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Priming of central- and peripheral mechanisms with heat and cutaneous capsaicin facilitates secondary hyperalgesia to high frequency electrical stimulation

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Abstract

Heat/capsaicin sensitization and electrical high frequency stimulation (HFS) are well known model of secondary hyperalgesia, a phenomenon related to chronic pain conditions. This study investigated whether priming with heat/capsaicin would facilitate hyperalgesia to HFS in healthy subjects.

Heat/capsaicin priming consisted of a 45 °C heat stimulation for 5 min followed by a topical capsaicin patch (4x4 cm) for 30 minutes on the volar forearm of 20 subjects. HFS (100 Hz, 5 times 1s, minimum 1.5 mA) was subsequently delivered through a transcutaneous pin electrode approximately 1.5 cm proximal to the heat/capsaicin application. Two sessions were applied in a crossover design; traditional HFS (HFS-HEAT/CAP) and heat/capsaicin sensitization followed by HFS (HFS+HEAT/CAP). Heat pain threshold (HPT), mechanical pain sensitivity (MPS) and superficial blood perfusion were assessed at baseline, after capsaicin removal, and up to 40 min after HFS. MPS was assessed with pinprick stimulation (128 mN and 256 mN) in the area adjacent to both HFS and heat/capsaicin, distal but adjacent to heat/capsaicin and in a distal control area. HPT was assessed in the area of heat/capsaicin. Larger sensitivity to 128 mN pinprick stimulation (difference from baseline and control area) was observed in the HFS+HEAT/CAP session than in the HFS-HEAT/CAP session 20 and 30 minutes after HFS. Furthermore, sensitivity was increased after HFS+HEAT/CAP compared to after heat/capsaicin in the area adjacent to both paradigms, but not in the area distal to heat/capsaicin. Results indicate that heat/capsaicin causes priming of the central- and peripheral nervous system, which facilitates secondary mechanical hyperalgesia to HFS.

New and noteworthy

High frequency electrical stimulation (HFS) and heat/capsaicin sensitization are well known models of secondary hyperalgesia. The results from the current study indicate that increased sensitivity to 128 mN pinprick stimulation can be obtained when HFS is delivered following an already established heightened central hyperexcitability provoked by heat/capsaicin sensitization.

Introduction

Chronic pain is a major world-wide problem (Breivik et al. 2006) and effective and individualized diagnosis and treatment is considered highly attractive but difficult due to partly unknown mechanisms (Woodcock et al. 2007). Many chronic pain patients (17-35%) experience wide-spread sensitivity (Schliessbach et al. 2013) and hyperalgesia, which is observed in pain conditions such as postoperative pain (Lavand'homme et al. 2008) and neuropathic pain (Maier et al. 2010). Hyperalgesia, defined by IASP as "increased pain from a stimulus that normally provokes pain" (iasp-pain.org), can be observed in the area of stimulation (primary hyperalgesia) and in surrounding areas (secondary hyperalgesia). There is an extensive amount of evidence that point to a central origin of secondary hyperalgesia, but peripheral mechanisms cannot be excluded (see reviews: (Ruscheweyh et al. 2011; Sandkuhler 2009; Treede et al. 1992)). One related mechanism is long-term potentiation (LTP) of spinal plasticity, but segmental and/or descending inhibitory control may also be involved (Ruscheweyh et al. 2011). Nociceptive LTP has been induced in spinal synapses of rodents by high frequency stimulation (HFS) of primary afferent fibers (Ikeda et al. 2003; Liu et al. 1997; Randic et al. 1993). It is therefore believed that LTP may develop after initial strong painful event such as injury or operation and play an important role in the development of chronic pain in humans (Ruscheweyh et al. 2011; Sandkühler 2007).

The most common and maybe the only way to investigate pain mechanisms in humans is by use of experimental pain models. The evidence will however be somewhat indirect, but with robust and well-controlled models, understanding in relation to human pain mechanisms can be gained. Perceptual correlates of LTP have been observed in humans as increased pain sensitivity after cutaneous high frequency stimulation (HFS) through special pin electrodes (Van Den Broeke et al. 2011; Klein et al. 2004; Xia et al. 2016). Increased sensitivity has been observed in the area of HFS (e.g. Klein et al. 2004; Lang et al. 2007; Magerl et al. 2018) and secondary mechanical hyperalgesia has been observed at surrounding sites (Van Den Broeke et al. 2011; van den Broeke and Mouraux 2014; Klein et al. 2004, 2008; Lang et al. 2007; Xia et al. 2016). Other human experimental models of secondary hyperalgesia involve intradermal capsaicin injection (Koltzenburg et al. 1992; Lamotte et al. 1991) and heat-burn (Dahl et al. 1993; Werner et al. 2001), inducing stable and lasting cutaneous sensitization. To avoid discomfort and invasiveness of the capsaicin injection and skin injury and blisters to the heat-burn, heat/capsaicin sensitization model was developed by combining two relatively low intensity stimuli (Dirks and Petersen 2003). The combined model did not reveal additive effect of the two stimuli (Dirks and Petersen 2003).

HFS and the heat/capsaicin sensitization models act through somewhat similar mechanisms, but some differences are also evident. The heat/capsaicin application acts selectively on the TRPV1-positive A- and C nociceptive fibers (Caterina and Julius 1999; Magerl et al. 2001) and causes primary heat hyperalgesia (peripheral sensitization) and secondary mechanical hyperalgesia (Dirks and Petersen 2003). The HFS model acts on all epidermal primary afferent fibers, i.e. both TRPV1-postive and TRPV1-negative Aδ- and C-fibers

(Henrich et al. 2015) and has also been shown to induce secondary hyperalgesia. Secondary hyperalgesia involves both spinal and supraspinal mechanisms (Sandkuhler 2009) and contrary to the fibers involved in its induction, the pain facilitation is obeyed by the TRPV1-negative A-fibers (van den Broeke et al. 2016; Magerl et al. 2001; Ziegler et al. 1999). The current study investigated the effectiveness and feasibility of a human experimental model combined of heat/capsaicin priming of the central nervous system followed by HFS. The hypothesis was that the combined model would cause facilitated secondary hyperalgesia mediated through synergistic/additive mechanisms from the two models.

