secondary hyperalgesia 2 cm outside of the capsaicin application area. These measures were performed 15 minutes before gabapentin/placebo administration (M0, t= -15) and 5 minutes after the capsaicin cream was removed (M6, t=185 min). The schedule of assessments for the HCW model, the pharmacological interventions and the assessments of the outcome measures are presented in figure 6.1.

6.2.5 SAFETY MONITORING

Safety and tolerability assessments consisted of continuous monitoring of heart rate, blood pressure and respiratory rate. During the infusion of the medication, a pulse oximeter probe was placed on one of the fingers for continuous monitoring of peripheral oxygen saturation. All adverse events reported by the subject during the course of the study were reported.

6.2.6 STATISTICAL ANALYSIS

All data are presented as group mean scores with Standard Error of the Mean (SE). For statistical analysis the software package SAS was used (SAS Institute Inc., Cary NC, USA).

A mixed model repeated measures procedure in SAS was used to create a statistical model with the variables treatment, period, treatment sequence, previous treatment, time point and treatment*time point as fixed effects. Repeated measures were: time point and period*time point. Because gabapentin was given before the capsaicin was applied, the time point M2 (120 min) after capsaicin application was included in the drug effect for gabapentin but not for remifentanil (remifentanil infusion started after M2). In the statistical model this was represented by weighting time point M2 with 1/100 for the remifentanil condition and 1 for the gabapentin condition.

Primary outcome measures were VAS ratings of continuous pain and extent of secondary hyperalgesia (in cm) and allodynia (in cm) along the midline of the arm. Secondary outcome measures were the NRS ratings to pinprick stimulation with 64 and 512 mN and surface area of secondary hyperalgesia (cm2) and allodynia (cm2). For all measurements we assessed the difference between treatment and placebo at all time points. Period effects of the capsaicin model itself were assessed for continuous pain, hyperalgesia and allodynia as well as for the Mechanical Detection Threshold (MDT) and Mechanical Pain Threshold (MPT) at before the beginning and end of the experimental procedures at each visit.
Table 6.1 Alertness scores on the Bond & Lader VAS. The three conditions are placebo (PLAC), gabapentin (1200mg, GABA) and remifentanil (0.05 μg/kg/min, REMI). Time point M1 is the baseline, time point M4 is at 150 min. Change indicates the difference M4 and M1. (n=24)

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>TIMEPOINT</th>
<th>ALERTNESS</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>name</td>
<td>mean</td>
</tr>
<tr>
<td>PLAC</td>
<td>-10 M1</td>
<td>57.9</td>
<td>3.04</td>
</tr>
<tr>
<td></td>
<td>150 M4</td>
<td>56.0</td>
<td>3.00</td>
</tr>
<tr>
<td></td>
<td>change M4-M1</td>
<td>-2.0</td>
<td>1.90</td>
</tr>
<tr>
<td>GABA</td>
<td>-10 M1</td>
<td>60.3</td>
<td>3.39</td>
</tr>
<tr>
<td></td>
<td>150 M4</td>
<td>51.8</td>
<td>3.18</td>
</tr>
<tr>
<td></td>
<td>change M4-M1</td>
<td>-8.5</td>
<td>2.84</td>
</tr>
<tr>
<td>REMI</td>
<td>-10 M1</td>
<td>59.4</td>
<td>3.20</td>
</tr>
<tr>
<td></td>
<td>150 M4</td>
<td>41.9</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td>change M4-M1</td>
<td>-17.4</td>
<td>3.14</td>
</tr>
</tbody>
</table>

