# Characterization and re-evaluation of experimental pain models in healthy subjects

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## Chapter 8

# THE CAPSAICIN-INDUCED HYPERALGESIA MODEL: VALIDATION AND INCORPORATION IN A MULTI-MODAL NOCICEPTIVE AND HYPERALGESIA PAIN TEST BATTERY TO DETECT ANALGESIC EFFECTS OF DRUGS IN HEALTHY SUBJECTS

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# INTRODUCTION

Continuing efforts are made to improve pain models, for example by simulation of clinical pain symptoms, such as hyperalgesia.[1-21] A frequently used hyperalgesia model is the capsaicin-induced hyperalgesia/allodynia model, in which application of the red chili pepper extract capsaicin induces a temporary hyperalgesia, resulting in a lower heat pain threshold, and a temporary mechanical allodynia.[5-7,19,21-23,26,28,37,51] Capsaicin is a highly selective agonist for transient receptor potential cation channels subfamily V member 1 (TRPV1). TRPV1 channels are transducers of physically and chemically evoked sensations.[29] The vanilloid 1 subtype is activated by noxious heat (≥ 43°C) and is expressed on C-fibers and on a subset of Aδ-fibers.[30,31] The transient effects of applying topical capsaicin are burning sensations, hyperalgesia, allodynia, and erythema. By periodically heating the zone of primary hyperalgesia to a non-painful temperature (prekindling and rekindling), a longer-lasting hyperalgesia can be maintained. Previous studies have shown that the topical application of capsaicin induces peripheral sensitization by primary mechanical allodynia/thermal hyperalgesia.[7,37] Primary hyperalgesia is caused by modulation of peripheral afferents and is therefore restricted to the site of injury, *i.e.*, peripheral sensitization. Capsaicin may also induce central sensitization by secondary mechanical allodynia, an increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input (secondary hyperalgesia/allodynia). The secondary hyperalgesia is thought to be a transient state of central sensitization where an increase in excitability of the dorsal horn neurons is induced, which can also be caused by nerve injury.[7,24] In addition, it triggers the release of pro-inflammatory agents at peripheral terminals, such as substance P and calcitonin gene-related peptide (CGRP).[32,33]

Thermal primary and secondary hyperalgesia, after application of capsaicin, can be evaluated via laser evoked potentials (LEP) using an EEG recording system. Laser stimulation (LS) uses energy to heat up the epidermis and parts of the dermis with brief and powerful stimuli.[34] This type of stimulation causes a characteristic double pain sensation, consisting of an initial sharp pinpricking-like pain (Aδ-fibers) and a second longer burning pain (C-fibers).[35-36] LS can stimulate the skin in a well-reproducible manner making it useful as a tool to elicit evoked potentials. Evoked potentials are monophasic deflections of spontaneous EEG and are time and phase locked on the onset of the stimuli.[27] These waveforms are typically characterized by their polarity, latency, amplitude, and measurement position on the scalp. Evoked potentials via EEG can be registered due to the brief nature of the stimulus of which the timing can be controlled and may provide important information on (central) pain processing.[25-27] Combined LS and EEG registration may quantify the pain stimulus in a more objective manner.

The use of multimodal, multi-tissue pain testing is a strategy to improve the predictive value of evoked pain models in clinical drug development. Effect outcomes, however, are only valuable when the pain models don't interfere with each other. The aim of the present study was to evaluate the validity and reliability of the capsaicin-induced hyperalgesia model. The model was evaluated in a multimodal pain test setting to determine whether the capsaicin-induced hyperalgesia model can contribute to the analgesic profile of the drug tested without influencing the other pain models.

# METHODS

## Study design and study drugs

This was a two part study which is registered in the European Clinical Trial database under No. 2017-000480-32. Part A was a method validation study of the capsaicin-induced hyperalgesia model. Part B was a double-blind, single dose, randomized, placebo-controlled, 3-way cross-over study. The latter study was setup to establish the usefulness of the capsaicin model, when used in the context of the pain test battery, to detect the effects of drugs used in the treatment of neuropathic pain. For both Part A and B screening occurred between 28 and 8 days before first study drug administration.

In Part A of the study, subjects were to attend the clinic on 2 occasions, with a wash-out period of at least 7 days. Subjects were divided in to 2 groups: those who were to receive pre-/rekindling (rekindle) or those who were not to receive pre-/rekindling (no-rekindle). Both groups followed the same schedule of assessments. Prekindling was performed immediately before application of the capsaicin patch (duration: 5 min; temperature: 45°C), rekindling was done immediately before the contact heat stimulation on the capsaicin treated skin at 0.5, 3, 5 and 8 hours post-capsaicin application (duration: 5 min; temperature: 40°C).

In Part B subjects were to attend the clinic on three separate occasions, with a wash-out period of at least 7 days. Each subject received on one occasion a single dose of duloxetine 60 mg, tramadol 100 mg, or placebo. Both tramadol and duloxetine are known to be effective in the treatment of neuropathic pain and are being used in the clinic for this indication.[38-41] The Pharmacy Department of

the Leiden University Medical Center prepared the study treatments and over-encapsulated all treatments to ensure blinding. A battery of human evoked pain models was used to demonstrate analgesic properties.

The study was approved by the Medical Ethics Committee Stichting Beoordeling Ethiek Biomedisch Onderzoek (Assen, The Netherlands) and was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with all International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines and the Declaration of Helsinki.

## Study participants

A total of 20 healthy male subjects (1:1 ratio rekindle: no-rekindle) between 18 and 45 years of age, were to be included in the study in Part A and 18 healthy male subjects in Part B. All subject provided written consent prior to any study activities. Subjects with a Fitzpatrick skin type I-IV, without widespread acne, tattoos or scarring on the volar forearms and who were willing and able to comply with all scheduled visits were included. Subjects indicating a NRS (0-10) > 8 after removal of the capsaicin or < 3 after LS were excluded. In part B, subjects were also excluded when they were not able to tolerate nociceptive assessment at screening or those who did not achieve tolerance at >80% of maximum input intensity for the mechanical, electrical or cold thermal nociceptive assessments, or who had less than 1°C decrease of the average pain detection threshold (PDT) between control and capsaicin treated skin during the contact heat pain model. Any condition that would affect sensitivity to pain or cold, or a confirmed significant allergic reaction (urticaria or anaphylaxis) to duloxetine or tramadol, or multiple drug allergies, would lead to exclusion.

## Evoked pain tests

Primary hyperalgesia to heat (contact heat and LEPs) and secondary hyperalgesia and mechanical allodynia (LEPs and pin-prick) were evaluated in the capsaicin-induced hyperalgesia/allodynia model in Part A. Only contact heat and LEPs (primary hyperalgesia) were assessed in Part B. For these assessments, a 3x3 cm surface on the right volar forearm was used for the application of occlusive topical 1% capsaicin cream FNA (Formulary of Dutch pharmacists) for 30 minutes, whereas the left volar forearm served as a non-sensitized control.

Pain detection thresholds to contact heat were measured using a 3x3 cm contact thermode (TSA-II, Medoc Ltd., St. Ramat Yishai, Israel) on the capsaicin treated skin and control area (initial temperature 32°C, ramp 0.5°C/s, cut-off 50°C, average of 3 stimuli). Measurements were performed twice pre-dose (baseline measurements were performed once before capsaicin application and once after capsaicin removal. Both baseline measurements were performed before study drug administration), and 0.5, 1, 3, 5, 8 and 10 hours post-capsaicin in Part A, and 0.5, 1, 2, 3, 4, 6, 8 and 10 hours post-study drug administration in part B.