Materials and Methods

Subjects

Twenty-one healthy volunteers participated in the experiment ((11 male, 10 female ranging from 19-43; mean age 23 years), which consisted of three experimental sessions. Data from two of the three sessions will be included in this study. Participants were excluded from the study if they had a history of psychiatric or neurological disorder, previous drug- or medication abuse, were pregnant or unable to cooperate. One subject was excluded from the study prior to participation according to the exclusion criteria. All subjects signed an informed consent form after being informed about the experimental procedure. Approval was obtained from the local ethical committee (N-20160076).

Conditioning stimulation

High frequency electrical stimulation (HFS)

In both sessions, the participants received trains of 100 Hz (pulse width; 2 ms) for 1 sec. repeated 5 times at 10 sec intervals with an intensity of 10 times perception threshold or a minimum of 1.5 mA. The electrical stimulator was a DS5 constant current stimulator (Digitimer LTd; Welwyn Garden City, UK). The stimulation was performed on the volar forearm, approximately 5 cm distal to the cubital fossa with a small-diameter pin electrode (Klein et al. 2004; Poulsen et al. 2020), which consisted of 15 blunt stainless steel pins protruding 1 mm from the base with a diameter of .2 mm. The cathodal pins were placed in a circle with a diameter of 10 mm. A rectangular electrode patch (9x5 cm, Pals Neurostimulation electrode; Axelgaard, Fallbrook, CA) placed on the dorsal forearm served as an anode.

Heat and Topical Capsaicin application

In one session, the participants were treated with a heat/capsaicin sensitization paradigm prior to the HFS. A 3x3 cm thermode (Pathway, Medoc Ltd., Ramat Yishai, IL) was placed 2.5 cm distal to the center of the electrode (see Fig. 1) to deliver thermal stimulation of 45 °C for 5 min. Subsequently, a 4x4 cm Qutenza 8% Capsaicin patch was placed on the same location for 30 minutes. The thermode was placed on top of the capsaicin patch at 32° to control for normal skin temperature. After removal of the patch, a Qutenza cleaning gel was applied for 1 minute. The remainder of the gel and capsaicin was removed using paper towels.

Conditioning- and test stimulation areas

The forearm was divided into areas for applying the different test- and conditioning stimuli, which were marked on the subjects. The drawing in Fig. 1 illustrates the areas where the two conditioning stimulation paradigms and pin prick stimulations were applied (A1, A2, A3). A1 was considered as the main test area where the pin prick sensitivity to either HFS (session 'HFS-HEAT/CAP') or the combined HFS and heat/capsaicin sensitization (session 'HFS+HEAT/CAP') could be compared (see protocol below). A2 was considered to represent an area, which was mainly sensitized due to the heat/capsaicin and A3 served as a non-conditioning control area.

Figure 1 – The areas used for conditioning – and test stimuli. All stimulation was performed on the volar forearm. The areas where high frequency stimulation (HFS) and heat/capsaicin were applied are marked with arrows and the three areas where pin prick stimulation was performed are referred to as A1, A2, and A3. The heat pain threshold was also measured in the area of heat/capsaicin.

Variables measured

Perception threshold

The perception threshold was identified using the method of limits to determine the HFS intensity. Participants were asked to press a button when they became aware of the presence or absence of a single 2 ms square pulse, which was delivered at 0.5 Hz. After each pulse, the amplitude was slowly increased or decreased 5% using a custom-made stimulation software (LabVIEW, National Instruments). The staircase procedure was repeated 3 times and the average of 3 upper and 3 lower values was calculated as the perception threshold.

Pain to HFS

Participants were asked to rate their sensation to each of the five pulse trains of the HFS on a Numerical Rating Scale (NRS) ranging from 0 (no sensation at all) to 10 (worst pain imaginable) with 5 being the pain threshold. Participants responded verbally and were free to use integers and decimals.

Mechanical pain sensitivity

In order to quantify the amount of secondary hyperalgesia (i.e. the increased sensitivity around the conditioning stimulation), mechanical pain sensitivity (MPS) of the subjects was evaluated by performing pin-prick stimulations with 128 mN and 256 mN at baseline (before any conditioning stimulation) and 10, 20, 30 and 40 minutes after HFS. Participants were stimulated three times within each area (A1, A2, or A3, see Fig. 1), after which they reported their average sensation on a NRS ranging from 0 (no sensation at all) to 10 (worst pain imaginable) with 5 being the pain threshold. Participants responded verbally and were free to use integers and decimals. All measurements were performed twice using a randomized order for both weight and location. The participants were asked to close their eyes or look away from the arm during the pin-prick stimulation.

Heat-pain threshold

To examine primary heat hyperalgesia (peripheral sensitization) to the heat/capsaicin paradigm, the heat pain threshold (HPT) was found in the area where the heat/capsaicin was applied at baseline, immediately after heat/capsaicin, and at 10, 20, 30 and 40 minutes after HFS. An increasing heat stimulus of 1°C/s was delivered from a baseline temperature of 32°C with the thermode (Medoc Ltd, Israel) until the subjects pressed a button indicating a change in sensation from warm to painful. This procedure was repeated three times with a randomized time from 5-20 s in between. The average of the three temperatures was used to report the HPT.

Superficial blood perfusion

The superficial blood perfusion was measured using a Full-Field Laser Perfusion Imaging (‘FLPI’, Axminster, Devon, UK). The forearm was placed on a black surface, 35 cm underneath the device. Single images were obtained at baseline, immediately after the heat/capsaicin and 10, 20, 30 and 40 minutes after HFS.

Protocol

Participants were familiarized with the staircase procedure for determining the perception threshold of the electrical pulses, the HPT and the pin-prick stimuli in a separate 30 minutes session, at least two days prior to the experimental sessions. The order of the two experimental sessions was randomized to avoid bias. To avoid interference of lateral dominance, the order of paradigms and dominant side was balanced across subjects. Each session started with a brief summary of the methods and time plan used in the upcoming session. In the beginning, the participants were seated comfortably on a chair, with their lower arm placed horizontally on a table in front of them. The two experimental sessions will be referred to as session ‘HFS+HEAT/CAP’ and ‘HFS-HEAT/CAP’.

