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**Effect of topical analgesia on desensitization following 8% topical capsaicin application**

**Running head: Topical analgesia does not affect capsaicin desensitization**

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## Abstract

To prevent pain associated with 8% capsaicin application, pretreatment with local anesthetics, such as EMLA (eutectic mixture of lidocaine 2.5% and prilocaine 2.5%), is considered an option. However, there is contradicting evidence regarding the effects of local analgesia on capsaicin-induced desensitization. In session 1, two skin areas in each forearm of 24 healthy volunteers were randomized to 2-hour pretreatment with EMLA/placebo cream. After pretreatment, 8% capsaicin patches were applied for 3 hours in one placebo and one EMLA pretreated area, obtaining the following four areas: Capsaicin+EMLA, Capsaicin+Placebo, EMLA alone, and Placebo. Pain intensity scores were assessed during the 3-h application of capsaicin. Warmth detection, heat pain sensitivity, and micro-vascular reactivity were measured after the removal of capsaicin. After 24 hours, in session 2, all tests were repeated followed by histamine application in each area to examine itch intensity and neurogenic flare.

Overall, EMLA caused significant reductions in capsaicin-induced pain compared with placebo ($p=0.007$) and enhanced the capsaicin-induced increase in superficial blood perfusion immediately after the 3-hour capsaicin application ($p<0.01$). Regardless of pretreatment, capsaicin induced heat hyperalgesia immediately after the application ($p<0.001$). 24 h post application, heat pain sensitivity was normalized. However, WDT increased significantly ($p<0.001$). Capsaicin tended to reduce the itch intensity and significantly reduced the neurogenic flare ($p<0.05$) induced by histamine compared with EMLA alone. The findings suggest that pre-treatment with topical analgesic cream reduces application site pain without interfering with the 8% topical capsaicin-induced desensitization.

**Perspective:** Pretreatment with local anesthetic EMLA cream might be considered a good therapeutic option to reduce the pain associated with 8% capsaicin application currently used for treatment of

neuropathic pain syndromes. This study also suggests the existence of a synergistic effect of capsaicin and EMLA on the process of neurogenic inflammation.

Key Words: Lidocaine, Prilocaine Drug Combination; Anesthetics, Local (LA); Quantitative Sensory Testing (QST); Pain; Itch

## Introduction

The transient receptor potential V1 (TRPV1) is a non-selective cation channel activated by heat and capsaicin, the pungent ingredient contained in hot chili peppers [35]. TRPV1 is expressed in the majority of polymodal nociceptors (thinly myelinated Aδ and unmyelinated C-fibers) [3,7,50] involved in the transduction of nociceptive and pruritic signals and in the pathophysiology of neuropathic pain [1]. Initially, the activation of TRPV1 by capsaicin induces an influx of $Ca^{2+}$ and $Na^{+}$ ions resulting in depolarization and thereby generating an action potential. Additionally, the activation of TRPV1 and the subsequent release of neuropeptides, such as CGRP and substance P, facilitate production of inflammatory mediators; thus producing an intense itchy and burning sensation [35]. Subsequently, a neurogenic flare arises characterized by a CGRP-induced vasodilation and a substance P-induced increase in permeability. Additionally, mast cells and neutrophils are also activated promoting inflammation [3,19,21].

The initial hypersensitization induced by capsaicin is followed by a long-lasting nerve desensitization [35]. In fact, prolonged exposure to a high concentration of capsaicin (>1%) is associated with intracellular enzymatic modifications, cytoskeletal breakdown, osmotic changes, and mitochondrial dysfunction leading to desensitization and nociceptive degeneration [1,2]. A reversible decrease in intraepidermal nerve fiber density has been detected upon capsaicin application [20,22].

Due to its ability to reduce neuronal excitability and responsiveness in TRPV1$^+$ fibers, topical capsaicin has been extensively studied as a treatment for nociceptive and pruritic diseases [2,44,54]. Previously, low-concentration capsaicin (0.025-0.1%) cream has been used for treatment of neuropathic pain syndromes and pruritus, but it requires frequent applications with low compliance. Hence, the treatment has been discarded [9,15,44]. High dose capsaicin (1-8%) requires infrequent applications, and currently topical 8% capsaicin is used clinically to treat neuropathic pain [10,29,51]. The strength of the capsaicin-induced desensitization may depend on the concentration of capsaicin used, application time, and penetration of the capsaicin through to the dermis [33,48].

The evidence regarding high concentration capsaicin as treatment of pruritus is limited, but recent findings suggest that topical 8% capsaicin may be a future antipruritic agent [2,51]. Moreover, evidence suggests that histaminergic itch transmission depends on co-option of TRPV1 receptor and is transmitted by fibers known to be capsaicin-sensitive in humans [42,51,57], in fact once histamine binds the histamine receptor H1, TRPV1 is activated downstream leading to the opening of sodium channels and subsequent itch sensation [8]. Previous studies have reported the effectivity of capsaicin pre-treatment in reducing histamine-induced neurogenic inflammation as direct consequence of substance P depletion, conduction block in nerve endings or decrease in intraepidermal nerve fiber density [12,32,47]. Primary adverse effects of topical capsaicin include pain and erythema in the application site causing discomfort for the patients [1,30]. To reduce this discomfort, administration of topical anesthetic is recommended [10,23,29]. However, although topical anesthetics may block the axonal conduction of nociceptors and consequently reduce the pain, evidence also exists that local anesthetics may block the TRPV1 receptor, thus preventing the neural desensitization induced by prolonged application of capsaicin [17,23]. Additionally, a study conducted by Leffler et al. showed that lidocaine may also potentiate capsaicin activated inward currents, indicating that lidocaine sensitized TRPV1 receptor, suggesting that local analgesics may both antagonize and potentiate TRPV1

activation [17,23,26,37]. Previously, pretreatment with the local anesthetic EMLA (eutectic mixture of lidocaine 2.5% and prilocaine 2.5%) for 1-2 h has been suggested to reduce capsaicin-induced pain, and studies have been conducted in minor study populations indicating a very time-limited pain relieving effect of EMLA [13,22,59].