6.3 RESULTS

6.3.1 TOLERABILITY AND SIDE-EFFECTS

All subjects completed the study and reported that the drugs, the capsaicin and all other procedures were tolerable. The most common side effects during the remifentanil infusion were: dizziness (n=14), sleepiness (n=11), hot flushes (n=8), transpiration (n=6), nausea (n=5) and itching (n=5). Most common side effects during the gabapentin were: sleepiness (n=5) and fatigue (n=4). No relevant cardiovascular or respiratory side-effects were observed throughout the study period. Because sleepiness was a common side-effect in both drugs one could expect a decreased alertness compared to the placebo condition. Alertness was measured on the Bond and Lader Visual Analogue Scale (BL-VAS) scale (Bond & Lader, 1974). The BL-VAS indicated that alertness was decreased after baseline for the gabapentin and the remifentanil condition (table 6.1). This effect was largest during the remifentanil infusion.
6.3.2 CONTINUOUS PAIN

The reported VAS ratings for continuous pain are presented in figure 6.2. At time point M2 ($t=120$ min) 30 minutes after capsaicin application, the mean pain rating was 26.2 (SE=3.67) for the placebo condition and 26.0 (SE=3.47) for the remifentanil condition (infusion was not started yet at M2). Over the time course of the challenge small decrease in reported pain was observed in the placebo condition. During the remifentanil infusion the pain was strongly reduced to a score of 15 (SE=2.25), 10 (SE=1.43) and 6 (SE=1.63) at 135, 150 and 165 min, respectively. All measurements after remifentanil were significantly lower than in the placebo condition ($p<0.001$). For gabapentin the pain reported at time points M2 (120 min), M3 (135 min), M4 (150 min) and M5 (165 min) were 23.2 (SE=3.27), 21 (SE=3.27), 19 (SE=2.65) and 14 (SE=2.04). Statistical analysis showed that only the last time point ($t=165$ min) was significantly lower than the placebo condition ($p<0.05$).

6.3.3 ALLODYNIA

6.3.3.3 MIDLINE MEASUREMENT

Results for alldynia for the three treatment conditions are presented in figure 6.3. The extent of alldynia at M2 ($t=120$ min) 30 min after the capsaicin application in the HCW model was 4.2 cm (SE=0.57) for the placebo condition and 3.8 cm (SE=0.71) for the remifentanil condition. For the gabapentin condition the extent of alldynia 2.9 cm (SE=0.53) was significantly lower compared to placebo ($p<0.05$).

In the placebo condition the extent of alldynia remained stable over time. All time points (except M2 for remifentanil) showed a reduced extent of alldynia for both the gabapentin and the remifentanil condition ($0.001 < p < 0.05$).
6.3.3.2 AREA MEASUREMENT

The area of allodynia at M2 (t=120 min) was 9.6 cm² (SE=1.88) for the placebo condition, and 13.4 cm² (SE=2.86) for the remifentanil condition. In the gabapentin condition the allodynia area was 12.1 cm² (SE=2.84) at the M2 time point (not significantly different from placebo). For the other time points after gabapentin the allodynia was reduced to 9.7 (SE=2.71), 7.3 (SE=2.16) and 5.8 cm (SE=1.51) at time points 135, 150 and 165 min, respectively. The difference between gabapentin and placebo was significant only at 135 min (p<0.05). Upon remifentanil infusion the allodynia area decreased to 7.24 cm² (SE=1.80), 7.1 cm² (SE=2.16) and 6.0 cm² (SE=2.10). The difference with placebo was significant at 150 min (p<0.05) and nearly significant at 135 min (p=0.06).

**FIGURE 6.3** Secondary Hyperalgesia and Allodynia
Mean and standard error of the mean (SE) for allodynia and hyperalgesia. Results are shown as the extent along the midline of the arm in cm (A and B) and the surface area in cm² (C and D). The three treatments are placebo (PLAC) in grey, gabapentin (1200mg, GABA) in blue and remifentanil (0.05 μg/kg/min, REMI) in red. Error bars represent SE. Significant difference between treatment and placebo are marked: *** p<0.001; ** p<0.01; * p<0.05. Near significant differences are marked: + (p<0.1). (n=24)
6.3.4 SECONDARY HYPERALGESIA

6.3.4.1 MIDLINE MEASUREMENT
The extent of hyperalgesia at M2 (t=120), 30 min after the capsaicin application was 8.6 cm (SE=0.69) for the placebo condition and 8.1 cm (SE=0.76) for the remifentanil condition. For the gabapentin condition the extent was 6.8 cm (SE=0.71), significantly smaller compared to placebo (p<0.05). The only other significant reduction of hyperalgesia compared to placebo was at 135 min in the gabapentin condition (p<0.01). There were no significant differences in hyperalgesia between remifentanil and placebo.

