



Aalborg Universitet

AALBORG UNIVERSITY
DENMARK

Offset analgesia

The role of peripheral and central mechanisms

Ligato, Davide; Petersen, Kristian Kjær; Mørch, Carsten Dahl; Arendt-Nielsen, Lars

Published in:
European Journal of Pain

DOI (link to publication from Publisher):
[10.1002/ejp.1110](https://doi.org/10.1002/ejp.1110)

Publication date:
2018

Document Version
Accepted author manuscript, peer reviewed version

[Link to publication from Aalborg University](#)

Citation for published version (APA):

Ligato, D., Petersen, K. K., Mørch, C. D., & Arendt-Nielsen, L. (2018). Offset analgesia: The role of peripheral and central mechanisms. *European Journal of Pain*, 22(1), 142-149. <https://doi.org/10.1002/ejp.1110>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- ? Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- ? You may not further distribute the material or use it for any profit-making activity or commercial gain
- ? You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us at vbn@aub.aau.dk providing details, and we will remove access to the work immediately and investigate your claim.

European Journal of Pain

Offset Analgesia: The Role of Peripheral and Central Mechanisms

--Manuscript Draft--

Manuscript Number:	EURJPAIN-D-16-00542R3
Article Type:	Original Manuscript
Corresponding Author:	Lars Arendt-Nielsen, DMSc, PhD Center for Sensory-Motor Interaction (SMI), Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Denmark DENMARK
First Author:	Davide Ligato, MSc
Order of Authors:	Davide Ligato, MSc Kristian Kjær Petersen, MSc, PhD Carsten Dahl Mørch, MSc, PhD Lars Arendt-Nielsen, DMSc, PhD
Abstract:	<p>Background: Offset Analgesia (OA) can be evoked by a three-heat-stimulus train (T1-T2-T3), with T1 (5s) and T3 (20s) having the same temperature (e.g. 48°C) and T2 (5s) being slightly higher (1-3°C). OA is defined as a disproportional pain reduction caused by the slight temperature decrease from T2 to T3. As the pain modulatory mechanisms behind OA are still poorly understood, the current study aimed to investigate the role of peripheral and central OA-mechanisms by applying heat stimuli to the same location and to different unilateral and bilateral locations.</p> <p>Method: Young healthy volunteers participated in the study. A 'standard-OA' paradigm (48-49-48°C) was applied to the non-dominant volar forearm (T1-T2-T3 applied on the same location). 'Unilateral-OA' trials were applied on three different locations of the non-dominant volar forearm (the same dermatome). 'Bilateral-OA' trials were applied by shifting T1-T2-T3 between dominant and non-dominant volar forearms. A constant stimulus of 48°C was applied as control for the evoked pain. The pain intensities were continuously recorded using an electronic visual analogue scale.</p> <p>Results: The largest pain intensity reduction was reported for the 'standard-OA' paradigm ($P < 0.001$) compared with the control stimulus. Both 'Unilateral-OA' and 'Bilateral-OA' trials caused a significant pain reduction ($P < 0.05$) compared with the control, however, the pain reduction was less than that evoked by 'standard-OA' ($P < 0.05$).</p> <p>Conclusion: This study showed that OA could be elicited when the stimuli were applied both to the same and to different locations (ipsi- and contralateral) indicating that peripheral as well as central mechanisms are involved in mediating OA.</p>

Abstract

Background: Offset Analgesia (OA) can be evoked by a three-heat-stimulus train (T1-T2-T3), with T1 (5s) and T3 (20s) having the same temperature (e.g. 48°C) and T2 (5s) being slightly higher (1-3°C). OA is defined as a disproportional pain reduction caused by the slight temperature decrease from T2 to T3. As the pain modulatory mechanisms behind OA are still poorly understood, the current study aimed to investigate the role of peripheral and central mechanisms by applying heat stimuli to the same location and to different unilateral and bilateral locations.

Method: Young healthy volunteers participated in the study. A 'standard-OA' paradigm (48-49-48°C) was applied to the non-dominant volar forearm (T1-T2-T3 applied on the same location). 'Unilateral-OA' trials were applied on three different locations of the non-dominant volar forearm (the same dermatome). 'Bilateral-OA' trials were applied by shifting T1-T2-T3 between dominant and non-dominant volar forearms. A constant stimulus of 48°C was applied as control for the evoked pain. The pain intensities were continuously recorded using an electronic visual analogue scale.

Results: The largest pain intensity reduction was reported for the 'standard-OA' paradigm ($P < 0.001$) compared with the control stimulus. Both 'Unilateral-OA' and 'Bilateral-OA' trials caused a significant pain reduction ($P < 0.05$) compared with the control; however, the pain reduction was less than that evoked by 'standard-OA' ($P < 0.05$).