However, previous studies have not investigated if EMLA pretreatment interferes with the capsaicin-induced desensitization essential for its therapeutic effect. This study aims to investigate whether capsaicin-induced cutaneous desensitization can be induced in an area pretreated with topical anesthetic cream. The hypothesis was that EMLA pretreatment could reduce capsaicin-induced pain without affecting the capsaicin-desensitizing action. Due to the potential antipruritic effect of capsaicin, the effect of EMLA and capsaicin on histaminergic itch intensity was investigated. As both histamine and capsaicin are known to induce neurogenic inflammation, the microvascular response was investigated to reveal if EMLA affected capsaicin- and histamine-induced neurogenic inflammation.

## Methods

*Subjects and Study Design*

A total of 24 (13 males and 11 females) healthy volunteers (27.5±6.5 years) were included in the study carried out at Aalborg University, Denmark. For the sample size calculation, an α-level of 0.05, a power of 0.9, and a smallest relevant difference of 30% were applied. Exclusion criteria included pregnancy, lactation, drug addiction, and medical intake as well as nociceptive conditions, dermatological, neurological, or musculoskeletal diseases. All subjects signed an informed consent. The protocol was approved by the regional Ethics Committee (N-20180034) in accordance with the Helsinki Declaration. The study was designed as a randomized, single blinded, controlled trial including two sessions separated by a 24 h interval. In both sessions, quantitative sensory tests (QSTs) were conducted along with neurogenic inflammatory response measurements to assess the effect of 3 h application of capsaicin combined with EMLA pretreatment or as monotherapy compared with control.

Session 1: In session 1, two skin areas in each forearm of 24 healthy volunteers were randomized to 2-hour pretreatment with EMLA or placebo cream. After the 2 hours of pretreatment, 8% capsaicin patches were applied for 3 hours in one placebo and one EMLA pretreated area, obtaining the following four areas: Capsaicin+EMLA, Capsaicin+Placebo, EMLA alone, and Placebo. Pain intensity scores were assessed during the 3-h application of capsaicin, followed by quantitative sensory tests: warmth detection, heat pain sensitivity, and neurogenic flare measurements (figure 1).

Session 2: Quantitative sensory tests and measurement of neurogenic flare response were repeated after 24 h and were followed by histamine application to examine the itch intensity and neurogenic flare (Figure 1).

*EMLA Pretreatment and Transdermal Capsaicin*

Two of four squared areas (4x4 cm) on the volar forearms on each subject were pretreated with topical 2.5% lidocaine/2.5% prilocaine cream (EMLA$^{R}$, AstraZeneca) for 2 h, while the last two areas were treated with a placebo cream (Neutral, Unilever, UK). After EMLA/placebo cream removal, capsaicin 8% patches (transdermal patch 8%, Qutenza, Astallas) were applied for 3 h in one of the areas treated with EMLA and in one of the areas treated with placebo cream. EMLA/placebo application and assessments were randomized between areas and right and left forearm. However, the two capsaicin patches were always applied in one area per arm. One area served as control and was not treated with neither EMLA nor capsaicin, only placebo cream. All application procedures were blinded to the test subject. During session one, EMLA and placebo cream were applied for 2 h with subsequent assessment of neurogenic flare response and QST measurements in the placebo and EMLA areas. Immediately after the measurements, two capsaicin 8% patches were applied inside the EMLA/placebo pretreated areas for 3 h. All tests were repeated after capsaicin patch removal (Figure 1).



*Assessment of Pain and Itch*

The subjects reported the sensation of pain using a numerical rating scale (NRS) ranging from 0 to 10, with 0 indicating "no pain" and 10 indicating "worst imaginable pain". An average pain NRS was collected retrospectively every hour during the 3 h capsaicin exposure.

In session two, the itch intensity was monitored for 9 min following the application of histamine dihydrochloride 1% using a 1 mm skin prick lancet. In the center of the pretreated area, a drop of histamine solution was placed and the lancet was applied using a weighted 120 g SPT device for 1-2 s. The itch intensity was assessed continuously using a computerized 100 mm VAS from 0 to 100 (eVAS Software, Aalborg University, Denmark) installed on a Samsung Note 10.1 Tablet (Samsung, Seoul, South Korea). VAS data were extracted once every 5 s. A VAS score of 0 indicated "no itch"

and 100 indicated "worst imaginable itch". The itch application was randomized between left and right arm to avoid bias during measurements. Immediately after the 9 min itch intensity assessment, the histamine-induced neurogenic inflammatory flare response was investigated.