Session ‘HFS-HEAT/CAP’: Each session started with baseline measurements including FLPI imaging, HPT and pin-prick stimulation. The perception threshold was found and subsequently the HFS was performed. 10, 20, 30 and 40 minutes after the HFS, the test measures were carried out; FLPI imaging, HPT and pin-prick stimulation.

Session 'HFS+HEAT/CAP': The same baseline measurements were performed as described in former session and the perception threshold was afterwards identified. Following that, the heat/capsaicin conditioning was applied, which lasted 35 minutes. After removal of the capsaicin patch, the test measures were carried out and following that the HFS was performed. The test measures were performed 10, 20, 30 and 40 minutes after HFS.

Figure 2 – Timeline for the experimental protocol. The gray shaded areas where only performed in session 'HFS+HEAT/CAPS', making that session 40 minutes longer. HPT = Heat pain threshold, MPS = Mechanical pain sensitivity, FLPI = Full-Field Laser Perfusion Imaging, HFS = High frequency stimulation

Data analysis

The perception threshold and intensity used to deliver HFS were both compared with a paired t-test between the two experimental sessions. The perceived sensation to the HFS paradigm, pinprick stimuli, and heat-pain thresholds were analyzed separately using repeated-measures analysis of variance (RM-ANOVA). In case of violation of sphericity, Greenhourse-Geisser correction was used. Normal distribution of the studentized residuals was evaluated by the Shapiro-Wilk test of normality ($p > 0.05$ indicated normal-distribution). Sidak correction of the p-value was applied for multiple comparisons and p values ≤ 0.05 were considered statistically significant.

Pain to HFS

A two-way RM-ANOVA was used to compare the pain ratings to HFS. The model included two within-subject variables: session (HFS+HEAT/CAPS and HFS-HEAT/CAPS) and stimulation no. (1, 2, 3, 4, 5).

Mechanical pain sensitivity

The pinprick stimuli ratings were normalized to baseline and the unconditioned control area (A3) and then compared between the experimental sessions (HFS+HEAT/CAPS and HFS-HEAT/CAPS) and time (10-, 20-, 30-, and 40 min after HFS) with a two-way RM-ANOVA for both weights. The ratings to pinprick stimuli were furthermore compared between areas (A1 and A2) and time (post Caps, 10-, 20-, 30-, and 40 min after HFS) for the HFS+HEAT/CAPS session using a two-way RM-ANOVA for both weights.

Heat-pain threshold

To evaluate the primary hyperalgesia caused by the heat/capsaicin sensitization paradigm, the heat pain threshold in the area of heat/capsaicin application was compared between baseline, immediately after capsaicin application and at the four time points after HFS using a one-way RM-ANOVA in the HFS+HEAT/CAPS session. Same analysis was used for the HFS-HEAT/CAPS session.

FLPI

This is the accepted author manuscript of the article:
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The FLPI variables include a grayscale image, a flux image and a colored image. Using the flux image, the superficial blood perfusion values (mean flux) were extracted in the area of HFS, i.e. a circular area of 1.5 cm^2 in diameter directly underneath the small diameter pin electrode (Moor FLPI Review). A two-way RM-ANOVA was used to compare the blood perfusion in the area of HFS (1.5 cm^2) between session (HFS+HEAT/CAPS and HFS-HEAT/CAPS) and time (baseline, 10-, 20-, 30-, and 40 min after HFS). The area of flare was furthermore calculated in the HFS+HEAT/CAPS session at the time point immediately after removal of capsaicin using a threshold of baseline + twofold standard deviation (Terkelsen et al. 2014).

Results

Intensity used for HFS

The perception threshold (mean ± standard error) were not different between the two sessions (119.86 μA± 12.98 μA and 109 μA ± 14.33 μA for session 'HFS-HEAT/CAP' and 'HFS+HEAT/CAP', respectively). As the minimum intensity was set to 1.5 mA the average intensities used for HFS were: 1.62 mA ± 0.08 mA and 1.63 mA ± 0.07 mA for session 'HFS-HEAT/CAP' and 'HFS+HEAT/CAP', respectively (n.s, $p = 0.95$).

Pain to HFS

Pain ratings to HFS (see Fig. 3) were higher when the subjects had received the heat/capsaicin paradigm prior to HFS (main effect, $F(1,19) = 5.130$, $p = 0.035$). The main effect of time was not significant ($F(2.50,47.45) = 2.85$, $p = 0.057$).

*Figure 3 – Sensitivity ratings on a NRS (0-10, 5: pain threshold) to the high frequency stimulation (HFS) in the two experimental sessions. Asterisk indicate significant main effect of paradigm, * $p < 0.05$.*

Mechanical pain sensitivity

Results for ratings to pinprick stimuli in the area between HFS and heat/capsaicin paradigms (A1) are shown in figure 4. Analysis of the pinprick ratings to low weight pin prick stimuli, 128 nM, revealed a significant interaction ($F(3,54) = 2.77$, $p = 0.05$), see Fig. 4. Post-hoc comparisons showed larger ratings to pinprick stimuli at 20- and 30 minutes after HFS in session 'HFS+HEAT/CAP' than in session 'HFS-HEAT/CAP' (20 min: $p = 0.017$, 30 min: $p = 0.041$).

For the 256mN pin prick stimulation, results showed a significant interaction between paradigm and time ($F(4,76) = 2.62$, $p = 0.032$). No differences were observed between the paradigms, but the interaction can be explained by higher pinprick ratings in 'HFS-HEAT/CAP' at 40 minutes after HFS compared to 20- and 30 minutes after HFS.