LEPs were recorded in a in a quiet room with minimal illumination. Both subject and investigator wore protective goggles. Laser stimuli (5 ms stimulus duration, 5 mm diameter, 2 Joules, random stimulus intervals of 6-8 seconds) were generated by a ND:YAP laser (neodymium:yttrium-aluminium perovskite, STIMUL 1340, Electronic Engineering). To avoid skin damage and nociceptor sensitization/habituation, the site of stimulation was moved after each laser stimulus.[25,45-46] LS was always performed on the secondary area first, then control area and last on the primary area in Part A, and on the control area first and then on the capsaicin treated area in part B. 20 stimulations were administered per location per stimulus block and were performed twice pre-dose (baseline measurements were performed once before capsaicin application and once after capsaicin removal. Both baseline measurement were before study drug administration), and 1, 3, 6 (part B only) and 8 hours post-dose. After each block of stimulations the subject was asked to indicate the most painful moment during this series on a numeric rating scale (0-10 with 0= 'no pain' and 10= 'worst pain imaginable'). LS was recorded using an EEG system (TMSi Polybench, Twente Medical Systems international (TMSi), Oldenzaal, the Netherlands). EEG data was collected with a cap using the 10-20 system. In order to minimize artefacts on the EEG recording, subject were instructed to focus, stay relaxed and keep the eyes open as much as possible. A reaction button had to be pushed as fast as possible after a stimulus was felt. Measurement parameters included the amplitude (μV) and latency (ms) of N2, P2 and N2P2 peaks.

Secondary allodynia was measured via mechanical pin-prick assessment with Von Frey filaments (Part A only). At each visit pre-capsaicin application, individualized pain perception was evaluated using Von Frey filaments ranging from 128-512 mN. The filament where the subject reported 'nearly painful' was then used to determine the area of secondary mechanical allodynia. Eight spokes dividing a circle in equal parts were used to assess secondary allodynia. Stimulation started 3.5-4 cm from the center of the primary area moving to the middle with steps of 5 mm. Once a change in sensation was felt, from nearly painful to painful, that point was determined to be the border of the secondary allodynia area. To minimize the risk of measuring false positive central sensitization, the primary area

was surrounded by a border of 5 mm in which no secondary allodynia measurements was performed. Due to a high variation and a relative high non-responder group in Part A of the study, analysis of this paradigm was not possible.

Part B nociceptive tasks were integrated in a range of pain assessments measuring different modalities of pain on different tissues, now included with the capsaicin-induced hyperalgesia model (no-rekindle), see Figure 1. The electrical stimulation, pressure stimulation and cold pressor assessment have been described previously.[42-44] The battery of evoked pain tasks also consisted of a paradigm quantifying conditioned pain modulation (CPM). These assessments were conducted twice pre-dose (double baseline) and 1, 2, 3, 4, 6 and 10 hours post-dose by trained personnel. A training session was included as part of the screening examination to familiarize subjects with the procedure. To eliminate the risk of tissue damage, all pain tasks have a maximum safety cut-off.

## Statistical analysis

Part A and B were analyzed separately.
Repeatedly measured pharmacodynamic data of Part A were analyzed with a mixed model analysis of covariance with fixed factors group, treatment, visit, time, group by treatment, group by visit, group by time, treatment by visit, treatment by time, visit by time, group by treatment by visit, group by treatment by time, group by visit by time, treatment by visit by time and group by treatment by visit by time and random factors subject, subject by treatment, subject by visit and subject by time and the average pre-value as covariate.

Repeatedly measured pharmacodynamic data of part B were analyzed with a mixed model analysis of variance with the fixed factors treatment, period, time and treatment by time, random factors subject, subject by treatment and subject by time and the average pre-value as covariate. Within each pain model the contrast of tramadol and duloxetine compared with placebo was calculated over the full testing period (0 -10 hours). Per variable results were generated with estimates of the difference of the different contrasts and a back transformed estimate of the difference in percentage for log transformed parameters, 95% confidence intervals (in percentage for log-transformed parameters) and Least Square Means (LSMeans) (geometric means for log transformed parameters), and the p-value of the contrasts.

All calculations of the pharmacodynamic parameters were performed using SAS for Windows version 9.4 (SAS Institute Inc., Cary, NC, USA). The main SAS procedure that was used in the analysis was "PROC MIXED". No adjustments for multiple comparisons were employed.



The test-retest reliability of the PDTS for the contact heat pain model was calculated using intra-class correlation (ICC). Calculations were based on all measurements performed on the control area of all subjects in Part A, and on all baseline measurements on the capsaicin and control area of the subjects in Part A. Assessment of variability included the calculation of the intra-subject coefficient variation (CV) of the PTT for the pressure stimulation, cold pressor and electrical stair of the current study and 4 previous studies conducted with these models: Study I-IV (Eudract no. 2014-003015-12, 2014-003553-34, 2014-004468-39 and 2015-003496-30).[63-66] Least squares means PDTS and PTTS per time point for all 5 studies were calculated.

# RESULTS

## Demographics and safety

21 male subjects were included in Part A and 18 male subjects were randomized to receive treatment in part B. One subject in Part A was replaced as he developed flu-like symptoms during occasion 1 and was unable to complete the study. This adverse event was not assessed as related to the application of the capsaicin or the study procedures. A total of 38 subjects completed the study and were included in the final analysis. Table 1 shows an overview of the subject characteristics. Table 2 shows an overview of the treatment related adverse events (AE) observed in part B. All AEs were transient and needed no medical intervention. One AE (dizziness) was moderate in intensity, while all others were mild.

## Part A

### Capsaicin-induced hyperalgesia contact heat model

LSMeans PDTS were measured in the rekindle and no-rekindle groups on each day the subjects attended the clinic. The time course of the heat PDTS is presented in Figure 2. Analysis of the contrasts showed a significant difference between the PDTS on the capsaicin treated skin versus the control area (LSMeans 40.5°C; estimate of the difference -3.85°C; 95%CI-4.45 – -3.26; p<.0001). Between the rekindle and no-rekindle groups no significant differences were observed, neither on the capsaicin treated skin (LSMeans 40.8°C; estimate of the difference 0.69°C; 95% CI -0.74-2.12; p=0.3205) nor on the control area (LSMeans 44.4°C; estimate of the difference 0.08°C; 95% CI -1.35-1.50; p=0.9116). The heat PDT's measured

on occasion 1 and occasion 2 were comparable (LSMeans 42.6°C; estimate of the difference 0.29°C; 95% CI -0.26-0.84; p=0.2886). The ICC on the control area was 0.784, for all baseline measurements 0.813, and for all data points (control and capsaicine) 0.700.

# Part B

## Capsaicin-induced hyperalgesia contact heat model

The heat PDT values were measured on the capsaicin treated skin and on the control skin (Figure 3A). Tramadol led to a significant increase in PDT for heat pain on the control area (LSMeans 44.9; estimate of the difference 1.08; 95% CI 0.42-1.75; p=0.0023) over the complete period. No significant difference between duloxetine and placebo was detected on the control area (p=0.1375). Neither tramadol nor duloxetine could be shown to lead to significant effects on primary heat hyperalgesia compared to placebo (tramadol: p=0.0753; duloxetine p=0.1915). A summary of the endpoints is presented in Table 3A and a time course profile of the heat PDT on the three treatments occasions is presented in Figure 4.

## Laser evoked potentials

On the control area, the placebo parameters were unchanged throughout the study period. Latency was not significantly affected by tramadol or duloxetine compared with placebo. Tramadol had a significant effect compared with placebo on the N2 amplitude (LSMeans -6.98 µV; estimate of difference 1.885 µV; 95% CI 0.61-3.16; p=0.0051), the N2P2 amplitude (LSMeans 15.07 µV; estimate of difference -3.106 µV; 95% CI -5.744 – -0.468; p=0.0226), and the reaction time (LSMeans 427.43 ms; estimate of difference 38.761 ms; 95% CI 13.426-64.097, p=0.0041), on the normal skin. A time course for the N2P2 amplitude has been depicted in Figure 4. Duloxetine had no significant effect compared with placebo on any of the endpoints in the normal skin. Also, there were no statistically significant differences from placebo on any of the endpoints on the capsaicin treated skin for tramadol or duloxetine. Perceptive thresholds after LEP were unaffected for both treatments compared with placebo (p>0.2). A summary of effects (LSMeans, estimate of the difference, 95% CI and p-values) are presented in Table 3B.