Conclusion: This study showed that OA could be elicited when the stimuli were applied both to the same and to different locations (ipsi- and contralateral) indicating that peripheral as well as central mechanisms are involved in mediating OA.

Title: Offset Analgesia: The Role of Peripheral and Central Mechanisms

Running title: Peripheral and Central Mechanisms in Offset Analgesia

Authors: Davide Ligato (DL), Kristian Kjær Petersen (KKP), Carsten Dahl Mørch (CDM), Lars Arendt-Nielsen (LAN)

Affiliation: SMI, Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Aalborg, Denmark

Original paper for: European Journal of Pain

Article type: Original article

Number of Figures and Tables: 5 figures and no tables

Number of Pages: 19 including title page and abstract)

Keywords: Offset analgesia, pain modulation, pain inhibition, pain pathways.

Acknowledgement: The Innovation Fund Denmark and Shionogi Science Program, Japan.

Conflict of interest: There are no conflicts of interest to declare

Significance: This study investigated offset analgesia by applying thermal painful stimuli to the ipsi- and bilateral forearms in healthy subjects and found that both peripheral and central mechanisms seem to mediate offset analgesia.

Corresponding Author:

Prof. Lars Arendt-Nielsen, Ph.D., DMSc.

SMI, Department of Health Science and Technology, Faculty of Medicine, Aalborg University,

Fredrik Bajers vej, 7 D3, 9220 Aalborg Øst, Denmark

Phone: +45 9940 8831, Fax: +45 9815 4008, E-mail: LAN@HST.AAU.DK

INTRODUCTION

Offset Analgesia (OA) is a disproportionately large reduction in perceived pain following a slight decrease in painful stimulus intensity (Grill and Coghill, 2002) and may be considered a potential novel mechanistic pain assessment tool for assessing peripheral or central pain modulatory processes (Arendt-Nielsen et al., 2015; Grill and Coghill, 2002; Hermans et al., 2016; King, 2014). Using a three-temperature-stimulus train (T1-T2-T3: T2 higher than T1; T3 equal to T1), Grill and Coghill discovered that a slight decrease (1-3°C) from T2 to T3 evokes a temporary analgesic effect with significantly lower pain ratings compared with control trials applying constant temperature stimuli (Grill and Coghill, 2002).

Changes in afferent information, occurring during certain stimulation frequencies, are amplified or attenuated, due to a filtering of the sensory information (Mørch et al., 2015). Even though the temporal aspects of pain perception are still not fully understood, the transduction and conduction delays differ between afferent fiber types (Treede et al., 1998), resulting in a complex filtering of the applied heat (Greffrath et al., 2007). These peripheral mechanisms will result in temporal filtering, but several studies have indicated that central mechanisms are also involved in temporal filtering of heat pain perception (Mørch et al., 2015; Yelle et al., 2008). Despite these dynamic processes could be confirmed in OA-trials, as OA reflects temporal filtering of sensory information (Mørch et al., 2015; Yelle et al., 2008), there is an ongoing debate regarding the role of peripheral and central mechanisms mediating OA. OA seems opioid-independent (Martucci et al., 2012a), and no effect is found on OA after oral Clonidine (α_2 -receptors agonists and Imidazoline-I₁-receptor agonist) (Nahman-Averbuch et al., 2016), suggesting that OA is different from conditioned pain modulation (CPM), which is reduced after Dexmedetomidine (α_2 -receptors agonists) (Baba et al., 2012).

Previous fMRI studies have shown that the Periaqueductal Gray (PAG) of the brainstem and Rostral Ventral Medulla (RVM) are activated by an OA-paradigm, but not during standard CPM-paradigms (Nahman-Averbuch et al., 2014), suggesting that OA and CPM activate different pain inhibitory systems. Moreover, several studies demonstrated that the facilitatory influences on spinal nociception that are emanated from the RVM are partly mediating the central sensitization (Millan, 2002, Vo and Drummond, 2014a), while the possible role of a peripheral mechanism involved in OA could explain differences in brain activation during OA and CPM (Nahman-Averbuch et al., 2014).

The aim of the present study was to systematically investigate the role of peripheral and central contribution to OA by applying a series of OA-trials by the use of thermal painful stimuli to similar and different locations (unilaterally and bilaterally). The hypothesis of the present study was that both peripheral and central mechanisms contribute to OA.