*Assessment of Neurogenic Flare Response*

The neurogenic inflammatory flare response was quantified by assessing the superficial blood perfusion (SBP) using Full-Field Laser Perfusion Imaging ('FLPI2', Moor Instruments, Axminster, Devon, UK) placed 25 cm above the skin surface. Images were obtained immediately after removal of the EMLA/placebo cream, 3 h post, and 24 h post capsaicin application, and 10 min after histamine provocation. FLPI software (MoorFLPI Full-Field Laser Perfusion Imager V5.0) was used to analyze the laser perfusion imaging data. During analysis, the average and peak SBP were quantified by marking the square areas of 4x4 cm as regions of interest. The average flux multiplied by 50% was set as lower bound to discard pixels that do not corresponded to the flare and to match the visual flare present in the pictures of each subject [34]. Mean and peak perfusion and flare size values were extracted to investigate the neurogenic inflammation caused by EMLA, capsaicin, and histamine.



*Thermal Assessments*

A modified QST protocol based on the guidelines of the German Research Network on Neuropathic Pain (DFNS) was used [39]. The thermal assessments were obtained using a pathway sensory testing device (Medoc Ltd, Israel). A thermode stimulator probe of 3x3 cm was placed on the 4x4 cm area and kept in place by Velcro tape. The instructions given to the subjects were based on a previous publication [34].

*Warm Detection Threshold and Heat Pain Threshold*

The starting temperature of 32°C was increased by 1°C/s until the subject perceived the relevant threshold and pressed a stop button. The temperature returned to baseline at a rate of 5°C/s. For warm detection threshold (WDT), the subject was instructed to press the button when a warm sensation or an increase in temperature occurred. For heat pain threshold (HPT), the subject was instructed to press the button when a painful sensation occurred.

*Painful Supra-threshold Heat Stimuli*

Subjects were instructed to rate the pain perceived in response to two supra-threshold heat pain stimuli (STHS) with an increasing ramp of 5 °C/s and reaching a 3 s plateau at 46 °C and decreasing at 5°C/s. Each stimulation started and ended at 32°C. The subjects reported the sensation of pain using an NRS ranging from 0 to 10.



*Statistical Analysis*

For all statistical analyses, a significance level of ≤0.05 was applied. Data are presented as mean ±SEM unless otherwise stated. For each parameter, the effect size has been reported as Cohen's d value for a within-subject design. The data analysis was conducted in SPSS (IBM SPSS statistics, version 23, Armonk, USA), while the graph plotting was realized using GraphPad Prism 8.1.2 (GraphPad Software Inc., CA, USA). Normality was inspected by using a Shapio-Wilk's test for normality. Sensory parameters and imaging data were analyzed using t-tests to compare EMLA vs placebo prior to capsaicin application and using within-subject repeated measures analysis of variance (RM-ANOVA) with the factors: treatment (placebo, EMLA, placebo+capsaicin, EMLA+capsaicin) and time (0 h, 24 h, after histamine). Peak, mean, and latency were extracted from itch and pain VAS recordings following histamine application. A comparison between the temporal itch and pain profile

of each treatment group was conducted using a one-way RM-ANOVA with the factor treatment (placebo, EMLA, placebo+capsaicin, EMLA+capsaicin). Sidak post hoc comparison was applied.

## Results

All participants completed the study procedures and all obtained data have been analyzed.

*Effect of EMLA Pretreatment on Capsaicin-induced Pain*

A significant interaction between time x treatment ($F(2, 44)=7.34$, $p=0.002$) and a significant effect of treatment ($F(1, 22)=8.831$, $p=0.007$) were found. The post hoc analysis showed that EMLA pretreatment effectively alleviated the capsaicin-induced pain within the first (effect size $d=0.78$) and the third hour of capsaicin patch application (Sidak; capsaicin+EMLA vs capsaicin+placebo, 1st h: $p<0.001$, 3rd h: $p<0.05$). Within the second hour of capsaicin patch application, EMLA pretreatment tended to be more effective than the placebo pretreatment in reducing the capsaicin-induced pain (Sidak; 2nd h: $p=0.053$, Figure 2).

*Effect of EMLA Pretreatment prior to Capsaicin application*

EMLA significantly increased both warm detection threshold (WDT) and heat pain threshold (HPT) compared with placebo prior to capsaicin application (WDT: $t(23)=-7.420$, $p<0.001$, $d= 1.5$; HPT: $t(23)=-8.094$, $p<0.001$, $d=1.7$; Figure 3A). Additionally, pain to supra-threshold heat stimuli (STHS) was significantly reduced by EMLA ($t(23)=3.600$, $p=0.002$, $d= 0.8$; Figure 3B, supporting the anesthetic effect of the EMLA pretreatment.

No differences in mean and peak superficial blood perfusion (SBP) were observed between placebo and EMLA prior to capsaicin application (mean: $t(23)=-1.755$, $p=0.092$; peak: $t(23)=-1.796$, $p=0.086$, Figure 3C).

## Thermal Assessments

After capsaicin application, a time x treatment interaction was present for WDT ($F(3, 66)=13.420$, $p<0.001$). Post hoc tests showed that EMLA significantly increased WDT compared with placebo (Sidak; $p=0.023$, $d=0.7$) even after three additional hours post removal of the cream. However, in capsaicin-treated areas no significant differences were observed. After 24 h, capsaicin significantly increased WDT regardless of pretreatment (Sidak; $p<0.001$, $d=0.5$), i.e. no difference in WDT between capsaicin-treated areas (Sidak; capsaicin+EMLA vs capsaicin+placebo, $p=0.846$). These results indicate that the anesthetic effect of EMLA lasted as long as 5 h post cream application in areas not treated with capsaicin, and furthermore EMLA did not affect the capsaicin-induced increase in WDT 24 h after the capsaicin application (Figure 3D).