## Mechanical, thermal (cold) and electrical stimulation models

Time courses and the analgesic profile for each treatment on the primary endpoints are shown in Figure 4 and 5. The results of the analyses for the PTT for the he pressure stimulation, cold pressor and electrical models are presented. Tramadol increased the PTT significantly compared with placebo for the pressure stimulation (LSMeans 50.5; estimate of the difference 18.7%; 95% CI 12.3%-25.5%; p<.0001), cold pressor (LSMeans 31.5; estimate of the difference 37.7%; 95% CI 24.3%-52.5%; p<.0001), and electrical stair model (LSMeans 25.8; estimate of the difference 17.6%; 95% CI 6.6%-29.8%; p=0.0023) over the complete 10 hours of testing. CPM (The delta PTT for electrical pain) effect was not demonstrated by either tramadol (LSMeans 1.71; estimate of the difference -0.241; 95% CI -1.119-0.636; p=0.5791) or duloxetine (LSMeans 1.70; estimate of the difference -0.243; 95% CI -1.127-0.640; p=0.5784). Duloxetine had no significant effect on any of the pain models. The effects of tramadol and duloxetine on evoked pain are depicted in Figure 3B and Table 3A.

## Reliability

The average PDT and PTT per time point per study were comparable (Table 4). The pressure stimulation, cold pressor and the electrical stimulation all had PTTs e in the range of the previous studies. The study-to-study variability (current study and studies I-IV)[34-36,45] of the mean PTT in the pressure stimulation, cold pressor and electrical stair were small. The pressure stimulation had a CV of 8.2% in the current study and 13.1%, 12.0%, 12.2% and 13.8% in the previous studies. The cold pressor CV ranged around 15% with an exception in Study I where the variation was higher (14.9%, 22.7%, 15.9%, 12.8% and 15.4%). The variability of the electrical stair was 14.3%, 16.5%, 14.4%, 11.2% and 16.7%.

# DISCUSSION

The capsaicin-induced hyperalgesia/allodynia model has been used in numerous experimental and clinical pain studies.[5-7,19,21-23,26,28,37,51] In this study we aimed to incorporate the capsaicin-model in a battery of multimodal pain testing. The validity and reliability of the capsaicin model was evaluated, as well as the analgesic profile of two known analgesics by testing the effects in comparison

with placebo on a range of experimental pain tests. Incorporation of this pain model should lead to a more complete analgesic profile of the compounds under investigation increasing the confidence in dose selection at an early stage of drug development and the probability of success in patient studies.[43,47-50]

Hyperalgesia to heat was robust and lasted for the full 10 hours of testing. This could be contributed to the capsaicin cream which was applied topically and not intra-dermally where skin trauma could influence heat hyperalgesia. It has been suggested that the model becomes more stable over a longer period of time due to a synergistic effect of heat (pre-/rekindling) and capsaicin.[19,21] This synergistic effect was not seen in the present study. Heat PDTs in Part A of the study in the rekindle versus no-rekindle group did not differ from each other on any time point nor between each visit. The rekindle interval in the present study might have been too long for a synergistic effect. Additionally, the two groups were evaluated with contact heat stimulation on the primary area which might have neutralized the synergistic effect. In future studies, it would be advantageous to compare the rekindle effect with a secondary allodynia model, for example with mechanical stimulation on the secondary area. In the present study this measurement paradigm proved to be too variable to analyze. The area of mechanical hyperalgesia did not increase in about half of the subjects, as it would have been expected.[19,26,51] Intra-subject variation within and between days of mechanical stimulation on the secondary area was too high in both groups giving reason to omit the assessment in Part B of the study.

A key learning from the current study is a better understanding of the influence of topical formulations on drug penetration. Capsaicin in cream and ethanol solutions are used to induce hyperalgesia, and intra-dermal injection are used to administer the capsaicin directly in to the target site.[5-7,19,21-23,26,28,37,51] Topical drug delivery depends on the ability to overcome biological barriers. Drug penetration and permeation through the skin are greatly influenced by the structural properties of the skin – mainly provided by the stratum corneum where the corneocytes, in a matrix of intercellular lipids, serve as the primary barrier of the skin, a regulator of water loss and prevent permeation of potentially harmful substances and microorganisms – and the physicochemical properties of the drug, like partition coefficient, molecular size, solubility and hydration.[67] Penetration of the active ingredient through the skin can be challenging, which might also have been the case in the present study. A low drug absorption could have resulted in a lower efficacy, reflected by the absence of secondary hyperalgesia in our study.

Chemical penetration enhancers (CPE) interact with the skin to stimulate drug flux and can be used to increase permeation of the topical drug.[76] CPEs are pharmacologically inactive compounds, or vehicles, that may partition and/or diffuse into the membrane and interact with the stratum corneum.[77] Ethanol is an often used CPE as it can permeate rapidly through the skin and interact with the skin in various ways. Ethanol can increase permeant solubility and delay depletion, resulting in a raise of the flux.[78] It Alters the tissue solubility, improving the drug partitioning into the membrane,[78] and ethanol increases the thermodynamic activity of the drug by a rapid evaporative loss from the application site. As ethanol disappears, the drug concentration may increase beyond the saturated solubility, yielding a supersaturated state with a greater driving force for permeation.[78] With this knowledge it can be expected that a topical formulation of capsaicin in an ethanol solution would have resulted in a better partition and solubility, increasing the efficacy and produce a stable secondary hyperalgesia. This has been confirmed is in studies using an ethanol solution.[5,6,23,25,28]

Demonstrating reliability is a distinctive feature in science as it verifies scientific evidence. In Part A the capsaicin model demonstrated a high between-day reliability assessed via primary hyperalgesia to heat. This is in line with the known literature where reliability of the capsaicin pain model mainly proved to be high.[21,26] Although these studies used intra-dermal injection of capsaicin and reliability was not assessed via primary heat hyperalgesia, but via secondary allodynia with mechanical stimulation[21] or ongoing pain ratings.[26] In opposition to Dirks et al., Geber et al. was not able to confirm the test-retest correlation of the areas of pin-prick hyperalgesia, since it was too poor to reach significance.[21,26] In the present study secondary mechanical allodynia also proved to be too variable. In Part B we incorporated the capsaicin model in the context of a test battery which has been used to determine the analgesic profile of new drugs in previous studies.[63-66] The capsaicin model did not interfere with the other pain models included in the test battery. The PDTs and PTTs of the pain models in the pain test battery were comparable to the ones observed in the previous studies (Study I-IV) conducted without the capsaicin model. This suggests that the newly incorporated model does not affect the results of the existing models which is an essential factor in multimodal pain testing. Several factor should be considered. First, the study design should have a cross-over design including a placebo arm. Sequence of treatment should be randomized (*e.g.*, Williams design). Repeated measures should be performed and analysis should be done with each subject's baseline recording of that study visit to deal with inter-subject variation.[50,52] Additionally, different test locations of each pain model can decrease the chance of interference. The volar forearms were used for the capsaicin-induced hyperalgesia

model, whereas the contralateral hand (compared with arm of capsaicin application) was being used to submerge in water for the cold pressor test, the anterior side of the lower legs were used for the electrical and pressure stimulation was performed on the contralateral lower leg. The latter does not automatically have to be the case since conditioned pain modulation is not limited by stimulus location.

Tramadol and duloxetine each showed a different analgesic profile where tramadol demonstrated an analgesic response on the thermal, electrical and the mechanical models, while duloxetine did not show any significant analgesic effects (Figure 5). Tramadol, the more broadly effective analgesic of the two, produces antinociception mainly by activation of the μ-opioid receptor and inhibition of monoamine reuptake, such as serotonin, but it also has an effect on various G protein coupled receptors, ion channels, and transporters. All mechanisms contribute to the analgesic effect by inhibiting pain transmission in the central nervous system. In literature, tramadol shows analgesic effects on cold[53-56] and heat,[57] electrical[58] mechanical models,[56] and even in chemical models.[59] In contrast to previous statements, where it is believed that opioids mainly attenuate pain intensities above the PDT,[47,54] our study did also demonstrate significant effects on heat PDT.