MATERIALS AND METHODS

Participants

Since pain inhibition is reduced in older adults (Riley et al., 2010), healthy young volunteers were recruited to participate in two sub-studies (Unilateral-trials: 12 females and 12 males; 1 left-handed, 23 right-handed, average age 27 ± 4 years; Bilateral-trials: 12 females and 12 males; 2 left-handed, 22 right-handed, average age: 28 ± 4 years). Eight participants participated in both sub-studies. The participants were excluded if they suffered from any concomitant pain problems, used any analgesics, lacked understanding of the procedures or had any history of alcohol or drug misuse. All participants were given oral and written information and signed written informed consent prior to

the initiation of the study. The study complied with the Helsinki Declaration and was approved by the local Ethical Committee (reference number: N-20120043).

Materials

Heat stimuli (rise and fall rate: 6°C/s) were applied using three individual Medoc Pathway systems (Medoc Ltd., Ramat Yishai, Israel) with a 30×30mm squared probe attached to the forearm with a Velcro strap. Three identical, equally calibrated, and computer-controlled thermal probes were used for the experiment. All experimental trials were divided into three time intervals of painful heat stimuli: T1 (5 seconds), T2 (5 seconds), and T3 (20 seconds). The baseline temperature was 35°C, and the temperatures during all trials were: T1=48°C, T2=49°C, and T3=48°C. The participants were asked to rate the overall pain intensity experienced on a continuous electronic visual analog scale (VAS; Aalborg University, Aalborg, Denmark) anchored at 0: no pain and 10: worst imaginable pain. Since primary afferents are known to have an adaptive behavior (Derbyshire and Osborn, 2009; Tillman et al., 1995), all trials were separated by five minutes to avoid adaptation, hyperalgesia or other carry-over effects on the site of the stimulation, as shown in a recent study (Vo and Drummond, 2014b).

Study Design

The study consisted of two sub-studies: sub-study 1 (Unilateral-trials) where the stimuli were applied at different locations (within the same dermatomes) of the non-dominant volar forearm, and sub-study 2 (Bilateral-trials) where stimulations were applied bilaterally within the same segment (volar forearm): Unilateral- and Bilateral- trials are sketched in Figure 1A and Figure 1B, respectively.

Sub-study 1: Unilaterally Evoked Offset Analgesia Trials

Three areas (A) on the non-dominant forearm were used for the painful stimuli: A1, A2, and A3, located 3cm, 7cm, and 11cm distal to the elbow joint, respectively (figure 1A). The three individual thermal stimulators were programmed to apply the different skin temperatures to the three areas using a single trigger for initiation of the various protocols. In total, nine trials were applied to each participant in sub-study 1 as described below.

Control Trials (Control)

Three control trials were conducted with a constant temperature of 48°C for 30s on one area (areas A1, A2, and A3), and the average pain ratings across the three trials were calculated (referred to as “Control”) and used for the analysis.

Offset Analgesia Unilateral Trials (UOA)

Three traditional-OA-trials were conducted according to the protocol proposed by Grill and Coghill (Grill and Coghill, 2002). The three-stimulus train was delivered individually to one area (areas A1, A2, and A3). The pain ratings across the three trials were averaged (referred to as “UOA”) and used for the analysis.

Different Location Unilateral Trials (P1-P3)

Three protocols (P1, P2, and P3) were constructed, applying heat to different locations to the non-dominant volar forearm as follows (figure 1A):

P1: A1 for T1, A2 for T2, and A1 for T3.

P2: A1 for T1, A1 for T2, and A2 for T3.

P3: A1 for T1, A2 for T2, and A3 for T3.

The sequence of all unilateral trials was randomized.

Sub-study 2: Bilaterally Evoked Offset Analgesia Trials

The stimuli were applied using two individual thermal stimulators programmed to apply different temperatures using a single trigger for initiation of the various protocols. The stimuli were applied to the volar forearms 3cm distal from the elbow joint (figure 1B). In total, eight trials were applied to each participant in sub-study 2 as described below.

Offset Analgesia Bilateral Trials (BOA)

Two traditional-OA-trials were applied to the non-dominant (ND) and the dominant (D) forearm. The averaged pain ratings were calculated across the two sessions (referred to as “BOA”) and used for the analysis.

Different Location Bilateral Trials (P4-P6)

Three protocols (P4, P5, and P6) were constructed, applying heat stimuli alternating between the two volar forearms (figure 1B). The three protocols were applied as follows:

P4: 1 (ND for T1, D for T2, and ND for T3) and 2 (D for T1, ND for T2, and D for T3).

P5: 3 (ND for T1, D for T2, and D for T3) and 4 (D for T1, ND for T2, and ND for T3).

P6: 5 (ND for T1, ND for T2, and D for T3) and 6 (D for T1, D for T2, and ND for T3).

Each protocol was ‘mirrored’ and thus applied twice. The average of the two ‘mirrored’ trials was used for further analysis. The sequences of the bilateral trials were randomized.