6.3.4.2 AREA MEASUREMENT
The area of hyperalgesia at M2 (t=120) 30 min after the capsaicin application was 34.9 cm² (SE=4.47) for the placebo condition and 36.0 cm² (SE=4.55) during remifentanil. After gabapentin the area was 27.0 cm² (SE=3.61), a significant reduction compared to placebo (p<0.05). At M3 (t=135 min) the reduced area of hyperalgesia after gabapentin was nearly significant (p=0.09). There were no significant differences for the surface area of hyperalgesia between placebo and remifentanil.

6.3.5 PERIOD EFFECTS
The present study also evaluated the applicability of the HCW model in cross-over designs. Therefore, we analysed the mean response on each visit independent from the treatment type received that day (table 6.4, figure 6.4). The continuous pain ratings showed a decrease after the first visit but were fairly stable between visits 2 and 3. Similar patterns were seen from the first to the second visit for the measures of allodynia and secondary
hyperalgesia. The decrease was more profound if the area was used compared to the midline measurement.

To test for any long lasting effects of the HCW model on the sensitivity of the skin after the two-week washout period we measured the mechanical detection threshold (MDT) and the mechanical pain threshold (MPT) at the area of capsaicin application (primary area) and the area where we expected secondary hyperalgesia (secondary area). The data indicated a small but significant desensitization of the skin at the primary and the secondary area as the mechanical pain threshold was increased at the beginning of visit 2 and 3 (figure 6.5). At the primary area the MPT was increased from 48.3 mN (SE=7), went up to 64.3 mN (SE=7) in the second visit and further increased to 78.5 mN (SE=7) in the last visit. Our analysis showed that this was a significant effect of period (p<0.01). At the secondary area a similar desensitization pattern was visible (p<0.05). Directly after the model application, at time point 180 min, the increased pain threshold was not present. The Mechanical Detection Threshold did not show any significant changes over the three visits.

![Figure 6.5](image)

**FIGURE 6.5** Period effects for the Mechanical Pain Threshold (MPT)
Group mean values independent of treatment for the MPT (force in mN) at the area of capsaicin application (primary area, A) and the area of hyperalgesia (secondary area, B). Period 1, 2, 3 refer to the three measurement days with 2 weeks in between visits. The ‘pre’ value is the baseline measure at t=-15 min; the ‘post’ value is the measurement at the end at t=180 min. Error bars represent SE. * indicates a significant period effect p<0.05. (n=24)

## 6.4 DISCUSSION
Capsaicin-induced sensitization is often used as an experimental pain model for hyperalgesia and allodynia observed in neuropathic pain patients. In the present study we performed a pharmacological validation of our recently developed Heat Capsaicin Warmth (HCW) model in healthy volunteers. Two different class drugs, gabapentin and remifentanil, known to be efficacious for neuropathic pain were used to test if the HCW model makes it possible to detect analgesic effects on continuous pain, and the extent of secondary hyperalgesia and allodynia.
As we expected the HCW model induced secondary hyperalgesia and allodynia as well as continuous pain similar to the data for the HCW model described in chapter 5 of this thesis. The development of secondary sensitization outside the area of capsaicin and heat application is regarded as a consequence of central sensitization of the central nervous system at the spinal level (Gottrup et al., 2004). This shows that the HCW model is able to mimic the mechanism of central sensitization seen in neuropathic pain patients.