Duloxetine is a serotonin noradrenalin reuptake inhibitor (SNRI). The antidepressant activity and pain inhibitory property of duloxetine is believed to be related to its potentiation of serotonergic and noradrenergic activity in the central nervous system where it increases descending pain inhibition by inhibiting reuptake of spinal noradrenalin and serotonin.[60,72,73] In humans, descending pain modulation can be evaluated with CPM. The pain stimulus, measured before and after another modulating pain stimulus, usually has an inhibiting effect, at least in healthy subjects.[71,74] In patients with painful diabetic neuropathy, duloxetine can improve CPM efficiency, Although this correlation was only seen in patients with a higher drug efficacy.[75] Neuropathic pain is efficaciously treated by duloxetine, but only in patients with inefficient CPM, and vice versa. Thus, patients that did not, or to a lesser extent, experience pain alleviation from duloxetine, did not show a significant change in CPM.[75] These patients already had an efficient CPM pretreatment. It is expected that the healthy subjects in our study have the same efficient CPM, explaining why there is a lack in significant CPM effects in the current study. An Alternative consideration is whether this specific CPM paradigm produces such a strong effect that it becomes difficult to increase the CPM after administration of a drug. It has been suggested that CPT temperatures between 4°C and 12°C can be sufficient to induce inhibition.[79-83] If so, duloxetine would not have been able to increase the CPM because the ceiling effect has already been reached. Finally, a lack of CPM effect could have to do with the timing of the dose administration which needs careful consideration since the duration of the cutaneous sensitization is fairly short. Recommended is to use compounds with the $T_{max}$ of 2 to 3 hours.[21] However, after ingestion of the duloxetine, there is a median two hour lag until absorption begins because of the enteric coating and the maximal plasma concentrations ($C_{max}$) of the drug occurs at approximately six hours.[68-69] Due to the pharmacokinetics of duloxetine it is possible that the hyperalgesic effect of the capsaicin might have already been worn off before duloxetine could reach its potential analgesic threshold. In addition, multiple doses of duloxetine administered over a longer period could potentially yield positive results since the analgesic onset only is observed within the first week of administration. In contrast, Schaffler et al.[28] used a similar design with a single-dose of duloxetine and was able to demonstrate a reduction in the peak-to-peak amplitude. Possibly this has to do with the study designs where the present study used a pre-defined fixed stimulation intensity in contrast to individualized intensities or with the type of laser (ND:YAP vs. CO2). Duloxetine has not been studied as extensively as tramadol in human evoked pain models. Three studies reported outcomes where duloxetine was tested in a heat model[28,61] and mechanical models.[62] The latter was tested in patients with central nerve pain and should therefore be interpreted differently than in healthy humans.

## CONCLUSIONS

The capsaicin-induced hyperalgesia/allodynia model leads to a stable primary hyperalgesia to heat after a 30 minute application of a 1% capsaicin cream, but not to a stable secondary hyperalgesia/allodynia to heat or mechanical stimulation. The test-retest analysis proved that the between-day variation was low. Moreover, the capsaicin model does not seem to interfere with other pain model after it was incorporated in the PainCart®, giving it validity in multimodal testing. Tramadol significantly affected multiple pain models in the PainCart, but duloxetine could not be demonstrated to affect the pain thresholds. For the detection of analgesic effects of duloxetine, it may be necessary to generate a stable secondary mechanical allodynia. Additional studies will now focus on improvement of the capsaicin model (*e.g.*, topical formulation) in such a way that primary and secondary hyperalgesia are both stable.

## REFERENCES


1 Treede RD, Meyer RA, Raja RA, Campbell JN. Peripheral and central mechanisms of cutaneous hyperalgesia. Prog Neurobiol. 1992;38(4):397-421.

2 Kidd BL, Urban LA. Mechanisms of inflammatory pain. Br J Anaesth. 2001;87(1):3-11..

3 Kilo S, Forster C, Geisslinger G, Brune K, Handwerker HO. Inflammatory models of cutaneous hyperalgesia are sensitive to effects of ibuprofen in man. Pain. 1995;62(2):187-193.

4 Andersen OK, Gracely RH, Arendt-Nielsen L. Facilitation of the human nociceptive reflex by stimulation of A beta-fibres in a secondary hyperalgesic area sustained by nociceptive input from the primary hyperalgesic area. Acta Physiol Scand. 1995;155(1):87-97.

5 Andersen OK, Felsby S, Nicolaisen L, Bjerring P, Jensen TS, Arendt-Nielsen L. The effect of ketamine on stimulation of primary and secondary hyperalgesic areas induced by capsaicin: a doubleblind, placebo-controlled, human experimental study. Pain 1996;66(1):51–62.

6 LaMotte RH, Lundberg LE, Torebjörk HE. Pain, hyperalgesia and activity in nociceptive C units in humans after intradermal injection of capsaicin. J Physiol. 1992;448:749-764..

7 Torebjörk HE, Lundberg LER, LaMotte RH. Central changes in processing of mechanoreceptive input in capsaicin-induced secondary hyperalgesia in humans. J Physiol. 1992;448 765–780.

8 Koltzenburg M, Lundberg LER, Torebjörk HE. Dynamic and static components of mechanical hyperalgesia in human hairy skin. Pain. 1992;51(2):207-219.

9 Zielgler EA, Magerl W, Meyer RA, Treede RD. Secondary hyperalgesia to punctate mechanical stimuli. Central sensitization to A-fibre nociceptor input. Brain. 1999;122(Pt 12):2245-2257.

10 Raja SN, Campbell JN, Meyer RA. Evidence for different mechanisms of primary and secondary hyperalgesia following heat injury to the glabrous skin. Brain. 1984;107(Pt 4):1179-1188.

11 Dahl JB, Brennum J, Arendt-Nielsen L, Jensen TS, Kehlet H. The effect of pre- versus postinjury infiltration with lidocaine on thermal and mechanical hyperalgesia after heat injury to the skin. Pain. 1993;53(1):43-51.

12 Moiniche S, Dahl JB, Kehlet H. Time course of primary and secondary hyperalgesia after heat injury to the skin. Br J Anaesth. 1993;71(2):201-205.

13 Pedersen JL, Kehlet H. Hyperalgesia in a human model of acute inflammatory pain: a methodological study. Pain. 1998;74(2-3):139-151.

14 Pedersen JL. Inflammatory pain in experimental burns in man. Dan Med Bull. 2000;47(3):168-195.

15 Kilo S, Schmelz M, Koltzenburg M, Handwerker HO. Different patterns of hyperalgesia induced by experimental inflammation in human skin. Brain. 1994;117(Pt 2):385-396.

16 Bickel A, Dorfs S, Schmelz M, Forster C, Uhl W, Handwerker HO. Effects of antihyperalgesic drugs on experimentally induced hyperalgesia in man. Pain. 1998;76(3):317-325.

17 Hoffmann RT, Schmelz M. Time course of UvA- and UvB-induced inflammation and hyperalgesia in human skin. Eur J Pain. 1999;3(2):131-139.

18 Kopper W, Likar R, Geisslinger G, Zeck G, Schmelz M, Sittl R. Peripheral antihyperalgesic effect of morphine to heat, but not mechanical stimulation, in healthy volunteers after ultraviolet-B irradiation. Anesth Analg. 1999;88(1):117-122.

19 Petersen KL, Rowbotham MC. A new human experimental pain model: the heat/capsaicin sensitization model. Neuroreport. 1999;10(7):1511-1516.

20 Dirks J, Fabricius P, Petersen KL, Rowbotham MC, Dahl JB. The effect of systemic lidocaine on pain and secondary hyperalgesia associated with the heat/capsaicin sensitization model in healthy volunteers. Anesth Analg. 2000;91(4):967-972.

21 Dirks J, Petersen KL, Rowbotham MC, Dahl JB. The heat/capsaicin sensitization model: a methodologic study in healthy volunteers. J Pain. 2003;4(3):122-128.

22 Hüllemann P, von der Brelie C, Manthey G, et al. Laser-evoked potentials in painful radiculopathy. Clin Neurophysiol. 2017;128(11):2292-2299.