For HPT, the time x treatment interaction was significant ($F(3, 57.135)=53.775$, $p<0.001$). After the 3 h capsaicin application, the capsaicin significantly reduced HPT regardless of pretreatment (Sidak; $p<0.001$, $d=2.1$) and no difference was observed between the capsaicin-treated areas (Sidak; capsaicin+EMLA vs capsaicin+placebo, $p=0.66$). After 24 h, HPT recovered and no differences in HPT were observed (Figure 3E).

For STHS, the analysis revealed a significant time x treatment interaction ($F(2.186, 48.094)=23.324$, $p<0.001$) with the post hoc analysis showing that capsaicin significantly increased pain to STHS after the 3 h capsaicin application regardless of pretreatment (Sidak; $p<0.001$, $d=2.2$). Thus, EMLA pretreatment did not alter the effect of capsaicin on pain to STHS. After 24 h, no differences were observed between the three areas (Figure 3F).

*Itch and Pain Evoked by Histamine*

For mean and peak itch intensity, a significant effect of treatment was observed (Mean; $F(3, 66)=5.31$, $p<0.002$, Peak; $F(3, 66)=5.473$, $p=0.002$). Yet, capsaicin only reduced itch compared with the EMLA-treated area (Sidak; Mean: EMLA vs capsaicin+EMLA: $p=0.02$, EMLA vs capsaicin+placebo: $p=0.01$, $d=0.74$; Peak: EMLA vs capsaicin+EMLA: $p=0.02$, EMLA vs capsaicin+placebo: $p=0.02$, $d=0.7$) and not placebo-treated areas (Sidak; $p<0.20$, Figure 3G-H).

*Effects of EMLA and Capsaicin on Neurogenic Inflammation*

For both mean and peak SBP, a significant effect of time x treatment was observed (Mean: $F(6, 81.703)=1.395$, $p<0.001$; Peak: $F(3.752, 82.539)=35.974$, $p<0.001$). After the 3 h capsaicin application, capsaicin significantly increased mean and peak SBP regardless of pretreatment (Sidak; Mean: $p<0.03$, $d=1$; Peak: $p<0.001$, $d=1.5$). Capsaicin areas pretreated with EMLA induced a significantly higher mean and peak SBP compared with the capsaicin area pretreated with placebo (Sidak; Mean: $p=0.003$, $d=0.8$; Peak: $p<0.001$, $d=1.3$). No significant differences in mean and peak SBP were observed when comparing EMLA and placebo. These results indicate that EMLA enhanced the capsaicin-induced increase in SBP. No significant differences in mean and peak SBP were identified 24 h after capsaicin application. After histamine application, capsaicin significantly reduced mean and peak SBP regardless of pretreatment compared with placebo (Sidak; Mean: $p<0.02$, $d=0.7$; Peak: $p<0.05$, $d=1$). However, when comparing capsaicin-treated areas with the EMLA area, only in the capsaicin area pretreated with placebo mean and peak SBP were significantly reduced (Sidak; Mean: capsaicin+EMLA vs EMLA: $p=0.12$, capsaicin+placebo vs EMLA: $p=0.03$, $d=0.6$ Peak: capsaicin+EMLA vs EMLA: $p=0.58$, capsaicin+placebo vs EMLA: $p=0.013$, $d=0.7$, Figure 3I-J).

*Effects of EMLA and Capsaicin on Histamine-induced Axon-reflex Flare*

After histamine application, a significant difference in flare size between treatments was evident ($F(1.826, 20.163)=78.198$, $p<0.001$). A post hoc analysis revealed that capsaicin significantly reduced the histamine-induced flare size regardless of pretreatment (Sidak; $p<0.001$, $d=2.8$). No significant differences were observed between placebo and EMLA, or between capsaicin pretreated with EMLA and capsaicin pretreated with placebo. These results indicate that capsaicin affected the flare size independently of EMLA pretreatment (Figure 3K).

In figure 4, all the individual values for each parameter at the different time points are presented, while figure 5 shows the temporal itch profile following histamine application (Figure 5A) and full-field laser perfusion-imaging (FLPI) pictures after histamine application in two subjects (Figure 5B).



## Discussion

This study found that topical analgesia (EMLA cream) did not interfere with the effect of capsaicin on thermal assessments. However, EMLA enhanced a capsaicin-induced increase in neurogenic inflammation immediately after capsaicin removal. EMLA induced evident anesthesia after the 2 h application and alleviated capsaicin-induced pain during the 3 h capsaicin application. 8% capsaicin induced hyperalgesia to heat immediately after patch removal, which was replaced by an impaired ability to detect warmth 24 h post capsaicin application. This desensitization was unaffected by the EMLA pretreatment. Capsaicin tended to reduce the itch intensity while reducing the histamine-induced neurogenic flare.