The HCW model is the only capsaicin model that has a (relatively) stable period of continuous pain combined with secondary hyperalgesia and allodynia. By inducing continuous pain the neuropathic pain the HCW model have a better resemblance with the pathophysiological symptoms in neuropathic pain compared to previous heat capsaicin models and intradermal capsaicin models. Only the electrical sensitization (ES) model described by Koppert et al. (2001) has similar sensory features. Still, there are some key differences between the HCW model and the ES model. The ES model directly activates nerve pathways bypassing any nerve endings. As a consequence the ES model is limited to testing analgesic compounds with a central mechanism of action (Koppert et al., 2001). Instead the HCW model stimulates peripheral nociceptors in the skin and therefore has the potential to test both peripherally and centrally acting analgesics.

### 6.4.1 EFFECT OF GABAPENTIN

Our pharmacodynamics results show that gabapentin reduced the extent of allodynia by 26 to 36% (varying over timepoints) and hyperalgesia by 21 to 24% compared to placebo. Continuous pain was reduced by 38% but only at the last time point (165 min).

Within our design gabapentin was given 90 min before application of the HCW model. This way the Tmax of gabapentin was to be expected around the 120 min time point. As such, the effect of gabapentin has to be considered as attenuating the development of alldodynia and hyperalgesia induced by the HCW model. Analgesic efficacy of gabapentin can already be present at the first time point (M2) during the HCW model application. This was confirmed by our data because both allodynia and secondary hyperalgesia were already lower compared to placebo at the first two time points (M2 and M3; 120 and 135 min).

This Tmax effect of gabapentin was not present for the continuous pain, which was only reduced at the last time point. Possibly it takes more time for gabapentin to affect the physiological mechanisms underlying continuous pain. Gabapentin has a central acting mechanism whereas the capsaicin induced continuous pain most likely has a peripheral origin possibly explaining this delay. However, many studies with experimental pain models have reported that gabapentin does not have an effect on acute and on-going pain (Gottrup et al., 2004; Gustorff et al., 2004; Segerdahl, 2006; Wallace & Schulteis, 2008). In contrast several studies have reported that gabapentin can reduce spontaneous chronic pain in neuropathic
pain patients (Dworkin et al., 2010; Moore et al., 2011).

The effects of gabapentin in the HCW model are in line with previous pharmacological studies using different types of experimental models for neuropathic pain. Experimental models using topical capsaicin (and heat) also showed a reduction of alldynia and hyperalgesia by gabapentin after gabapentin (Dirks et al., 2002; Mathiesen et al., 2006). These studies did not report any lasting continuous and therefore no effect of gabapentin on this aspect of the model could be compared.

For alldynia a similar result was obtained in an intradermal capsaicin model (Gottrup et al., 2004) where gabapentin reduced brush alldynia. However, this study failed to show an effect of gabapentin on secondary hyperalgesia and continuous pain. In our study we also observed that the effect of gabapentin was most prominent for brush alldynia. However, we were able to detect a clear reduction of the development of hyperalgesia in the gabapentin condition. This indicates that the HCW model might have a greater pharmacological sensitivity than intradermal capsaicin models. Furthermore, we also found that gabapentin diminished the continuous pain. Due to large differences in pharmacological design of the two studies it is difficult to directly compare these differences in results. Gottrup and colleagues (Gottrup et al., 2004) used a 15 day administration period with a titrated dose up to 800 mg gabapentin three times a day whereas we used a single dose of 1200 mg.

Similar to our study, in the heat-capsaicin model study described by Dirks (Dirks et al., 2002) a single dose of 1200 mg gabapentin was used. They reported a 86% reduction of hyperalgesia and a 69% reduction of alldynia. Whereas in the HCW model we found a maximum reduction of 24% and 36% respectively, more similar to the 31% reduction of alldynia found by Gottrup and colleagues with the intradermal capsaicin model. A clear difference in the designs between these studies is the timing of the gabapentin administration. In the HCW model and the intradermal capsaicin study the gabapentin is given before the capsaicin administration whereas in the Dirks study gabapentin is administered after the capsaicin has been applied. It might be that gabapentin is more active when the nociceptive system is already sensitized (Gottrup et al., 2004). However, even if given before the capsaicin application gabapentin reduces the sensitization that is comparable to effects seen in clinical trials with gabapentin (Backonja et al., 1998; Rowbotham et al., 1998; Rice et al., 2001; Serpell & Neuropathic pain study group, 2002; Backonja & Glanzman, 2003) (see (Backonja & Glanzman, 2003) for review).