*Figure 4 – The ratings (NRS difference from baseline and control area) to pinprick stimulations at 10 min, 20 min, 30 min, and 40 min after high frequency stimulation (HFS) for the two experimental sessions in A1 (area between the paradigms). HFS+HEAT/CAP: Heat/capsaicin priming before HFS, HFS-HEAT/CAP: HFS without heat/capsaicin priming. Left) 128 mN pinprick stimulation, right) 256 mN pinprick stimulation. Asterisks indicate differences between paradigms from post hoc comparison with Sidak correction, *, p < 0.05.*

The results on pinprick ratings in the HFS+HEAT/CAP session are shown for areas A1 (between heat/capsaicin and HFS applications) and A2 (distal to heat/capsaicin application) and time points including the ratings immediately after heat/capsaicin and at the four time points after HFS in Fig. 5. For the low weight, 128mN, interaction between time and area was observed ($F(4,76) = 3.03$, $p = 0.023$). The interaction is explained by larger ratings 20-40 minutes after HFS than immediately after capsaicin for area A1 but not for area A2 (see Fig, 5). No differences were found between the areas at the individual time points. No statistical effects were observed for the 256 mN pinprick stimulation.

*Figure 5 – The sensitivity ratings (NRS difference from baseline and control area) to pinprick stimulations in the HFS+HEAT/CAP session after capsaicin removal (Post caps), and 10 min, 20 min, 30 min, and 40 min after high frequency stimulation (HFS) in areas A1 and A2. A1: Area between HFS and heat/capsaicin application, A2: area distal to heat/capsaicin application. Left) 128 mN pinprick stimulation and right) 256 mN pin prick stimulation. Asterisk indicate post hoc differences for area A1 with Sidak correction, * p < 0.05, ** p < 0.01.*

Heat pain threshold

Analysis of HPT (see Fig. 6) in the heat/capsaicin sensitized area revealed a main effect of time ($F(2.52,47.95) = 45.94$, $p < 0.001$). Post hoc comparisons showed a decrease in HPT for all post-treatment measurements compared to baseline ($p < 0.001$). There was furthermore a tendency for the HPT to increase linearly from the moment capsaicin was removed until the end of the session (Fig. 6). No differences were observed in HPT in the 'HFS-HEAT/CAP' session.

*Figure 6 – The heat pain threshold (HPT) in the area of heat/capsaicin. Left) HPT in session 'HFS+HEAT/CAP' at baseline, directly after removal of the capsaicin patch (Post caps), and 10, 20, 30 and 40 minutes after high frequency stimulation (HFS). Right) HPT in session 'HFS-HEAT/CAP' at baseline and 10, 20, 30 and 40 minutes after HFS. Asterisk indicate post hoc difference from baseline, *** p < 0.001.*

Superficial blood perfusion

The superficial blood perfusion (the mean flux within the area underneath the HFS electrode) is shown in Fig. 7. Analysis revealed a two-way interaction between session and time ($F(2.59,40.24) = 12.99$, $p < 0.001$), which is explained by a larger blood perfusion in the 'HFS+HEAT/CAP' session 10 and 20 minutes after HFS compared to session 'HFS-HEAT/CAP'. At 30 and 40 minutes after HFS, the average flux had decreased in both sessions, and there were no observed differences between the sessions. The area of flare in the 'HFS+HEAT/CAP' immediately after heat/capsaicin application was 39.92 ± 3.40 cm^2.

*Figure 7 – The superficial blood perfusion (mean flux) in a 1.5 cm^2 circular area of high frequency stimulation (HFS) (mean ± standard error) at baseline and 10, 20, 30, and 40 minutes after HFS for the two sessions. Asterisks indicate significant differences from post hoc comparison with Sidak correction, ** $p < 0.001$.*

Discussion

This study showed that priming the central- and peripheral nervous system with a heat/capsaicin application increased pain ratings to high frequency electrical stimulation and it further indicated an enhancement of the amount of secondary hyperalgesia in the area between the application areas of the two paradigms.

Mechanisms involved

The mechanisms underlying secondary hyperalgesia, observed in many chronic pain conditions, are believed to involve facilitated primary afferent input, which causes sensitization of nociceptive neurons in the spinal cord (Iannetti 2013 – find better ref?). The current study is the first study to the authors knowledge, which combines heat/capsaicin and HFS experimental models of secondary hyperalgesia where heat/capsaicin was used to prime the central- and peripheral nervous system, followed by HFS. The two methods are believed to act through somewhat overlapping mechanisms. Possible mechanisms involved in the induction and facilitated pathways in the two models and the combined model will be discussed.

Heat/capsaicin induced secondary hyperalgesia

The heat/capsaicin application acts selectively on the capsaicin-sensitive (TRPV1-responsive) fibers, which counts approximately 80% of the peripheral nociceptors (Michael and Priestley 1999) including most C-fibers (Schmelz et al. 2000) and type II A-fiber mechano-heat nociceptors (type II AMHs) (Ringkamp et al. 2001). Two A-type nociceptors, namely the high-threshold mechanoreceptors (HTMs) and type 1 AMHs have not been shown to respond to the action of capsaicin (Magerl et al. 2001; Ziegler et al. 1999). A prolonged application of capsaicin causes desensitization of the TRPV1-responsive fibers (Henrich et al. 2015), which is also used as a treatment of neuropathic pain (Finnerup et al. 2015) but as some afferent fibers, likely involved in neuropathic pain syndrome, are insensitive to capsaicin, the treatment may be partially ineffective (Magerl et al. 2001; Sindrup and Jensen 1999). Short-term action of capsaicin causes sensitization of the capsaicin-sensitive fibers and induces secondary mechanical hyperlgesia as pain ratings to pin prick stimulation and pin prick evoked potentials are increased in a capsaicin sensitized state (Iannetti et al. 2013; Lamotte et al. 1991). Secondary hyperalgesia to capsaicin injection was abolished during A-fiber block, but not during desensitization of capsaicin-responsive fibers (Magerl et al. 2001). Therefore the A-fiber nociceptors, which are not responsive to capsaicin (type 1 AMHs and HTMs) are believed to be involved in the facilitated pathway (Magerl et al. 2001). The mechano-insensitive C-fibers or „silent C-fibers“ are also believed to be involved in secondary hyperalgesia to capsaicin (Serra et al. 2004).