*Anesthetic Effect of EMLA on Capsaicin-Induced Pain*

Pain is a well-known side effect associated with capsaicin application [16,18,51] caused by activation of TRPV1 receptors. Local anesthetics, such as EMLA, block voltage-gated $Na^+$ channels leading to disruption of the influx of $Na^+$ currents [26,31]. In previous studies, the anesthetic effect of EMLA on both low and high concentration capsaicin-induced pain showed that EMLA pretreatment reduced the capsaicin-associated pain in healthy subjects significantly within a limited time period [13,22,59]. Knolle et al. investigated the effect of EMLA on 1 h 8% capsaicin-induced pain. Only within the first 15 min of application, EMLA significantly reduced the capsaicin-induced pain [22]. Another study by Fuchs et al. investigated a 2 h EMLA pretreatment demonstrating a time-limited pain relief in a 6 h 1% capsaicin application period. Within the first 15-30 min after capsaicin application both average and peak pain ratings were significantly reduced in EMLA-pretreated areas compared with vehicle, but no differences were observed after 30 min [13]. Yosipovitch et al. showed that 1 h EMLA pretreatment significantly reduced the burning sensation induced by 0.075% capsaicin [59]. These studies suggest that EMLA pretreatment is mostly effective on low concentration capsaicin. Also in clinical settings, EMLA has been shown to relieve capsaicin-induced pain in post-herpetic neuralgia patients [54].

The present findings suggest an extended period of EMLA-induced analgesia on high concentration capsaicin-induced pain in healthy subjects compared with previous findings since EMLA induced a clear anesthetic effect after 2 h application; thus reducing the capsaicin-induced pain during the 3 h capsaicin application.

A reason for the time-limited effect of EMLA may be that as long as the dermal concentration of capsaicin is low, the EMLA-induced $Na^+$ block is effectively preventing excitation, explaining the significant effect of EMLA on low-concentration capsaicin [59]. It has been shown that a 2 h EMLA pretreatment significantly induced analgesia to needle insertion 2-3 h after cream removal. The

maximal depth of analgesia was observed during a 60 min period after a 2 h EMLA treatment, and the analgesic effect subsided gradually but would last for up to 6 h [5]. This supports the duration of the EMLA effect throughout the 3 h capsaicin application in the present study. Furthermore, evidence suggests that local analgesics may both antagonize TRPV1 activation and potentiate the inward current flow upon TRPV1 activation [17,23,26,37] suggesting an ability of local anesthetics to interfere with capsaicin-induced desensitization. The ability of local anesthetics to activate TRPV1 may contribute to the unsuccessful alleviation of capsaicin-induced pain in previous studies.

According to these previous studies, EMLA has a time-limited analgesic effect on capsaicin-induced pain [13,22], while the depth of cutaneous analgesia may vary based on application time [52]. A study conducted by Walgreen et al. showed that after 1 hour of EMLA application, a skin biopsy could be performed with acceptable pain at a skin depth of 1-2 mm. The skin depth was increased up to 3 mm after 2 h of EMLA application and up to 6 mm after 4 h treatment, showing that EMLA is able to anaesthetize free nerve endings located in the epidermis and dermis [52].

Not surprisingly, all participants experienced pain during the 3 h capsaicin application; with an evident variation between subjects. The lowest and highest overall mean pain intensities reported were 1.3/10 and 9.7/10 in the capsaicin area pretreated with placebo versus 0.7/10 and 8.0/10 in the capsaicin area pretreated with EMLA. In the present study, the statistical analysis showed no differences in pain ratings between genders as reported by another study investigating the anesthetic effects of EMLA and cooling on capsaicin-induced pain [22]. Nevertheless, high interindividual differences in capsaicin-induced pain intensities have previously been reported [2]. In clinical studies using high-concentration capsaicin and local anesthetic pretreatment, dropouts occurred due to capsaicin-related adverse events as opposed to the present study [18,53] in which the participants were young, healthy volunteers.

*Effects of EMLA and Capsaicin on Warm Detection and Heat Pain Sensitivity*

Warm detection and heat pain thresholds were increased and pain to supra-threshold heat stimuli was decreased significantly in EMLA-treated areas prior to capsaicin application indicating profound anesthesia in accordance with previous findings [59]. This is most likely due to the EMLA-induced $Na^+$ channel block in the warm and heat sensitive primary afferents [26,31]. These afferents are thought to terminate as free nerve endings in the epidermis and dermis where they can be anaesthetized by EMLA [14,36].

In the present study, the warm detection thresholds were not affected by capsaicin until 24 h after capsaicin application, in line with previous studies [43,51]. It is well known that the TRPV1 receptor responds to temperatures (43°C) above the detection temperature for warm stimuli [27,51]. It is also known that in C-fibers the TRPV1 receptor is often co-expressed with the TRPV3 receptor responsible for detecting innocuous temperature (33°C), explaining the observed results [41,45,58]. The impaired sensation of warmth is presumably caused by desensitization of the warm detection nociceptors. Upon TRPV1 activation by high concentration capsaicin, the inwards $Ca^{2+}$ flux leads to mitochondrial dysfunction and osmotic swelling resulting in impaired nociceptor function and degeneration [1]. Yet, the heat pain thresholds were not increased 24 h post capsaicin application. This may be due to capsaicin-insensitive TRPV2 receptors expressed in type I Aδ-fibers normally activated at temperatures >50°C, but during a sustained heat stimulus the heat threshold decreases to 44-49°C [28,41,49]. In accordance with the present findings, previous studies have also shown that warm detection threshold is the most profoundly desensitized parameter [51].

Immediately after the capsaicin application, the heat pain thresholds were significantly reduced and supra-threshold heat stimuli ratings were significantly increased in capsaicin areas regardless of pretreatment, supporting the interpretation that capsaicin sensitizes TRPV1 and lowers the thermal

threshold of activation of TRPV1 [46]. Similar results indicating capsaicin-induced heat pain sensitization are available [11,43].