23 Domnick C, Hauck M, Casey KL, Engel AK, Lorenz J. C-fiber-related EEG-oscillations induced by laser radiant heat stimulation of capsaicin-treated skin. J Pain Res. 2009;2:49-56.

24 Laird JM, Bennett GJ. An electrophysiological study of dorsal horn neurons in the spinal cord of rats with an experimental peripheral neuropathy. J Neurophysiol. 1993 Jun;69(6):2072-85.

25 Hullemann P, von der Brelie C, Manthey G, et al. Reduced laser-evoked potential habituation detects abnormal central pain processing in painful radiculopathy patients. Eur J Pain. 2017;21(5):918-926.

26 Geber C, Fondel R, Krämer HH, Rolke R, Treede RD, Sommer C, Birklein F. Psychophysics, flare, and neurosecretory function in human pain models: capsaicin versus electrically evoked pain. J Pain. 2007 Jun;8(6):503-14.

27 Mouraux A, Iannetti GD. Across-trial averaging of event-related EEG responses and beyond. Magn Reson Imaging. 2008;26(7):1041-1054.

28 Schaffler K, Nicolas LB, Borta A, et al. Investigation of the predictive validity of laser-EPs in normal, UvB-inflamed and capsaicin irritated skin with four analgesic compounds in healthy volunteers. Br J Clin Pharmacol. 2017;83(7):1424-1435.

29 Hauck M, Domnick C, Lorenz J, Gerloff C, Engel AK. Top-down and bottom-up modulation of pain-induced oscillations. Front Hum Neurosci. 2015;9:375.

30 Frølund F, Frølund C. Pain in general practice: pain as a cause of patient-doctor contact. Scand J Prim Health Care. 1986;4(2):97-100.

31 Le Bars D, Dickenson AH, Besson JM. Diffuse noxious inhibitory controls (DNIC). I. Effects on dorsal horn convergent neurones in the rat. Pain. 1979;6(3):283-304.

32 Kakigi R. Diffuse noxious inhibitory control. Reappraisal by pain-related somatosensory evoked potentials following $CO_2$ laser stimulation. J Neurol Sci. 1994;125(2):198-205.

33 Yarnitsky D, Arendt-Nielsen L, Bouhassira D, et al. Recommendations on terminology and practice of psychophysical DNIC testing. Eur J Pain. 2010;14(4):339.

34 Plaghki L, Mouraux A. How do we selectively activate skin nociceptors with a high power infrared laser? Physiology and biophysics of laser stimulation. Neurophysiol Clin. 2003;33(6):269-277.

35 Price DD. Selective activation of A-delta and C nociceptive afferents by different parameters of nociceptive heat stimulation: a tool for analysis of central mechanisms of pain. Pain. 1996;68(1):1-3.

36 Price DD. Psychological and neural mechanisms of the affective dimension of pain. Science. 2000;288(5472):1769-1772.

37 Mohammadian P, Andersen OK, Arendt-Nielsen L. Correlation between local vascular and sensory changes following tissue inflammation induced by repetitive application of topical capsaicin. Brain Res. 1998;792(1):1-9.

38 Dworkin RH, O'Connor AB, Audette J, et al. Recommendations for the Pharmacological Management of Neuropathic Pain: An Overview and Literature Update. Mayo Clin Proc. 2010;85(3 Suppl):S3-14.

39 Moulin D, Boulanger A, Clark AJ, et al. Pharmacological management of chronic neuropathic pain: revised consensus statement from the Canadian Pain Society. Pain Res Manag. 2014;19(6):328-335.

40 Bohlega S, Alsaadi T, Amir A, et al. Guidelines for the pharmacological treatment of peripheral neuropathic pain: expert panel recommendations for the middle East region. J Int Med Res. 2010;38(2):295-317.

41 Hadley GR, Gayle JA, Ripoll J, et al. Post-herpetic Neuralgia: a Review. Curr Pain Headache Rep. 2016;20(3):17.

42 Hay JL, Okkerse P, van Amerongen AG, Groeneveld GJ. Determining Pain Detection and Tolerance Thresholds Using an Integrated, Multi-Modal Pain Task Battery. J Vis Exp. 2016(110).

43 Okkerse P, van Amerongen G, de Kam ML, et al. The use of a battery of pain models to detect analgesic properties of compounds: a two-part four-way crossover study. Br J Clin Pharmacol. 2017;83(5):976-990.

44 Okkerse P, Alvarez-Jimenez R, Hay JL, et al. No evidence of potentiation of buprenorphine by milnacipran in healthy subjects using a nociceptive test battery. Eur J Pain. 2017;21(3):494-506.

45 Greffrath W, Baumgartner U, Treede RD. Peripheral and central components of habituation of heat pain perception and evoked potentials in humans. Pain. 2007;132(3):301-311.

46 Price DD, Hu JW, Dubner R, Gracely RH. Peripheral suppression of first pain and central summation of second pain evoked by noxious heat pulses. Pain. 1977;3(1):57-68.

47 Enggaard TP, Poulsen L, Arendt-Nielsen L, et al. The analgesic effect of codeine as compared to imipramine in different human experimental pain models. Pain. 2001;92(1-2):277-282.

48 Olesen AE, Staahl C, Arendt-Nielsen L, Drewes AM. Different effects of morphine and oxycodone in experimentally evoked hyperalgesia: a human translational study. Br J Clin Pharmacol. 2010;70(2):189-200.

49 Olesen AE, Brock C, Sverrisdóttir E, Larsen IM, Drewes AM. Sensitivity of quantitative sensory models to morphine analgesia in humans. J Pain Res. 2014;7:717-726.

50 Staahl C, Reddy H, Andersen SD, Arendt-Nielsen L, Drewes AM. Multi-modal and tissue-differentiated experimental pain assessment: reproducibility of a new concept for assessment of analgesics. Basic Clin Pharmacol Toxicol. 2006;98(2):201-211.

51 Petersen KL, Jones B, Segredo V, Dahl JB, Rowbotham MC. Effect of remifentanil on pain and secondary hyperalgesia associated with the heat–capsaicin sensitization model in healthy volunteers. Anesthesiology. 2001;94(1):15-20.

52 Montgomery DC. Repeated measures. In: Montgomery DC, editors. Design and analysis of experiments. New York: John Wiley & Sons Inc; 2001:624–629.

53 Enggaard TP, Poulsen L, Arendt-Nielsen L, Brøsen K, Ossig J, Sindrup SH. The analgesic effect of tramadol after intravenous injection in healthy volunteers in relation to CYP2D6. Anesth Analg. 2006;102(1):146-150.

54 Poulsen L, Arendt-Nielsen L, Brøsen K, Sindrup SH. The hypoalgesic effect of tramadol in relation to CYP2D6. Clin Pharmacol Ther. 1996;60(6):636-644.

55 Noehr-Jensen L, Zwisler ST, Larsen F, Sindrup SH, Damkier P, Brosen K. Escitalopram is a weak inhibitor of the CYP2D6-catalyzed O-demethylation of (+)-tramadol but does not reduce the hypoalgesic effect in experimental pain. Clin Pharmacol Ther. 2009;86(6):626-633.

56 Laugesen S, Enggaard TP, Pedersen RS, Sindrup SH, Brøsen K. Paroxetine, a cytochrome P450 2D6 inhibitor, diminishes the stereoselective O-demethylation and reduces the hypoalgesic effect of tramadol. Clin Pharmacol Ther. 2005;77(4):312-323.

57 Truini A, Panuccio G, Galeotti F, et al. Laser-evoked potentials as a tool for assessing the efficacy of antinociceptive drugs. Eur J Pain. 2010;14(2):222-225.

58 Filitz J, Ihmsen H, Günther W, et al. Supra-additive effects of tramadol and acetaminophen in a human pain model. Pain. 2008;136(3):262-270.

59 Hummel T, Hummel C, Friedel I, Pauli E, Kobal G. A comparison of the antinociceptive effects of imipramine, tramadol and anpirtoline. Br J Clin Pharmacol. 1994;37(4):325-333.