Statistical Analysis

The statistical analysis was performed using SPSS (version 24.0, IBM Corporation, New York, USA). The analgesic effects took place after the decrease of the temperature from T2 to T3 (49°C to

48°C) (Grill and Coghill, 2002). The average pain ratings following the decrease from T2 to T3, i.e. in the time interval between 16s and 20s (due to the delay of the thermodes to reach the target temperature and the responses latency) were calculated (figure 2). The time window used for statistical analysis of OA-effect was adapted based on the study conducted by Grill and Coghill (2002).

Initially, the sex differences of OA were investigated independently for each protocol using independent t-tests. Secondly, differences between the protocols (CONTROL 1-3, UOA 1-3, BOA 1-2, P 4-6 and the mirrored protocol) were investigated independently for each protocol using repeated measures Analysis of Variance (rmANOVA) and averages were calculated, since no differences were found. Thirdly, two rmANOVAs were applied to investigate the difference in pain ratings between the protocols. The sub-studies were analyzed separately with *paradigm* (sub-study1: CONTROL, P3, P2, P1, UOA, and sub-study2: CONTROL, P6, P5, P4, BOA) as main factor. The CONTROL paradigm was the same for both sub-studies. Finally, the OA-effect was calculated as the differences in pain ratings between the control and each of the four protocols in both sub-studies, and the OA-effect was compared between protocols within each sub-study using rmANOVA. The Bonferroni post-hoc test was applied in case of significant findings. The data are presented as mean \pm standard error of mean (SEM). $P < 0.05$ was considered significant.

RESULTS

Eight subjects participated in both the sub-study 1 and sub-study 2 and these demonstrated no significant differences (rmANOVA: $F(1-2,22-44) < 0.64$, $P > 0.53$) in pain ratings to any of the paradigms or age (independent t-test: $P > 0.19$) compared to subjects who only participated in one sub-study.

No significant differences in the VAS ratings between females and males were found for any of the protocols ($P > 0.14$).

Unilaterally Evoked Offset Analgesia Trials

The mean VAS scores of the three CONTROL- and the three UOA- trials showed no significant difference (rmANOVA: $F(3,21)=0.14$, $P > 0.9$, and rmANOVA: $F(2,22)=4.24$, Bonferroni: $P > 0.1$, respectively). Therefore, the mean values for CONTROL and UOA were taken, as shown in figure 2A and figure 2B, respectively.

The mean VAS scores of the unilateral trials are shown in figure 3A. Significantly lower mean VAS scores (rmANOVA: $F(4,20)=14.01$, $P < 0.001$) compared with the control trials were found for P1 (Bonferroni: $P < 0.001$), P2 (Bonferroni: $P < 0.05$), and UOA (Bonferroni: $P < 0.001$), but not for P3 (Bonferroni: $P > 0.07$; figure 4A). Further, the mean VAS scores were significantly lower for UOA compared with P2 (Bonferroni: $P < 0.001$) and P3 (Bonferroni: $P < 0.01$).

Bilaterally Evoked Offset Analgesia Trials

The mean VAS scores of the two BOA-trials showed no significant difference (rmANOVA: $F(1,23)=0.15$, $P > 0.8$). Therefore, the mean values for BOA were taken, as shown in figure 2C.

The mean VAS scores of the bilateral trials are shown in figure 3B. Significantly lower mean VAS scores (rmANOVA: $F(4,20)=11.26$, $P < 0.001$) compared with the control trials were found for P4 (Bonferroni: $P < 0.05$) and BOA (Bonferroni: $P < 0.01$), but not for P5 (Bonferroni: $P > 0.2$) or P6 (Bonferroni: $P > 0.9$; figure 4B). Further, the mean VAS scores were significantly lower for BOA compared with P4 (Bonferroni: $P < 0.05$), P5 (Bonferroni: $P < 0.001$), and P6 (Bonferroni: $P < 0.001$). Finally, the mean VAS scores of P4 were significantly lower compared with P6 (Bonferroni: $P < 0.01$).

Offset Analgesic Effect

The calculated OA-effects are shown in figure 5. Significantly lower unilateral OA-effects (rmANOVA: $F(3,21)=6.98$, $P<0.001$) were seen for P2 (Bonferroni: $P<0.01$) and P3 (Bonferroni: $P<0.05$), but not for P1 (Bonferroni: $P<0.9$) compared with UOA. Significantly lower bilateral OA-effects (rmANOVA: $F(3,21)=15.97$, $P<0.001$) were seen for P4 (Bonferroni: $P<0.05$), P5 (Bonferroni: $P<0.01$), and P6 (Bonferroni: $P<0.001$). Further, for the bilateral OA-effects, P6 was found to be significantly lower compared with P4 (Bonferroni: $P<0.01$).