There are some controversies in the literature regarding heat hyperalgesia to capsaicin. Primary hyperlagesia to heat is well established (Hughes et al. 2020; Lamotte et al. 1991) as also observed with decreased HPT in the current study. A 1-2 cm zone of hyperalgesia to heat has been observed in few studies, which may still be within the primary hyperalgesic area (Lamotte et al. 1991; Torebjörk et al. 1992). One study however observed desensitization to heat stimuli in the area of injection (Ali et al. 1996), but the area of stimulation with heat may affect the response, since a small laser was used in the study by (Ali et al. 1996) compared to the 3x3 contact heat thermode applied in the current study. Secondary heat hyperalgesia has to the author knowledge not been observed (Ali et al. 1996; Hughes et al. 2020).

A recent study observed a drop in electrical pain threshold in primary and secondary area of capsaicin injection (Hughes et al. 2020) supporting the increased pain to HFS observed in the secondary area in the current study.

HFS induced secondary hyperalgesia

HFS through small diameter pin electrodes acts on both C and Aδ fibers as reduced pain ratings to HFS were recently shown during block of capsaicin-responsive fibers with long-term capsaicin desensitization and during A-fiber block (Henrich et al. 2015). HFS causes secondary hyperalgesia (e.g. Van Den Broeke et al. 2011; Klein et al. 2004), which recently was shown to be heavily reduced under block of TRPV1-positive fibers and reduced to some extent during A-fiber block (Henrich et al. 2015). This indicates HFS acts on both TRPV1-positive and TRPV1-negative fibers, which both contribute to the development of secondary hyperalgesia (Henrich et al. 2015). Slightly different from the capsaicin model, where the facilitated pathway in secondary hyperalgesia is likely mediated by the TRPV1-negative fibers, which include both HTMs and type 1 AMHs (Magerl et al. 2001), van den broeke and colleagues recently proposed that the secondary hyperalgesia to pin prick stimulation after HFS was only mediated by the HTMs and not by type 1 AMHs. This was based on a study where perception to long-lasting heat stimuli was not increased in the area of secondary hyperalgesia (van den Broeke et al. 2016). They have further proposed that nonnociceptive somatosensory input could also contribute to the enhanced responses to mechanical pinprick stimuli, since enhanced vibrotaticle event related potentials (ERPs) (van den Broeke and Mouraux 2014) and ERP to transcutaneous electrical nerve stimulation (TENS) (van den Broeke et al. 2010) have been observed.

Similar to the capsaicin model there are some controversies in the literature regarding heat hyperalgesia to HFS**.** One study has showed secondary heat hyperalgesia to thermonociceptive laser stmuli after HFS but thermonociceptive ERPs were unaffected by HFS (van den Broeke and Mouraux 2014). They suggetsed that the secondary heat hyperalgesia could be mediated by the quicly adapting, heat-sensitive C-fibers and not type II AMHs (van den Broeke and Mouraux 2014). In the current study, no differences were observed in HPT in the secondary area of HFS, which is in line with studies where no change in heat sensitivity was observed (Lang et al. 2007; Xia et al. 2016).

Synergistic mechanisms of heat/capsaicin and HFS

The results indicate that greater secondary hyperalgesia is obtained in the combined model than when only HFS is applied (Fig. 4) and also when only heat/capsiacin is applied as increased sensitivity was observed after HFS in the area proximal to capsaicin but not in the area distal to capsaicin (Fig. 5). This was however only shown for 128 mN pinprick stimulation, but results for 256 mN followed a similar trend.

In the current study, the largest blood perfusion and drop in HPT were observed immediately after capsaicin removal. Both of these effects diminished in the following time period opposite to the increased sensitivity to pinprick in the HFS+HEAT/CAP session, which was maximized 30 minutes after HFS (Fig. 4). A likely explanation of the difference between the paradigms is that the heat/capsaicin priming caused an increased sensitivity/activity in the central cells, which is in line with the general blief that secondary hyperalgesia is centrally mediated (Lamotte et al. 1991; Schmelz et al. 2000).

Another possibility is that increased peripheral sensitization following heat/capsaicin facilitates the induction of secondary hyperalgesia, which maintains for at least 30 minutes despite a concurrent decrease blood perfusion and increase in HPT. This could be explained by the larger blood perfusion in the HFS+HEAT/CAP session. Pain ratings to HFS performed shortly after capsaicin removal were furthermore facilitated in the combined model (Fig. 3). This is likely due to priming of the capsaicin-responsive fibers with heat and capsaicin, which are also a part of the fibers mediating HFS induced pain (Henrich et al. 2015). Whether this peripheral priming could have caused delayed central priming cannot be excluded.

Stimulation methods/parameters

In this study, two experimental pain paradigms were applied sequentially. As both methods cause moderate to high discomfort, participant discomfort had to be considered. The participants rated the pain to heat/capsaicin relatively mild (data not shown) compared to severe pain ratings observed after capsaicin injection (Lamotte et al. 1991) and it is unknown whether a more robust priming had been caused by capsaicin injection. Neither was rekindling of the heat/capsaicin method was performed (Dirks and Petersen 2003) and therefore unclear whether maintaining increased peripheral sensitization would have affected the results. To further limit discomfort, a relatively low intensity for HFS was also applied compared to studies from Van den Broeke and colleagues (van den Broeke et al. 2016; Van den Broeke et al. 2019; Gousset et al. 2020), which could have affected the amount of secondary hyperalgesia.

Implications

The current model is considered to have wide range of potential implications both within experimental and clinical purpose. It is first of all considered to improve existing models of neuropathic pain causing both primary heat hyperlagesia and facilitated secondary hyperalgesia. In relation to clinical applicability, the model can simulate a sensitized state of the central nervous system, which is more prone to the HFS-induced sensitization. The model can therefore be considered to resemble patients in a vulnerable state (experimentally senstized by capsaicin) who are more prone to developing long lasting pain after injury (experimental HFS). Based on these speculations, the current model is considered highly useful within pain diagnostics, pharmacological testing, or even for prediction of postoperative pain.