60 Westanmo AD, Gayken J, Haight R. Duloxetine: A balanced and selective norepinephrine- and serotonin-reuptake inhibitor. Am J Health Syst Pharm. 2005;62(23):2481-2490.

61 López-Solà M, Pujol J, Hernández-Ribas R, et al. Effects of duloxetine treatment on brain response to painful stimulation in major depressive disorder. Neuropsychopharmacology. 2010;35(11):2305-2317.

62 Vranken JH, Hollmann MW, van der Vegt MH, et al. Duloxetine in patients with central neuropathic pain caused by spinal cord injury or stroke: a randomized, double-blind, placebo-controlled trial. Pain. 2011;152(2):267-273.

63 Loudon P, Siebenga P, van Amerongen G, Gormal D, Gore K, Dua P et al. Demonstration of an anti-hyperalgesic effect of a novel pan Trk inhibitor PF-06273340 in a battery of human evoked pain models. Br J Clin Pharmacol. 2018 Feb;84(2):301-309.

64 van Amerongen G, Siebenga P, de Kam ML, Hay JL, Groeneveld GJ. Effect profile of paracetamol, Δ9-THC and promethazine using an evoked pain test battery in healthy subjects. Eur J Pain. 2018 Aug;22(7):1331-1342.

65 van Amerongen G, Siebenga P, Gurrell R, Dua P, Whitlock M, Gorman D, et al. Analgesic potential of PF-06372865, an $\alpha_2/\alpha_3/\alpha_5$ subtype selective $GABA_A$ partial agonist, demonstrated using a battery of evoked pain tasks in humans.

66 Siebenga PS, van Amerongen G, Hay JL, McDonnell A, Gorman D, Butt RP, et al. The use of pain models to detect the analgesic properties of PF-05089771, a selective $Na_V1.7$ inhibitor, in healthy subjects.

67 Current Science, Skin Permeation, and Enhancement Approaches. In: Topical and transdermal drug delivery : principles and practice. Eds.: Benson Hae & Watkinson AC. John Wiley & Sons, Inc., Hoboken, New Jersey, USA. 2012, p3 -43.

68 Lantz RJ, Gillespie TA, Rash TJ, et al. Metabolism, excretion, and pharmacokinetics of duloxetine in healthy human subjects. Drug Metab Dispos. 2003;31:1142Y1150.

69 Bellingham GA, Peng PW. Duloxetine: a review of its pharmacology and use in chronic pain management. Reg Anesth Pain Med. 2010 May-Jun;35(3):294-303.

70 Wolverton, SE. Comprehensive Dermatologic Drug Therapy. WB Saunders. 2001. pp 563-572.

71 Chen AC, Treede RD, Bromm B (1985) Tonic pain inhibits phasic

pain: evoked cerebral potential correlates in man. Psychiatry Res 14:343–351

72 Iyengar S, Webster AA, Hemrick-Luecke SK, Xu JY, Simmons RM. Efficacy of duloxetine, a potent and balanced serotonin-norepinephrine reuptake inhibitor in persistent pain models in rats. J Pharmacol Exp Ther 2004;311:576–845.

73 Smith T, Nicholson RA. Review of duloxetine in the management of diabetic peripheral neuropathic pain. Vasc Health Risk Manag 2007;3:833–44.

74 Yarnitsky D (2010) Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): its relevance for acute and chronic pain states. Curr Opin Anaesthesiol 23:611–615.

75 Yarnitsky D, Granot M, Nahman-Averbuch H, Khamaisi M, Granovsky Y (2012) Conditioned pain modulation predicts duloxetine efficacy in painful diabetic neuropathy. Pain 153: 1193–1198.

76 Hadgraft J . Passive enhancement strategies in topical and transdermal drug delivery . Int J Pharm 1999;184:1–6.

77 Marjukka Suhonen TA, Bouwstra J, Urtti A. Chemical enhancement of percutaneous absorption in relation to stratum corneum structural Alterations . J Control Release 1999;59:149–161 .

78 Chemical permeation Enhancement. In: Enhancement in drug delivery. Eds.: Touitou E & Barry BW. Taylor and Francis Group. Baco Raton, FL, USA. 2011; p233-254.

79 Lewis GN, Rice DA, McNair PJ. Conditioned pain modulation in populations with chronic pain: a systematic review and meta-analysis. J Pain 2012;13:936–44.

80 Granot M, Weissman-Fogel I, Crispel Y, Pud D, Granovsky Y, Sprecher E, et al. Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) paradigm: do conditioning stimulus painfULNess, gender and personality variables matter? PAIN 2008;136:142–9.

81 Nir RR, Yarnitsky D, Honigman L, Granot M. Cognitive manipulation targeted at decreasing the conditioning pai perception reduces the efficacy of conditioned pain modulation. PAIN 2012;153:170–6.

82 Olesen SS, van Goor H, Bouwense SA, Wilder-Smith OH, Drewes AM. Reliability of static and dynamic quantitative sensory testing in patients with painful chronic pancreatitis. Reg Anesth Pain Med 2012;37:530–6.

83 Valencia C, Fillingim RB, Bishop M, Wu SS, Wright TW, Moser M, Farmer K, George SZ. Investigation of central pain processing in post-operative shoulder pain and disability. Clin J Pain 2014;30:775–86.



**Table 1** – Summary of Demographic Characteristics.

| | Part A | Part B |
|---|---|---|
| Number of subjects | 21 | 18 |
| Sex | Male | Male |
| AGE | | |
| Mean (SD) | 24.2 (4.9) | 26.1 (5.1) |
| Range | 19 – 42 | 20 – 38 |
| RACE | | |
| White | 17 | 13 |
| Asian | 1 | 0 |
| Hispanic | 1 | 1 |
| Mixed | 1 | 0 |
| Other | 1 | 2 |
| WEIGHT (KG) | | |
| Mean (SD) | 78.2 (11.6) | 78.0 (10.3) |
| Range | 52.4 – 107.2 | 64.0 – 102.6 |
| Height (cm) | | |
| Mean (SD) | 184.7 (7.3) | 185.2 (6.1) |
| Range | 166.7 – 200.4 | 175.8 – 197.9 |
| BMI | | |
| Mean (SD) | 23.2 (2.7) | 22.8 (2.8) |
| Range | 18.8 – 29.8 | 19.4 – 28.6 |

Body mass index was defined as weight/(height × 0.01)² / SD = standard deviation.

**Table 2** – Treatment-related Adverse Events. Subjects were counted only once per treatment in each row. Included all data collected since the first dose of study drug.

| MedDra (v19.1) Preferred Term | Placebo | Tramadol | Duloxetine |
|---|---|---|---|
| Nausea | | 4 | 5 |
| Somnolence | 1 | 4 | 2 |
| Dizziness | | 3 | 4 |
| Fatigue | 1 | | 3 |
| Diarrhoea | | | 2 |
| Vomiting | | 1 | 1 |
| Dry mouth | | 1 | |
| Muscular weakness | | 1 | |
| Abdominal pain | | 1 | |
| Headache | | 1 | |
| Stress | | | 1 |
| Retching | | | 1 |

MEDDRA = Medical Dictionary for Regulatory Activities; V = version.