DISCUSSION

Painful heat stimulation applied to different areas evoked an OA response, both when applied unilaterally (P1 and P2) and bilaterally (P4), indicating that a painful input from different receptive field areas is centrally integrated and capable of evoking OA. The study further showed that OA can be elicited when the stimuli are applied to different areas, both unilaterally and bilaterally. The traditional unilaterally generated OA, within same dermatome, is the most potent. The study confirmed that peripheral as well as central mechanisms contribute to the initiation of OA.

OA-Effect across Test Paradigms

The current study found the largest OA-effect using the traditional OA-paradigm proposed by Grill and Coghill (2002). This paradigm has demonstrated OA in many studies using painful heat stimulations, with temperature stimuli ranging from 41-42-41°C (Derbyshire and Osborn, 2009) to 49-50-49°C (Grill and Coghill, 2002; Martucci et al., 2012a; Nahman-Averbuch et al., 2014; Yelle et al., 2009).

In contrast to previous OA studies, the location of the stimuli was alternated to investigate to which extent the spatio-temporal filtering in the nociceptive system was able to produce an OA response. Complex spatio-temporal integration in the nociceptive system has previously been demonstrated (Mørch et al., 2010; Trojan et al., 2006). Yelle et al., 2008 reported that pain reduction was found when the pain was induced proximally (but not distally) to a second painful stimulus (Yelle et al., 2008). Similarly, the present study found that OA could be evoked when T3 was applied proximally to T2 (the P1). Furthermore, OA could be evoked when T2 was applied bilaterally to T1 and T3 (the P4), but not when applied unilaterally to T1 or T3 (P6 and P5, respectively). The analgesic behavior revealed in the current study suggests a synergy between the central mechanisms integrating temperatures applied to different locations and the peripheral mechanisms, as the OA-effect was larger when the temperatures were applied to the same location.

Previous studies have found the area of the receptive fields of C-fibers to range from, e.g. 6mm² on the hand (vanHees and Gybels, 1981), 35mm² on the toes (Schmidt et al., 1997), and 213mm² on the lower leg (Schmidt et al., 1997). The current study applied the stimuli outside of these ranges and still an OA response (the P1 and P2) was evoked, which could indicate that OA is not only peripherally, but also centrally mediated.

The complementary mechanisms behind OA and its pain attenuation still remain unclear, but impaired OA-effects have been observed in different groups of chronic pain patients. The chronic neuropathic pain patients showed reduced OA-effect (Niesters et al., 2011b; Suzan et al., 2015), which could imply that the peripheral pathologies reduce OA and that peripheral mechanisms are involved in OA. In addition, the reduced OA-effect has also been observed in patients with central sensitization, such as fibromyalgia (Julien et al., 2005; Oudejans et al., 2015). These studies in chronic pain patients indicate that OA is an integral feature of the pro-/anti- nociceptive balance of the healthy pain system, suggesting an impairment of the involved pain modulatory network.

Gender Differences in Offset Analgesia

Gender differences to endogenous pain modulatory responses have been found for both healthy participants and patients with chronic pain (Staud et al., 2003). Niesters et al., 2011, reported an age-dependent gender-effect with healthy males showing greater OA-effects compared with females (Niesters et al., 2011b). Further, Honigman et al., 2013 found that the analgesic effect of OA could be increased by adding a CPM paradigm in addition to the OA paradigm, but this was only found in healthy males (Honigman et al., 2013). In contrast, Naugle et al., 2013 and Niester et al., 2011 found no sex effects in OA (Naugle et al., 2013; Niesters et al., 2011a). The current study found no gender differences in young healthy participants, but further studies need to confirm or disprove the presence of gender differences in OA.

Possible Mechanisms behind OA

Contrary to CPM, where a local test stimulus is modulated by an extra-segmental stimulus (Yarnitsky et al., 2015), the pain modulation in OA occurs due to changes in the stimuli intensities at the local site of the body. During the Bilateral-trials, we have acknowledged that a CPM-effect could influence the VAS scores of the subjects. Their scores could be influenced by the bilateral stimulation; on the contrary, it could also be that subjects were unable to rate the felt pain as rapidly as they do during Unilateral-trials. Further, a recent study found no CPM-effect on a contact heat test stimuli during a contact heat conditioning stimuli, if stimuli were shorter than 20 seconds (Granovsky et al., 2016). In the current study, OA-effect was analyzed from 16s to 20s, to avoid that the occurring pain relieving effect was CPM.