Other studies using non-capsaicin based experimental models combined with a single dose gabapentin administration failed to show a significant anti-sensitization effect of gabapentin in the UV-burn model (Werner et al., 2001) and the Electrical stimulation (ES) model (Segerdahl, 2006). The fact that we could detect clear effects of gabapentin at this dose underlines that the HCW model has a high sensitivity to detect analgesic efficacy.
Although the ES model did not show analgesic efficacy after a single dose of gabapentin, it did show a significant reduction of the area of hyperalgesia (15% reduction) after multiple dose treatment with 4 doses 600 mg gabapentin over a 24 hours period (Segerdahl, 2006). Also the continuous pain in the ES model was affected by gabapentin. The electrical current was adjusted until a pain rating of 5 out of 10 was reached. For the gabapentin condition a stronger current was needed to reach this level of continuous pain. This does support our finding that gabapentin can reduce experimental continuous pain. This supports the idea that next to neuropathic pain conditions gabapentin might be used for post-operative pain and other acute pain states to reduce ongoing pain and prevent possible sensitization of the nervous system (Dirks et al., 2002).

6.4.2 EFFECT OF REMIFENTANIL

The results of our present study showed that remifentanil reduced the extent of allodynia by 29 to 36%. Continuous pain was reduced by 36 to 65% over time. No significant effect of remifentanil was observed for hyperalgesia. Previous studies using topical capsaicin showed reduced areas of alldynia and hyperalgesia. (Petersen et al., 2001; Hood et al., 2003; Petersen et al., 2003). Several studies have investigated the effects of remifentanil in the ES model. These studies show that remifentanil reduces the rating of continuous pain and the area of hyperalgesia compared to placebo (Koppert et al., 2003; Singler et al., 2007; Lenz et al., 2011). This further supports our data that confirm that remifentanil has a strong impact on continuous pain ratings. The results from our experimental model are in line with data from clinical trials that morphine can reduce both continuous pain and sometimes also alldynia in post herpetic neuralgia patients (Rowbotham et al., 1991; Watson & Babul, 1998; Raja et al., 2002), diabetic neuralgia patients (Gimbel et al., 2003; Watson et al., 2003) and other neuropathic pain patients (Morley et al., 2003; Rowbotham et al., 2003; Portenoy et al., 2007) (for review see (Eisenberg et al., 2006; Dworkin et al., 2007)).

At present it is not determined why we failed to find an effect of remifentanil on the extent of hyperalgesia in contrast to the previous reports. Possibly our dose of 0.05 μg/kg/min remifentanil was too low to induce changes in hyperalgesia but was strong enough to affect allodynia. In a previous remifentanil study by Hood et al. (2003) the reduction in the area of alldynia was twice as large compared to the effect for hyperalgesia. Another explanation could be that other studies measured hyperalgesia with Von Frey Filaments that exerted much stronger forces of 256 and 450 mN compared to our 64 mN. Possibly, the impact of the 64 mN was not strong enough to pick up changes in perception induced by remifentanil. To measure the hyperalgesia along the midline we used a filament with a rounded tip whereas the other studies use a flat tip filament (Touch Test, Stoelting, Illinois, USA). The rounded tip of our filaments could have stimulated mostly A-β fibres whereas
flat type high force filaments will also activate A-δ fibres. A previous study reported that remifentanil influences A-δ fibres but not A-β fibres (Gustorff et al., 2001).