TABLE 3A – Summary of the primary endpoints in Part B.

| | Tramadol - Placebo 0-10hr | | | | | Duloxetine - Placebo 0-10hr | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 95% CI | | | | | 95% CI | | |
| | LSMean | Estimate of the difference | Lower | Upper | p-value | LSMean | Estimate of the difference | Lower | Upper | p-value |
| Heat thermode - normal skin PDT | 44.9 | 1.08 | 0.42 | 1.75 | **0.0023** | 44.3 | 0.5 | -0.17 | 1.16 | 0.1375 |
| Heat thermode – capsaicin skin PDT | 41.9 | 0.76 | -0.08 | 1.59 | 0.0753 | 41.7 | 0.57 | -0.3 | 1.44 | 0.1915 |
| Electrical stair PTT | 25.8 | 17.6% | 6.6% | 29.8% | **0.0023** | 23.0 | 4.5% | -5.3% | 15.2% | 0.3646 |
| Cold pressor PTT | 31.5 | 37.7% | 24.3% | 52.5% | **<.0001** | 23.5 | 2.7% | -7.2% | 13.7% | 0.5944 |
| Pressure stimulation PTT | 50.5 | 18.7% | 12.3% | 25.5% | **<.0001** | 44.0 | 3.4% | -2.2% | 9.4% | 0.2242 |

TABLE 3B – Summary of laser evoke potential endpoints in Part B. Clusters of analgesic effects for tramadol and duloxetine compared with placebo. Significant effect are in bold.

| | Tramadol - Placebo 0-10hr | | | | | Duloxetine - Placebo 0-10hr | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 95% CI | | | | | 95% CI | | |
| | LSMean | Estimate of the difference | Lower | Upper | p-value | LSMean | Estimate of the difference | Lower | Upper | p-value |
| NORMAL SKIN | | | | | | | | | | |
| Amplitude N2 | -6.98 | 1.885 | 0.61 | 3.16 | **0.0051** | -8.32 | 0.541 | -0.73 | 1.812 | 0.3919 |
| Amplitude P2 | 8.14 | -1.207 | -2.752 | 0.337 | 0.1190 | 9.21 | -0.131 | -1.687 | 1.424 | 0.8626 |
| Amplitude N2P2 | 15.07 | -3.106 | -5.744 | -0.468 | **0.0226** | 17.66 | -0.516 | -3.153 | 2.122 | 0.6923 |
| Latency N2 | 203.36 | 5.947 | -2.383 | 14.276 | 0.1525 | 203.14 | 5.727 | -2.590 | 14.044 | 0.1668 |
| Latency P2 | 319.32 | 5.813 | -2.494 | 14.120 | 0.1628 | 314.16 | 0.653 | -7.608 | 8.915 | 0.8724 |
| Reaction time | 427.43 | 38.761 | 13.426 | 64.097 | **0.0041** | 393.24 | 4.574 | -20.739 | 29.887 | 0.7136 |
| CAPSAICINE SKIN | | | | | | | | | | |
| Amplitude N2 | -7.74 | 0.352 | -1.101 | 1.805 | 0.6097 | -8.47 | -0.376 | -1.837 | 1.084 | 0.5883 |
| Amplitude P2 | 7.74 | -0.409 | -1.812 | 0.993 | 0.5519 | 9.22 | 1.067 | -0.385 | 2.519 | 0.1426 |
| Amplitude N2P2 | 15.41 | -0.605 | -2.875 | 1.665 | 0.5864 | 18.04 | 2.032 | -0.285 | 4.348 | 0.0829 |
| Latency N2 | 208.54 | 2.691 | -3.484 | 8.867 | 0.3807 | 205.51 | -0.341 | -6.534 | 5.853 | 0.9113 |
| Latency P2 | 323.86 | -5.074 | -15.125 | 4.978 | 0.3092 | 321.38 | -7.557 | -17.596 | 2.481 | 0.1339 |
| Reaction time | 401.11 | 17.514 | -5.632 | 40.659 | 0.1327 | 380.960 | -2.630 | -25.701 | 20.440 | 0.8174 |

CI= Confidence Interval; LSMeans = Least squares Means; PDT = Pain Detection Threshold; PTT= Pain Tolerance Threshold

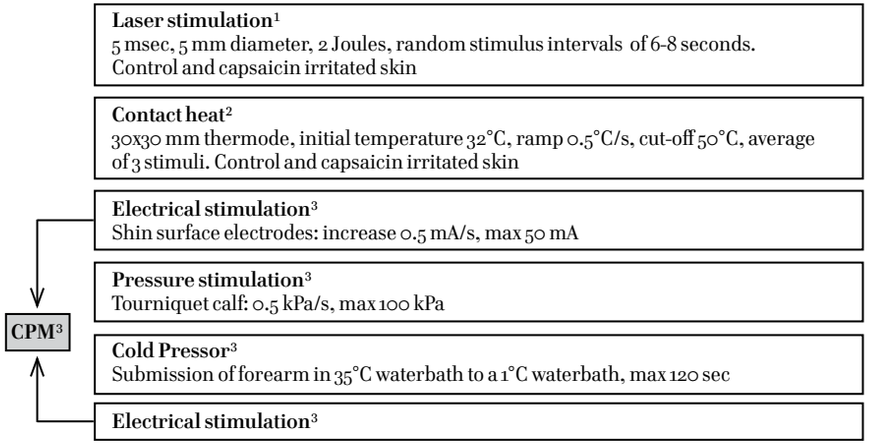


**Table 4** – Pain thresholds per time point per study. Overview of the average pain detection and tolerance thresholds per study per measurement time point. PDT is given first, then PTT.

| | Time (hr) | Present study | Study I | Study II | Study III | Study IV |
|---|---|---|---|---|---|---|
| Pressure stimulation PDT/PTT (kPa) | 1 | 11.4/41.8 | 12.6/40.5 | 21.0/48.6 | 12.5/34.5 | 17.0/40.5 |
| | 2 | 11.0/42.3 | 11.9/37.8 | 16.5/51.1 | 12.3/33.1 | 16.6/40.3 |
| | 3 | 12.2/44.5 | 13.0/40.5 | 18.3/51.4 | 12.5/35.2 | 16.7/41.7 |
| | 4 | 12.6/42.4 | 11.3/37.1 | 19.7/51.3 | 12.7/33.2 | 15.7/38.3 |
| | 6 | 11.5/43.0 | 13.1/38.6 | 23.0/50.4 | 12.0/33.5 | 16.2/38.7 |
| | 10 | 12.6/41.4 | 11.6/39.1 | 21.8/47.7 | 12.3/33.0 | 17.7/37.8 |
| Cold pressor PDT/PTT (sec) | 1 | 4.0/23.0 | 4.7/18.4 | 8.8/25.3 | 4.2/16.6 | 3.4/14.1 |
| | 2 | 3.9/23.5 | 4.2/17.9 | 7.2/26.6 | 4.4/17.5 | 3.5/13.6 |
| | 3 | 3.7/22.9 | 4.9/19.2 | 8.2/27.3 | 4.0/17.5 | 3.2/13.6 |
| | 4 | 3.8/22.2 | 5.3/18.4 | 7.0/28.5 | 4.1/17.4 | 2.7/13.0 |
| | 6 | 3.1/23.7 | 4.4/17.7 | 7.6/24.8 | 3.7/16.1 | 3.1/13.4 |
| | 10 | 3.4/21.9 | 3.6/17.4 | 6.3/24.2 | 3.7/15.6 | 3.2/12.9 |
| Electrical stair PDT/PTT (mA) | 1 | 6.0/22.3 | 6.6/19.3 | 10.8/22.2 | 7.9/19.7 | 9.0/20.9 |
| | 2 | 5.1/21.8 | 6.0/19.2 | 7.6/21.5 | 9.6/20.3 | 8.0/22.3 |
| | 3 | 6.5/21.7 | 7.2/18.8 | 8.3/22.4 | 8.5/20.0 | 9.5/21.5 |
| | 4 | 6.3/21.8 | 7.0/19.5 | 10.4/23.0 | 8.3/20.8 | 8.9/22.2 |
| | 6 | 5.6/22.2 | 5.0/17.8 | 10.1/23.4 | 8.8/20.6 | 10.2/22.3 |
| | 10 | 5.3/22 | 5.6/18.3 | 10.6/22.8 | 9.9/20.7 | 10.8/22.7 |