Whereas CPM is considered an integration of spatial mechanisms, OA implies temporal filtering mechanisms (Yelle et al., 2008). To investigate the interaction between spatial and temporal

mechanisms, a recent review (Hermans et al., 2016) suggested adding a spatial component to an OA-paradigm. The current study added a spatial component to the OA paradigms by stimulating bilaterally the forearms and confirmed an OA-effect, which could indicate central mechanisms to be involved in OA. Further, Honigman et al., 2013 found that applying a CPM-paradigm in parallel with an OA-paradigm enhanced the OA-effect (Honigman et al., 2013), which suggests that OA consists of both temporal and spatial mechanisms. OA is believed to involve central mechanisms that evoke an analgesic response in a spatially localized area; this theory is confirmed by a study that showed that spatial summation was not reported when OA was simultaneously tested with two thermodes (Yelle et al., 2008).

Moreover, spatial interactions were also reported during paired stimulus paradigms; in fact, OA was observed to modulate pain intensities, which were evoked by a discrete distal stimulus, while pain was terminated when tested from a proximal stimulus (Yelle et al., 2008). To date, peripheral mechanisms are not able to fully explain the latter asymmetric special interactions due to the activation of distinct afferent fiber populations (vanHees and Gybels, 1981; Jorum et al., 1989; Raja et al., 1999, Yelle et al., 2008).

To understand the peripheral contribution in OA, Martucci et al. 2012, studied the OA responses before and after peripherally induced sensitization by capsaicin cream, but found no differences (Martucci et al., 2012b), indicating that a strong peripheral sensitization does not change the OA effect, supporting the central contribution. In a direct comparison, Nahman-Averbuch et al., 2014 found that OA produced reduced activity in the SI cortex, but was associated with greater activation in the brainstem, the anterior insula, dorsolateral prefrontal cortex, intra-parietal sulcus, and inferior parietal lobule relative to CPM (Nahman-Averbuch et al., 2014), suggesting that different mechanisms are involved in OA and CPM.

To investigate the drug modulation of OA, Niesters et al., 2011 administered ketamine and found no effect on OA in healthy volunteers (Niesters et al., 2011b), suggesting that the NMDA-receptors are not involved in OA. Further, Martucci et al., 2012 found no effect on OA after remifentanyl or naloxone intake in participants with opioid-induced hyperalgesia (Martucci et al., 2012a), suggesting that OA is not opioid dependent. Other central pain mechanisms such as impaired CPM and facilitated temporal summation have been found to improve after duloxetine (Yarnitsky et al., 2012) or ketamine (Graven-Nielsen et al., 2000) administration. Future studies should investigate the specific pharmacological basis for OA. Furthermore, no animal OA-models have been developed to study the more fundamental aspects of OA, which would be important to investigate the involvement of the central pathways and transmitter systems.

LIMITATIONS

The temperatures of the thermodes and the very superficial skin were computer-controlled in the current study and it was assumed that the skin temperature followed the temperature of the thermodes. However, Magerl and Treede, 1996 found a delay in increasing pain after increasing heat stimuli of approx. 30 seconds (Magerl and Treede, 1996). In contrast, Frahm et al. 2010 investigated this phenomenon in a computer-model, and found that changes in temperature reached the nociceptors and was perceived as painful within seconds (Frahm et al., 2010). Future studies are encouraged to assess the skin temperature during offset analgesia paradigms using different areas of stimulation.

CONCLUSION

The study suggests that peripheral as well as central factors are involved in mediating OA. The central and peripheral components still need to be elucidated, and the possible role as a pain inhibitory pathway for, e.g. drug intervention should be further explored.

ACKNOWLEDGEMENTS

The authors would like to thank the Innovation Fund Denmark (j.no. 136-2014-5) and Shionogi Science Program, Japan, for providing the support to conduct the study.

There are no conflicts of interest to declare.

AUTHOR CONTRIBUTION

DL, KKP, CDM, and LAN designed the study. DL acquired and analyzed the data. All authors critically reviewed and approved the submitted work.