Conclusion

This study showed the combined model of heat/capsaicin and HFS causes greater mechanical pinprick sensitivity to 128 mN pinprick stimulation than HFS without priming with heat/capsaicin, and, increased pinprick sensitivity in A1 (area between the paradigms) was observed after HFS compared to after immediate removal of capsaicin. This increase is likely explained by the addition of HFS rather then time as this increase was not observed distally to capsaicin outsside the area of HFS induced secondary hyperalgesia. The two models may therefore cause synergistic peripheral and/or central mechanisms facilitating hyperalgesia and mimicking widespread increase in pain observed in many chronic pain conditions.

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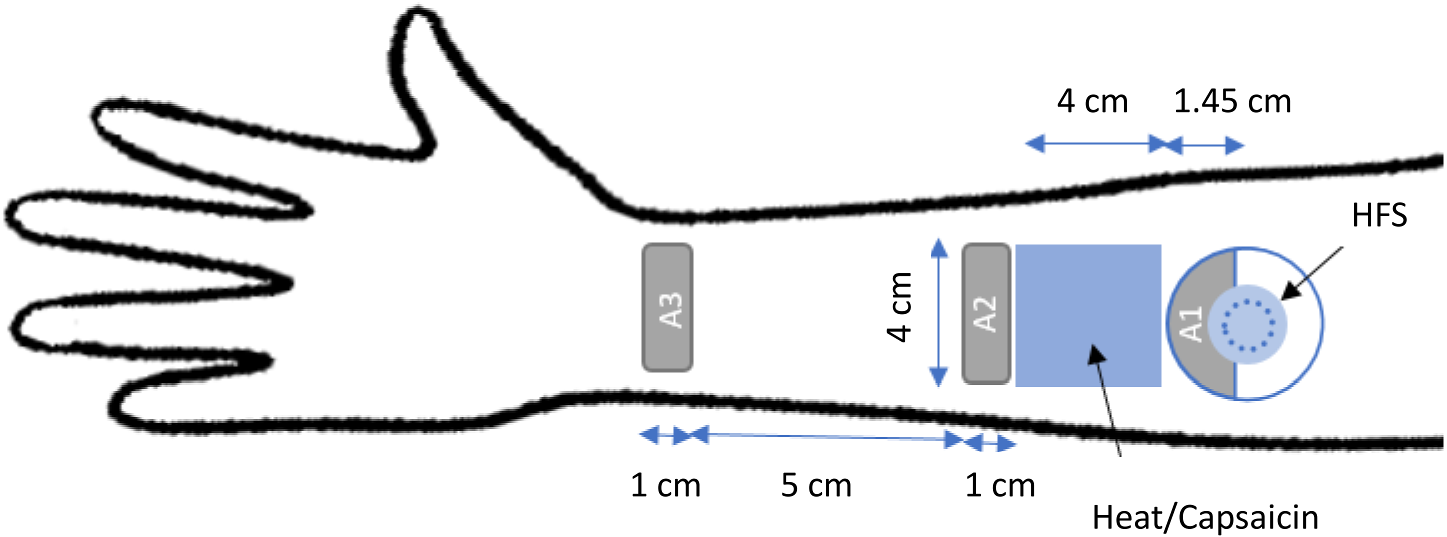

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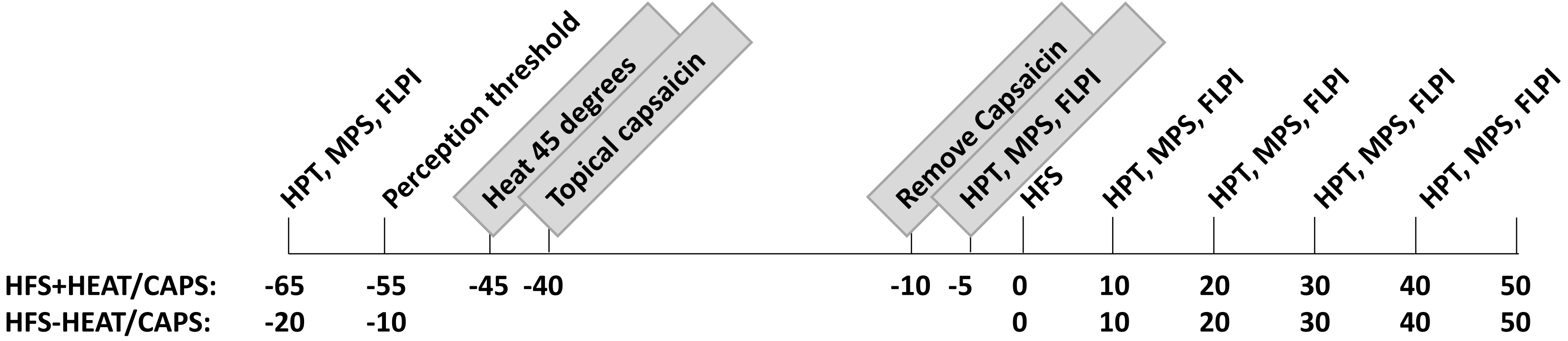