*Itch Evoked by Histamine*

Several studies have reported that histaminergic itch depends on functional interaction between H1R, the histamine receptor, and TRPV1 on mechano-insensitive c-fibers (CMi), explaining the antipruritic potential of capsaicin. This is also confirmed by the study of Roberson et al. on neurons culture from adult mice showing that histaminergic itch is mediated by $TRPV1^+$ fibers since it can be suppressed by silencing capsaicin-activated neurons [38]. Moreover, a study conducted by Schmelz et al. on humans reported CMi fibers with a sustained response to histamine were also responsive to capsaicin [42]. The antipruritic effect of capsaicin on histamine-sensitive fibers was not entirely consistent in the present study as no difference between capsaicin areas and placebo areas was identified. Yet, capsaicin significantly reduced the itch intensity compared with EMLA. However, it is clearly indicated by the temporal itch profiles that capsaicin tends to reduce histamine-induced itch regardless of pretreatment. Since histamine was only applied 24 h post EMLA pretreatment, any effect of the EMLA pretreatment should be abolished as emphasized by the insignificant differences between capsaicin areas regardless of pretreatment and between EMLA areas and placebo areas. The half-life is 70 min for prilocaine and 110 min for lidocaine after IV administration. Obviously, skin bioavailability might slow down the elimination; however, prilocaine and lidocaine are eliminated after 24 h [56]. If this is not the case, EMLA may have modulated the pruriceptors in an unknown, itch-intensifying manner.

A study by Simone et al. showed that treatment with capsaicin 0.075% cream did not show any difference in itch response after intradermal injection of histamine [43]. More recent findings suggest that 1h application of a capsaicin 8% patch failed to reduce the peak itch intensity. Conversely, a 24

h application of capsaicin 8% effectively reduced histamine-evoked peak itch intensity [2] indicating that both application time and concentration of capsaicin influence the effect on histaminergic itch. The inhibitory effect of capsaicin on histamine-evoked itch may be explained by degeneration of $TRPV1^+$ fibers as these fibers are involved in pruriception [25,32].

*Neurogenic Inflammation Caused by Capsaicin and Histamine*

Capsaicin significantly increased the mean and peak superficial blood perfusion immediately after the 3 h application indicating an increase in neurogenic inflammation caused by release of vasoactive neuropeptides CGRP and substance P [1,40]. Even though the results prior capsaicin application indicated that EMLA did not affect the superficial blood perfusion, EMLA enhanced the capsaicin-induced increase in superficial blood perfusion after the 3 h capsaicin application. Previously, it has been suggested that lidocaine increased the flare area in skin treated with intradermally injected capsaicin [24], and it has been shown that lidocaine induces TRPV1-dependent release of CGRP [26]. Similarly, EMLA has been shown to induce erythema in untreated skin 2 h after cream removal [4]. Reduced neurogenic inflammation upon administration of histamine in capsaicin-treated skin has previously been reported and may be a result of depletion of both CGRP and substance P or of nociceptive degeneration [2,6,32,43,51]. A more recent study revealed that extended application of lidocaine on healthy human skin induced a significant degeneration of epidermal nerve fibers similar to capsaicin [55] which may also contribute to the reduction in neurogenic inflammation observed in the present study.

*Limitations*

Limitations of this study are related to the impossibility to perform participant and investigator blinding since application of capsaicin was associated with mild-to-moderate pain. Likewise, the QST

measurements after the EMLA pretreatment exposed the different pretreatments as the warm detection and heat pain threshold were substantially increased in EMLA treated areas.

*Conclusion*

The present findings suggest that topical analgesia (EMLA cream) has a significant inhibitory effect on capsaicin pain application without interfering with the thin fiber desensitizing effects of capsaicin. Interestingly, the capsaicin-neurogenic inflammation was intensified by EMLA immediately after the capsaicin application. This may be due to a synergistic effect of capsaicin and EMLA on neurogenic inflammation. However, stronger evidence is required to prove the theory. Capsaicin tended to reduce the histamine-induced itch intensity. Yet, the antipruritic effect of capsaicin needs further research.

*Author Contributions*

LAN and SLV designed the study. JDC collected and analyzed the data and wrote the manuscript draft. All authors discussed the results and commented on and approved the manuscript.



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## Figure legend

Figure 1
The study design included two sessions separated by a 24 h interval. The application and order of the experimental areas were randomized. The four 4x4cm squared areas were marked A-D. The placebo and EMLA creams were applied for 2 h. Baseline measurements were conducted in EMLA and placebo areas after the 2 h cream application. Capsaicin patches were applied for 3 h including pain ratings every 1 h. Experimental assessments included measurement of superficial blood perfusion (SBP), warm detection threshold (WDT), heat pain threshold (HPT), and pain to suprathreshold stimulation (STHS).