References

- Arendt-Nielsen, L., Skou, S.T., Nielsen, T.A., Petersen, K.K. (2015). Altered Central Sensitization and Pain Modulation in the CNS in Chronic Joint Pain. *Curr Osteoporos Rep* 13, 225–234.
- Baba, Y., Kohase, H., Oono, Y., Fujii-Abe, K., Arendt-Nielsen, L. (2012). Effects of dexmedetomidine on conditioned pain modulation in humans. *Eur J Pain (United Kingdom)* 16, 1137–1147.
- Derbyshire, S.W.G., Osborn, J. (2009). Offset analgesia is mediated by activation in the region of the periaqueductal grey and rostral ventromedial medulla. *Neuroimage* 47, 1002–1006.
- Frahm, K.S., Andersen, O.K., Arendt-Nielsen, L., Mørch, C.D. (2010). Spatial temperature distribution in human hairy and glabrous skin after infrared CO₂ laser radiation. *Biomed Eng Online* 9, 69.
- Graven-Nielsen, T., Kendall, S.A., Henriksson, K.G., Bengtsson, M., Sørensen, J., Johnson, A., Gerdle, B., Arendt-Nielsen, L. (2000). Ketamine reduces muscle pain, temporal summation, and referred pain in fibromyalgia patients. *Pain* 85, 483–491.
- Grill, J.D., Coghill, R.C. (2002). Transient analgesia evoked by noxious stimulus offset. *J Neurophysiol* 87, 2205–2208.
- Van Hees, J., Gybels, J. (1981). C nociceptor activity in human nerve during painful and non painful skin stimulation. *J Neurol Neurosurg Psychiatry* 44, 600–607.
- Hermans, L., Calders, P., Van Oosterwijck, J., Verschelde, E., Bertel, E., Meeus, M. (2016). An Overview of Offset Analgesia and the Comparison with Conditioned Pain Modulation: A Systematic Literature Review. *Pain Physician* 19, 307–326.
- Honigman, L., Yarnitsky, D., Sprecher, E., Weissman-Fogel, I. (2013). Psychophysical testing of spatial and temporal dimensions of endogenous analgesia: Conditioned pain modulation and offset analgesia. *Exp Brain Res* 228, 493–501.
- Julien, N., Goffaux, P., Arsenault, P., Marchand, S. (2005). Widespread pain in fibromyalgia is related to a deficit of endogenous pain inhibition. *Pain* 114, 295–302.
- King, C.D. (2014). Conditioned pain modulation and offset analgesia: Different avenues to inhibit pain. *Pain* 155, 2444–2445.
- Magerl, W., Treede, R.D. (1996). Heat-evoked vasodilatation in human hairy skin: axon reflexes due to low-level activity of nociceptive afferents. *J Physiol* 497, 837–848.
- Martucci, K.T., Eisenach, J.C., Tong, C., Coghill, R.C. (2012a). Opioid-independent mechanisms supporting offset analgesia and temporal sharpening of nociceptive information. *Pain* 153, 1232–1243.

Martucci, K.T., Yelle, M.D., Coghill, R.C. (2012b). Differential effects of experimental central sensitization on the time-course and magnitude of offset analgesia. *Pain* 153, 463–472.

Mørch, C.D., Frahm, K.S., Coghill, R.C., Arendt-Nielsen, L., Andersen, O.K. (2015). Distinct temporal filtering mechanisms are engaged during dynamic increases and decreases of noxious stimulus intensity. *Pain* 156, 1906–1912.

Mørch, C.D., Andersen, O.K., Quevedo, A.S., Arendt-Nielsen, L., Coghill, R.C. (2010). Exteroceptive aspects of nociception: Insights from graphesthesia and two-point discrimination. *Pain* 151, 45–52.

Nahman-Averbuch, H., Dayan, L., Sprecher, E., Hochberg, U., Brill, S., Yarnitsky, D., Jacob, G. (2016). Pain Modulation and Autonomic Function: The Effect of Clonidine. *Pain Med* 17, 1292–1301.

Nahman-Averbuch, H., Martucci, K.T., Granovsky, Y., Weissman-Fogel, I., Yarnitsky, D., Coghill, R.C. (2014). Distinct brain mechanisms support spatial vs temporal filtering of nociceptive information. *Pain* 155, 2491–2501.

Naugle, K.M., Cruz-Almeida, Y., Fillingim, R.B., Riley, J.L. (2013). Offset analgesia is reduced in older adults. *Pain* 154, 2381–2387.

Niesters, M., Dahan, A., Swartjes, M., Noppers, I., Fillingim, R.B., Aarts, L., Sarton, E.Y. (2011a). Effect of ketamine on endogenous pain modulation in healthy volunteers. *Pain* 152, 656–663.

Niesters, M., Hoitsma, E., Sarton, E., Aarts, L., Dahan, A. (2011b). Offset analgesia in neuropathic pain patients and effect of treatment with morphine and ketamine. *Anesthesiology* 115, 1063–1071.

Oudejans, L.C.J., Smit, J.M., van Velzen, M., Dahan, A., Niesters, M. (2015). The influence of offset analgesia on the onset and offset of pain in patients with fibromyalgia. *Pain* 156, 2521–2527.

Riley, J.L., King, C.D., Wong, F., Fillingim, R.B., Mauderli, A.P. (2010). Lack of endogenous modulation and reduced decay of prolonged heat pain in older adults. *Pain* 150, 153–160.