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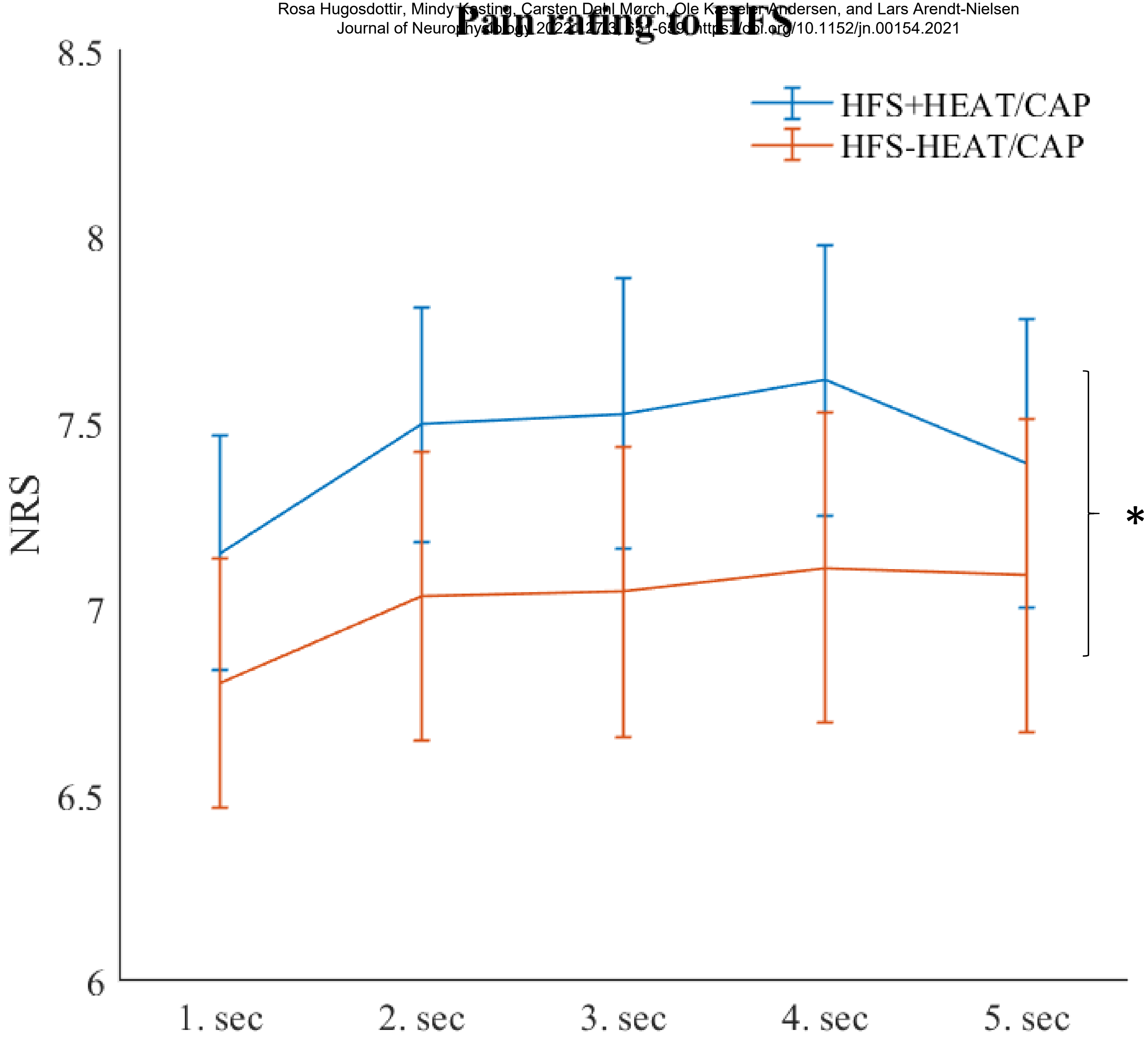

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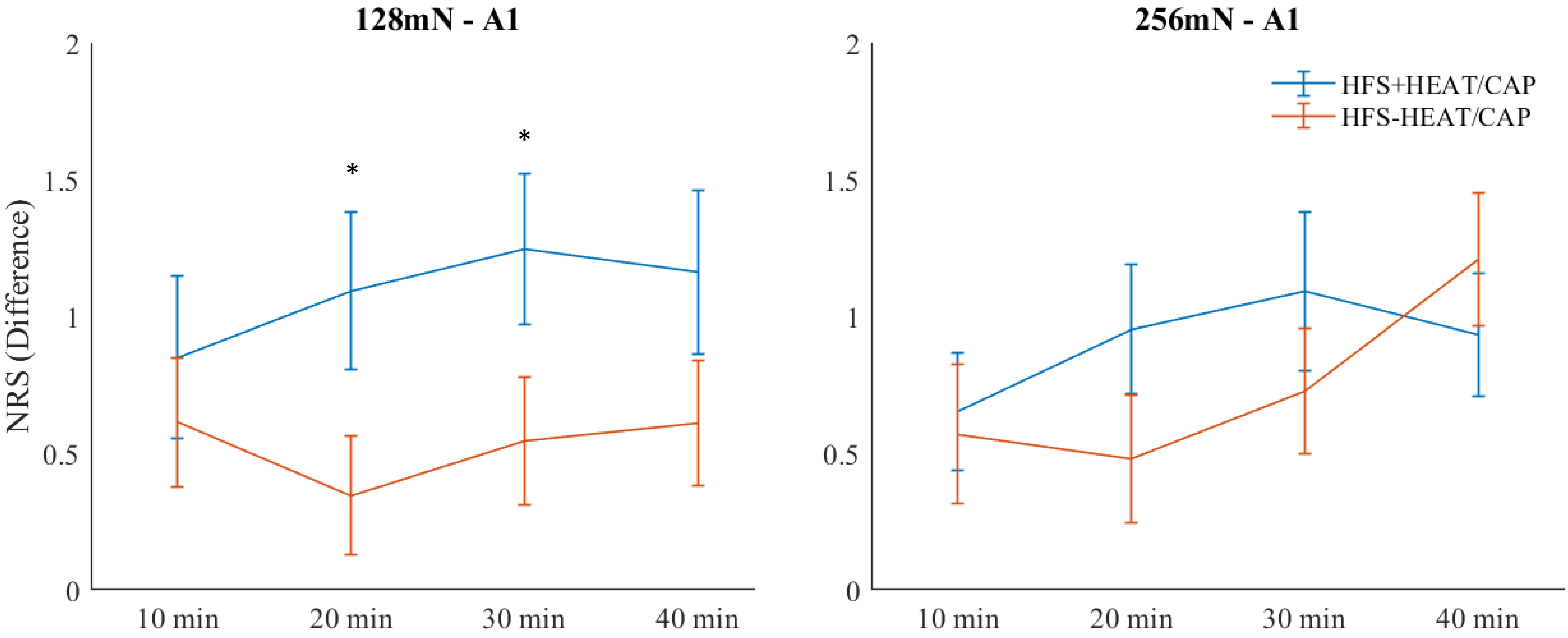