Figure 2
Pain scores during the 3 h capsaicin application. Overall, EMLA significantly reduced capsaicin-induced pain compared with placebo pretreated areas ($p=0.007$). The black line shows mean pain intensity for capsaicin + placebo pretreatment (Capsaicin). The grey line shows mean pain intensity for capsaicin + EMLA pretreatment (Capsaicin+EMLA). Mean±SEM shown. *: $p<0.05$, ***: $p<0.001$

Figure 3
Effects of EMLA and capsaicin on thermal assessments, histamine-evoked itch and flare, and the microvascular inflammatory response. A) EMLA significantly increased both WDT and HPT prior to capsaicin application ($p<0.001$). B) EMLA significantly reduced pain to STHS prior to capsaicin application ($p=0.002$). C) EMLA had no effect on neither mean nor peak SPB prior to capsaicin application compared with placebo. D) Capsaicin significantly increased the warm detection threshold (WDT) regardless of pretreatment ($p<0.001$) 24 h after the 3 h capsaicin application (24 h). E) Heat pain threshold (HPT) was significantly decreased in capsaicin-treated areas immediately after the 3 h capsaicin application (0 h) ($p<0.001$). F) Immediately after the 3 h application of capsaicin, a significantly higher pain intensity was measured to suprathreshold stimulation (STHS) regardless of pretreatment ($p<0.001$). G) The mean itch intensity induced by histamine was significantly reduced in capsaicin areas compared with EMLA-treated areas ($p<0.024$). H) Capsaicin pretreated with EMLA significantly reduced the mean peak itch intensity compared with EMLA ($p<0.024$). I-J) After the 3 h capsaicin application, EMLA significantly enhanced the capsaicin-induced increase in superficial blood perfusion (SBP) (Mean: capsaicin+EMLA vs capsaicin+placebo: $p=0.003$; Peak: capsaicin+EMLA vs capsaicin+placebo: $p<0.001$). After histamine application, capsaicin reduced the SBP (Sidak; Mean: $p<0.05$; Peak: $p<0.05$). K) Capsaicin significantly reduced the histamine-induced flare regardless of pretreatment ($p>0.001$). Fig. D-K: Δ-value calculated by subtracting the result from the placebo area. Horizontal dashed line indicates placebo value. I-K: mean±SEM shown. VAS: visual analogue scale; NRS: numerical rating scale; CAP+EMLA: capsaicin pretreated with EMLA; After hist: after histamine application. * indicates a significant difference from placebo area; # indicates a significant difference from EMLA area; ^ indicates a significant difference from both

placebo and EMLA areas; § indicates a significant difference from capsaicin areas; */#/^/§: $p<0.05$; **/##/^^/§§: $p<0.01$; ***/###/^^^/§§§: $p<0.001$.

Figure 4

Individual values for each parameter at the different time points. A) Warm detection threshold, B) Heat pain threshold, C) Pain intensity to suprathreshold stimulation, D) Mean itch intensity, E) Mean peak itch intensity, F) Flare size, G) Mean superficial blood perfusion, H) Mean peak superficial blood perfusion. The horizontal black bar represents the mean value.

Figure 5

Itch evoked by histamine 24 h after the 3 h capsaicin application in areas treated with placebo, EMLA, and capsaicin. A) Temporal itch profiles for EMLA (light blue line), capsaicin pretreated with EMLA (grey line), placebo (blue line), and capsaicin pretreated with placebo (black line). To improve the graphical visuals, SEM is presented using the frequency: 0.05 Hz. Data are presented as mean±SEM. B) Representative full-field laser perfusion-imaging (FLPI) pictures taken after histamine application in two subjects showing that capsaicin reduced the histamine-induced flare size. S1: subject 1; S2: subject 2; CAP: capsaicin