Schmidt, R., Schmelz, M., Ringkamp, M., Handwerker, H.O., Torebjörk, H.E. (1997). Innervation territories of mechanically activated C nociceptor units in human skin. *J Neurophysiol* 78, 2641–2648.

Staud, R., Robinson, M.E., Vierck, C.J., Price, D.D. (2003). Diffuse noxious inhibitory controls (DNIC) attenuate temporal summation of second pain in normal males but not in normal females or fibromyalgia patients. *Pain* 101, 167–174.

Suzan, E., Treister, R., Pud, D., Haddad, M., Eisenberg, E. (2015). The effect of hydromorphone therapy on psychophysical measurements of the descending inhibitory pain systems in patients with chronic radicular pain. *Pain Med (United States)* 16, 168–175.

Tillman, D.B., Treede, R.D., Meyer, R. a, Campbell, J.N. (1995). Response of C fibre nociceptors in the anaesthetized

monkey to heat stimuli: correlation with pain threshold in humans. *J Physiol* 485, 767–774.

Trojan, J., Stolle, A.M., Kleinböhl, D., Mørch, C.D., Arendt-Neilsen, L., Hözl, R. (2006). The saltation illusion demonstrates integrative processing of spatiotemporal information in thermoceptive and nociceptive networks. *Exp Brain Res* 170, 88–96.

Yarnitsky, D., Bouhassira, D., Drewes, A.M., Fillingim, R.B., Granot, M., Hansson, P., Landau, R., Marchand, S., Matre, D., Nilsen, K.B., Stubhaug, A., Treede, R.D., Wilder-Smith, O.H.G. (2015). Recommendations on practice of conditioned pain modulation (CPM) testing. *Eur J Pain* 19, 805–806.

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.

Yelle, M.D., Oshiro, Y., Kraft, R.A., Coghill, R.C. (2009). Temporal filtering of nociceptive information by dynamic activation of endogenous pain modulatory systems. *J Neurosci* 29, 10264–10271.

Yelle, M.D., Rogers, J.M., Coghill, R.C. (2008). Offset analgesia: A temporal contrast mechanism for nociceptive information. *Pain* 134, 174–186.

FIGURE LEGENDS

Figure 1: Areas of thermal application and stimulation protocols (P1-6). Summary of the experimental offset analgesia (OA)-protocols with different heat stimuli at different time points (T1, T2, and T3) in different areas (A1, A2, and A3) unilaterally on the volar non-dominant forearm or bilaterally on the non-dominant (ND) and dominant (D) forearms. The temperatures were set to T1=48°C (5s), T2=49°C (5s), and T3=48°C (20s).

A) The heat stimulation was applied to different areas in three unilateral protocols.

B) The heat stimulation was alternated between the two forearms in the bilateral protocols.

Figure 2: Colored lines represent the pain intensity (VAS scores, 0-10 cm) of painful stimuli. VAS: Visual Analogue Scale.

A) Unilateral-paradigms for CONTROL applied to area A1 (CONTROL1, red), to area A2 (CONTROL2, blue), to area A3 (CONTROL3, green), and average value (dashed black).

B) Unilateral-paradigms applied to area A1 (UOA1, red), to area A2 (UOA2, blue), to area A3 (UOA3, green), and average value (UOA, black).

C) Bilateral-paradigms applied to the dominant forearm (BOA1, red), to the non-dominant forearm (BOA2, blue), and average value (BOA, black).

Figure 3: Colored lines represent the pain intensity (VAS scores, 0-10 cm) of painful stimuli. VAS: Visual Analogue Scale.

A) Unilateral-paradigms for CONTROL (dashed black), P3 (green), P2 (blue), P1 (red), and Unilateral Offset Analgesia (UOA, black).

B) Bilateral-paradigms for CONTROL (dashed black), P6 (green), P5 (blue), P4 (red), and Bilateral Offset Analgesia (BOA, black).

Figure 4: Mean pain intensity (VAS scores, 0-10 cm) following the slight temperature decrease of A) unilaterally and B) bilaterally applied offset analgesia (OA) stimulation protocols (P1-P6) and standard unilateral (UOA) and bilateral (BOA) offset analgesia paradigms. Significantly different from the control is marked as “*”, and different from UOA and BOA marked is as “#”.

Figure 5: OA-effects of unilateral (P1, P2, P3 and UOA) and bilateral (P4, P5, P6 and BOA) protocols. Significance levels compared with UOA (blue gradient colors) marked as “*” and BOA (red gradient colors) marked as “#”. VAS: Visual Analogue Scale. The OA-effect indicates VAS differences from the different protocols compared with the control stimulus.