6.4.3 PERIOD EFFECTS OF THE HCW MODEL

We observed that there were significant period effects for the measurements of continuous pain, allodynia and secondary hyperalgesia. All outcome measures were somewhat reduced after the first visit. This study was especially designed to validate the HCW model in a 3-way cross-over setting. Because we had anticipated possible period effects in such a design we chose a two week washout period between the applications of the model. Because of this long wash-out period we are confident that the period effects are not caused by any carry-over effects of the administered drugs itself. Instead, they more likely are related to the repeated application of the HCW model. It is known that the effects of capsaicin on sensory perception can last as long as 6 weeks (Simone et al., 1998). The results described in that study show that using an intradermal capsaicin paradigm the nerve function in the skin was reduced for up to six weeks and resulted in a strong desensitization at the injection site. Although we used a topical capsaicin application instead of intradermal capsaicin, similar effects on the peripheral nervous system of the arm could have been present. Among other reasons, we chose a 2 week wash-out period in order to reduce carry over effects from the previous HCW application while at the same time we tried to keep the study duration within reasonable limits to ensure that the design would be suitable in a phase I/IIa research environment. Because of this two week wash-out period it could have been that the nerve function of the subjects was not fully recovered after each application of the HCW model and a desensitization of the skin was present. This was confirmed by the measurements of the Mechanical Pain Threshold (MPT) at the beginning and end of each visit. The results showed that at the beginning of the 2nd and the 3rd visit the MPT was increased indicating a desensitization induced by the capsaicin application of a previous period. This desensitization did however not limit the sensitization effects of the HCW model, because at every visit this desensitization was not present at the end of the session after the HCW model application. This indicates that the HCW model was inducing secondary sensitization every time the model was applied, independent of any long term desensitization. Of course if possible, the period effects could be reduced by increasing the length in between the HCW applications and/or using different areas on the skin (e.g. separate arms).

Another influence that cannot be ruled out and that could have caused a period effect is the psychological familiarising effect. Subjects will get more familiar with the HCW model and its procedures each time it is applied. We tried to prevent or minimise this effect by familiarizing the subjects with the capsaicin application and the different measurements during the screening session. However, we cannot exclude this psychological aspect as a
variable contributing to the period effects.

Overall, the significant treatments effects showed that the HCW model is applicable and effective in a 3-way cross-over design. The analgesic and anti-sensitization effects of gabapentin and remifentanil were well detected even in the presence of some period effects from the multiple applications of the HCW model.

### 6.4.4 MIDLINE VS. SURFACE AREA

One of the goals of this study was to compare two methods for the measurement of allostynia and hyperalgesia i.e. the extent in cm along the midline of the arm versus the surface area in cm² on the arm. Our results show that the midline measurement gave better results. Compared to the area, the midline measurement resulted in 7 vs. 2 significant differences for allostynia and 2 vs. 1 significant time points for hyperalgesia. Furthermore, the midline measurement is a much more practical measurement to implement within a trial design. It takes less time and has a much lower sensory impact on the skin of the subjects. Also, it is difficult to measure allostynia and hyperalgesia in the horizontal directions of the arm for the area calculations and these measurements give a lot of ‘zero’ measurements inducing unwanted variance into the analysis. When you take all these considerations into account we propose that the use of a single midline measurement is preferable over a surface area measurement for allostynia and secondary hyperalgesia.

### 6.4.5 CONCLUSION

We conclude that the HCW model is a capsaicin model for neuropathic pain symptoms with good sensitivity to detect drug effects on allostynia, hyperalgesia and continuous pain. Gabapentin decreased the extent of allostynia and hyperalgesia while remifentanil reduced continuous on-going pain and allostynia but not hyperalgesia.

In this study we have shown the potential for using the HCW model in a crossover study design with a simple method for measuring allostynia and hyperalgesia. Therefore, we believe that the HCW model is applicable in early clinical development of novel compounds for neuropathic pain that are targeting mechanisms of central sensitization as well as continuous pain.
REFERENCES