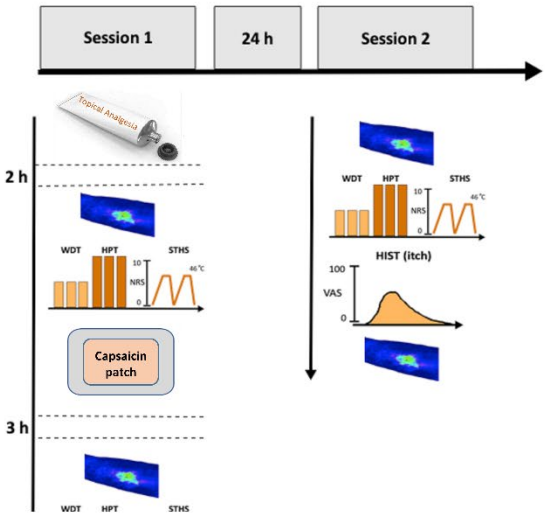


Figure 1

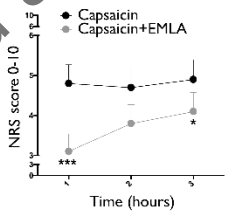


Figure 2

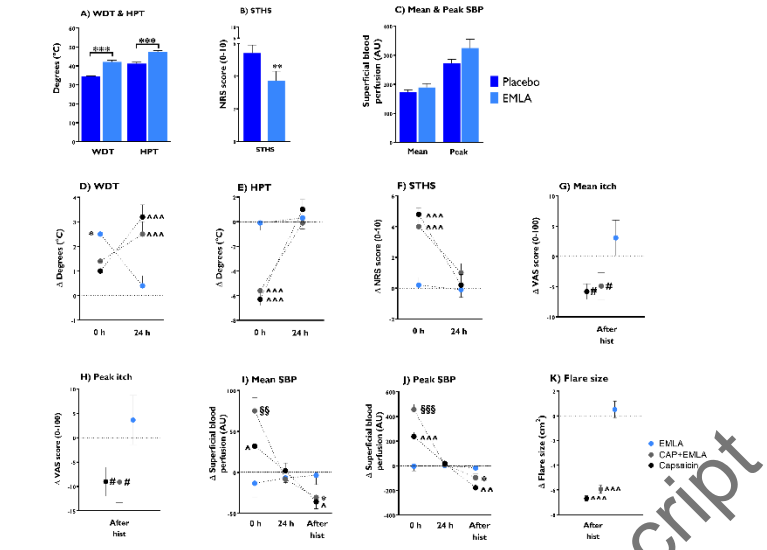


Figure 3

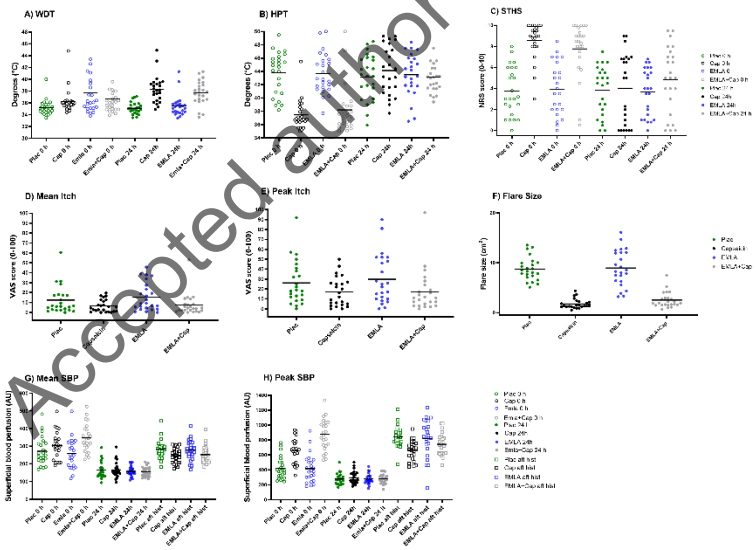


Figure 4

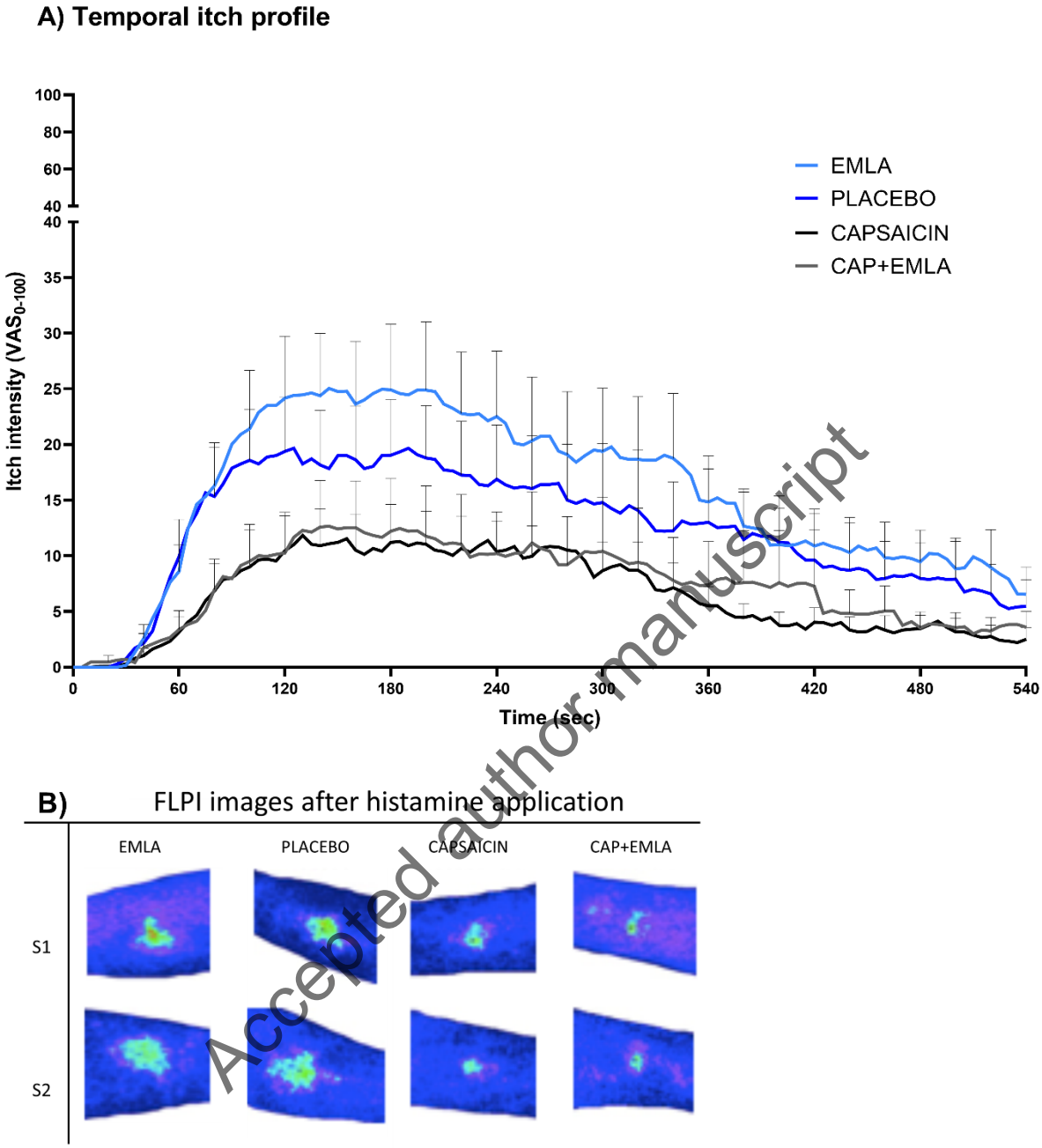




Figure 5