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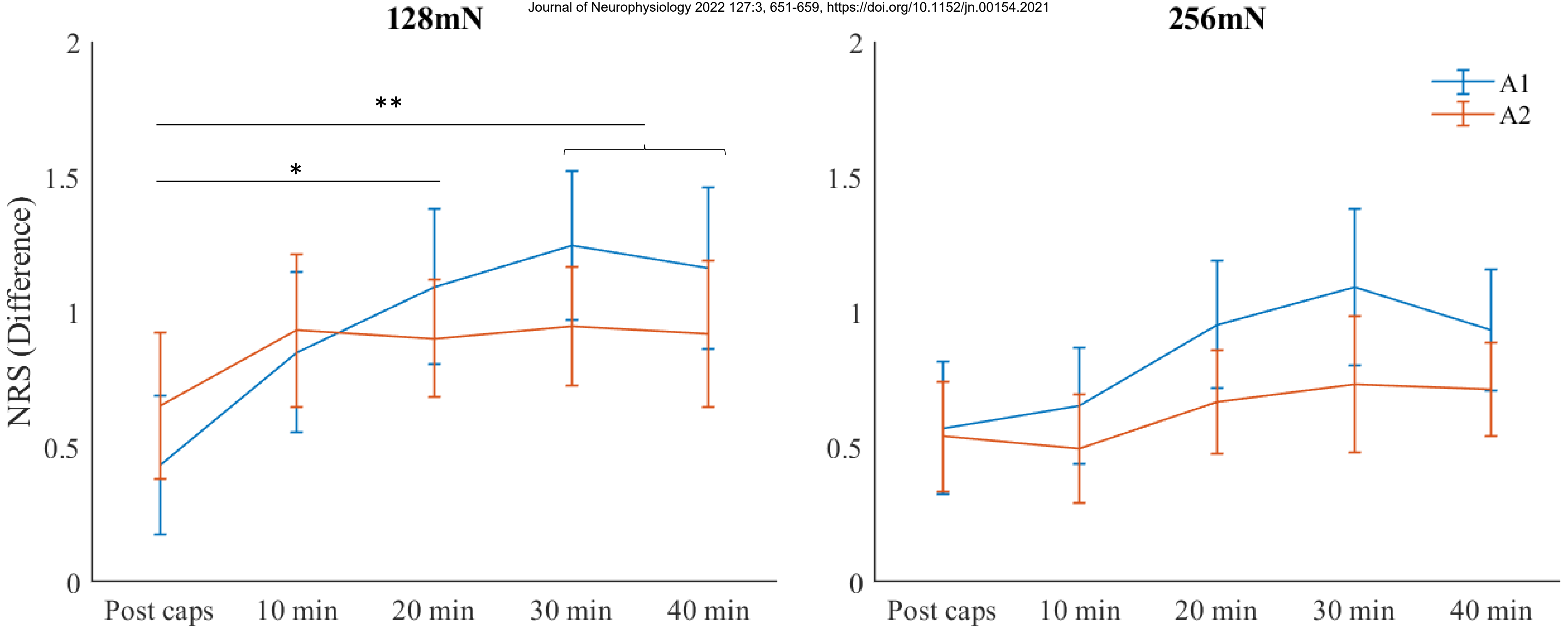

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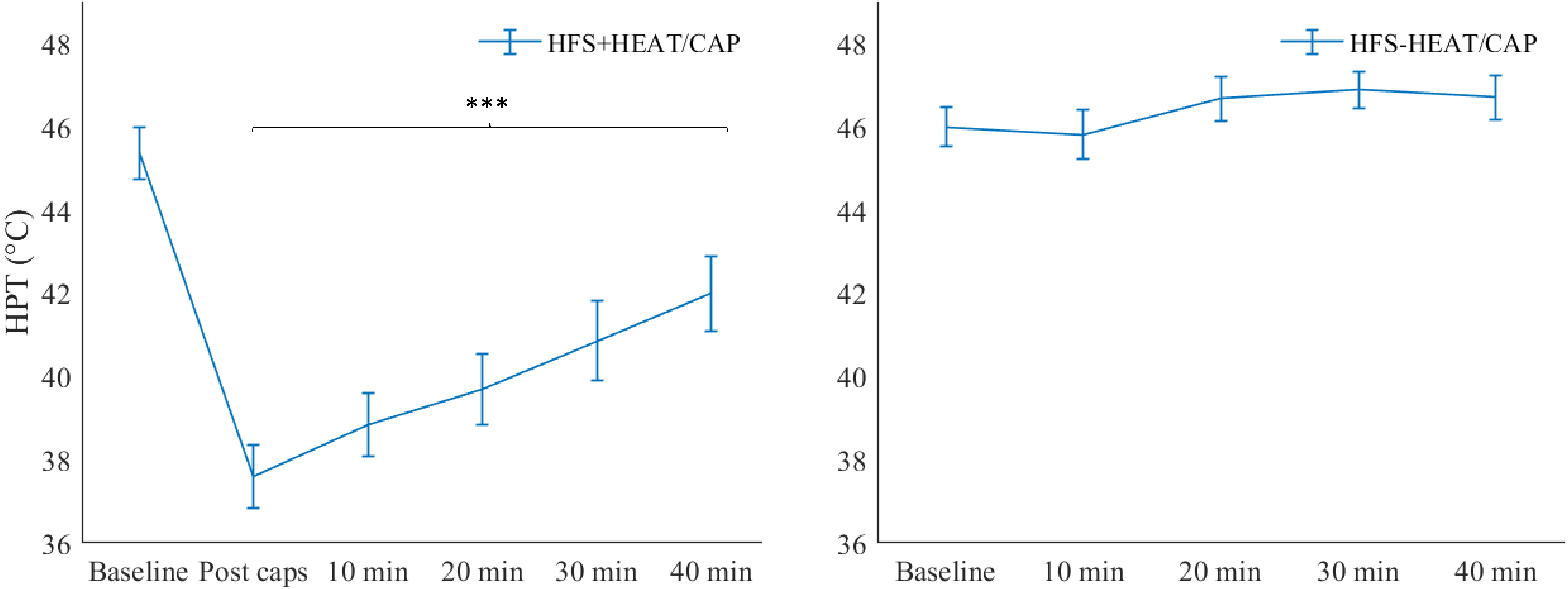

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