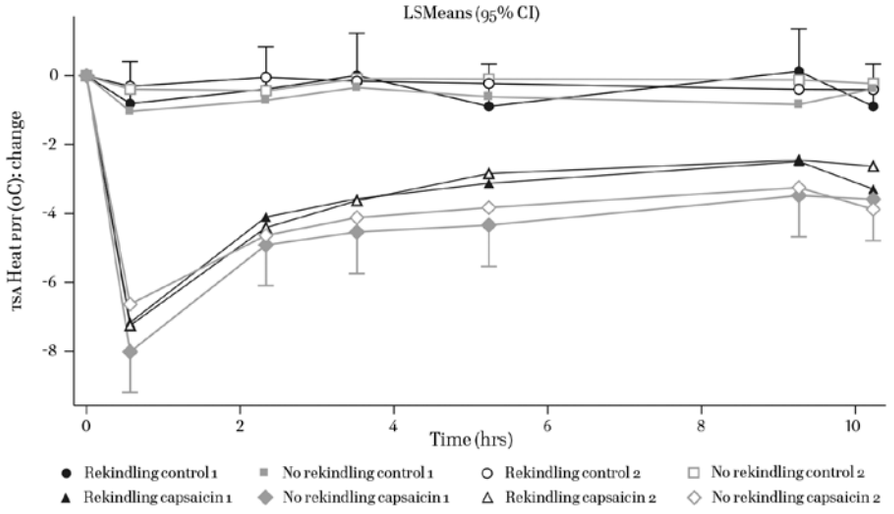
kPa= kilopascal; mA= milliampere; PDT= Pain Detection Threshold; PTT= Pain Tolerance Threshold; SEC= seconds.

**Figure 1** – Order of pain assessments in Part B.



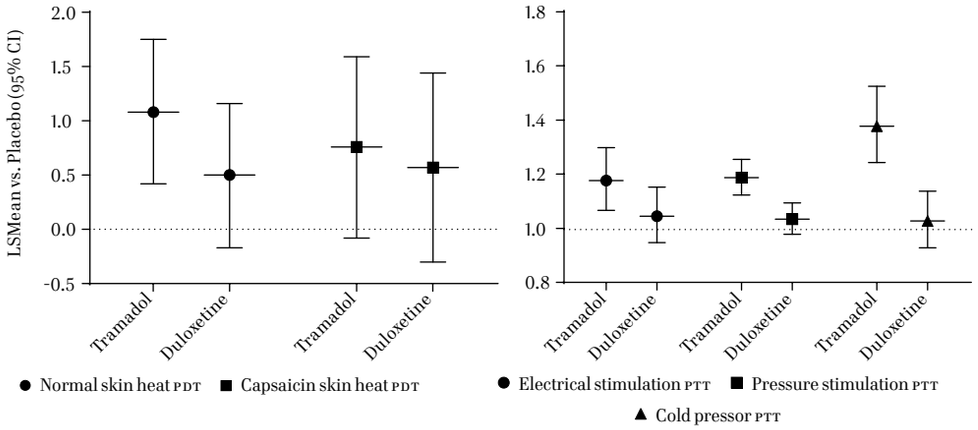

1 Laser stimulation was performed 1, 3, 6 and 8 hours post-dose. / 2 Contact heat was performed 0.5, 1, 2, 3, 4, 6, 8 and 10 hours post-dose. /3 Electrical stimulation, pressure stimulation, cold pressor and CPM were performed 1, 2, 3, 4, 6 and 10 hours post-dose. / CPM = Conditioned Pain Modulation

**Figure 2** – Time course of contact heat pain assessment in Part A. Change from baseline graph of the least squares mean PDTs over time on the capsaicin treat area and control area in the rekindle and no-rekindle groups for visit 1 and 2.




C = Celsius; CI = Confidence Interval; LSMeans = Least squares Means; PDT = Pain Detection Threshold;

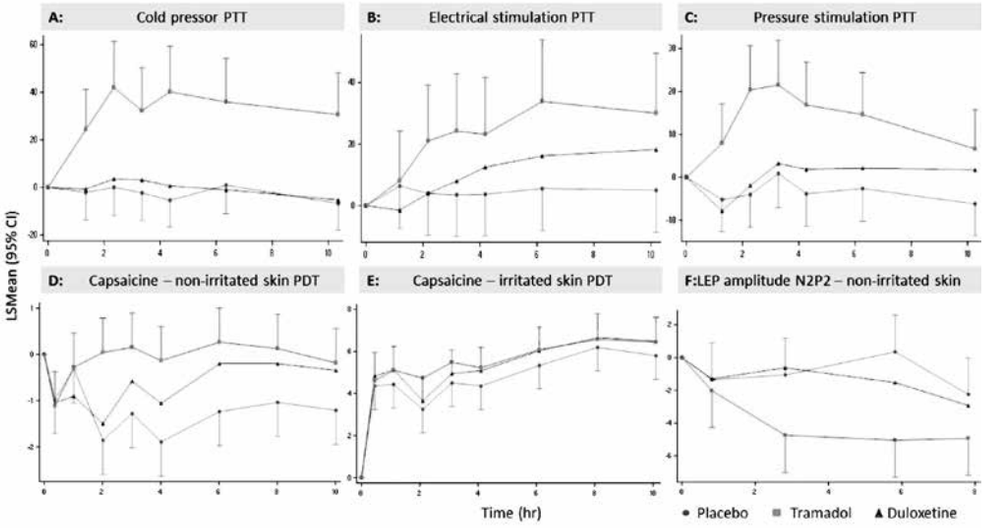
**Figure 3** – Primary analysis results Part B. Comparison of tramadol and duloxetine versus placebo with LSMeans and 95% confidence interval over the 0 – 10 hour period. Figure 3A is presented as the absolute differences to placebo, Figure 3B as changes to placebo in percentages. The dotted line represents no significant effect.




CI = Confidence Interval; LSMeans = Least Squares Mean; PDT = Pain Detection Threshold; PTT = Pain Tolerance Threshold.

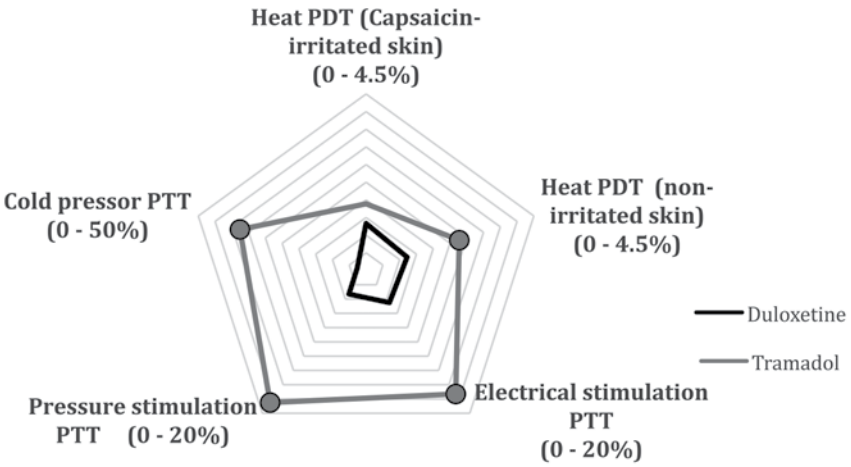


**Figure 4** – Time course of treatment effect on the pain models in Part B. Overview of change from baseline time profiles for battery of evoked pain tasks. (A) cold pressor in seconds (PTT); (B) electrical pain task in mA (PTT); (C) pressure pain task in kPa (PTT); (D) capsaicin-induced hyperalgesia model on the capsaicin irritated skin assessed with the contact heat paradigm in°C (PDT); (E) capsaicin-induced hyperalgesia model on the non-irritated (control) skin assessed with the contact heat paradigm in°C (PDT); (F) laser evoked potential on the non-irritated (control) skin N2P2 amplitude in µV.




Lines with Circles (●) = placebo; lines with squares (■) = tramadol; lines with triangles (▲) = duloxetine. PTT = pain tolerance threshold; PDT = pain detection threshold.

**Figure 5** – Star plot of effects of tramadol 100mg and duloxetine 60mg on the different pain assessments. Star plot of effects of tramadol 100 mg and duloxetine 60 mg on the pre-defined primary endpoints. Values shown are differences compared with placebo. Values marked with a dot are significantly different ($p<0.05$) compared with placebo.




PDT = Pain Detection Threshold, PTT = Pain Tolerance Threshold.