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Published in:
European Journal of Pain

DOI (link to publication from Publisher):
10.1002/ejp.1250

Publication date:
2018

Document Version
Accepted author manuscript, peer reviewed version

Article Type: Original Manuscript

Electrical stimulation for evoking Offset Analgesia: A human volunteer methodological study

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Running title:

Electrical evoked offset analgesia

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Category:

Original article

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ejp.1250

Funding Sources:

Acknowledgement: Shionogi Science Program and TaNeDS Europe.

Conflicts of interest disclosures:

None

Significance:

Electrical stimulation can elicit offset analgesia in humans, indicating that this perceptual modification can be obtained even bypassing peripheral receptors.

ABSTRACT

Background: Offset analgesia (OA) is a disproportionally large decrease in the pain perception in response to a small decrease in the stimulation intensity. Traditionally, heat stimulation has been used to evoke OA. The aim of this study was to investigate if OA could be evoked by electrical stimulation.

Methods: Healthy volunteers (N=24) underwent two OA experimental sessions consisting of heat stimuli intensities of 48-49-48°C (traditional OA-paradigm) and electrical stimuli at 150-180-150% of the electrical pain perception (EPP) threshold. The three stimuli were delivered for 5 seconds (STIM1), 5 seconds (STIM2), and 20 seconds (STIM3), respectively. The sessions were randomized to the dominant or non-dominant volar forearm. Two control-sessions were performed with 30s constantly heat (48°C) and electrical stimuli (150% of the EPP) (CONTROL-STIM). In all sessions, the pain intensities were constantly rated on a Visual Analog Scale (VAS, 0-10).

Results: Significantly reduced STIM3 VAS ratings as compared to the CONTROL-STIM were reported for heat (1.81±0.54; P<0.001) and electrical (2.12±0.42; P<0.001) stimuli. The degrees of OA produced by heat and electrical stimuli were similar. A significantly positive correlation was found between thermal and electrical OA-effects (r=0.48, P<0.02).

Conclusions: These findings demonstrate that electrical stimulation can elicit significant OA in humans indicating that the peripheral receptors can be bypassed and still evoke OA. Application of the electrical-OA model may be of interest for further basic and clinical investigations as a potential new biomarker for central pain inhibition and provide the option to back-translate the technology to animals to understand the underlying neurobiology.

INTRODUCTION

Grill and Coghill demonstrated that a small decrease in the temperature during tonic painful heat stimulation resulted in a disproportionally large decrease in the pain perception, and they termed the phenomenon: 'Offset Analgesia' (OA) (Grill & Coghill, 2002).

The underlying mechanisms of OA are unknown but both peripheral and central pain mechanisms have been suggested to provide the pain inhibitory effect (Hermans *et al.*, 2016). Compared with conditioning pain modulation, OA is not dependent on NMDA- (Niesters, Dahan, *et al.*, 2011; Niesters, Hoitsma, *et al.*, 2011) or opioid-receptors (Martucci, Eisenach, *et al.*, 2012; Suzan *et al.*, 2015). In addition, the analgesic effect of OA and

conditioning pain modulation adds to each other (Honigman *et al.*, 2013), indicating that these two paradigms rely on different inhibitory mechanisms.

OA representing a neural temporal filtering phenomenon (Mørch *et al.*, 2015) and the temporal aspects of pain perception are often investigated as summation to repeated electrical stimuli where the nociceptive responses increase during a series of repeated stimuli and hence may represent the initial phase of the wind-up responses measured in animal dorsal horn neurons (You *et al.*, 2003). The facilitation and inhibition of the nociceptive system outlast the stimulation period investigated and may be related to long-term potentiation (LTP) and depression (LTD) (Klein *et al.*, 2004).

It is still unclear at which level of the nervous system the temporal filtering occurs during OA. Functional magnetic resonance imaging has revealed increased activity in the brain areas often related to pain processing such as the periaqueductal gray, thalamus, and insula (Derbyshire & Osborn, 2009; Yelle *et al.*, 2009). However, these central processes may likewise be driven by changes in the peripheral drive during OA. Indeed, substantial temporal filtering of the noxious heat stimulation (traditionally used for generating OA) can occur at the peripheral receptor level, e.g. adaptation (Tillman *et al.*, 1995; Treede *et al.*, 1998).

Even though most OA-studies are controlled for adaptation to a constant heat stimulus (Hermans *et al.*, 2016), receptor adaptation may be a non-linear phenomenon that is amplified through the central nervous system. The present study used electrical stimulation, which bypassed the peripheral receptor transduction mechanisms, and hence was used to investigate if the OA-phenomenon could be evoked by such a driving input. For preferential activation of the thin epidermal nerve fibers, the present study applied electrical stimulation

through an electrode consisting of a circular array of small-area pin electrodes (Mørch *et al.*, 2011). An electrical OA model may possibly be translated into pre-clinical studies.

MATERIALS AND METHODS

Study population

Young healthy volunteers were recruited (12 females and 12 males, 21 right-handed; average age: 26±4 years). The participants were excluded if they suffered from any concomitant pain problems, used any analgesics, lacked understanding of the procedures of the study, or had any history of alcohol or drug abuse. All participants were given oral and written information, and signed written informed consent were obtained from all of them 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). All experiments were conducted by Davide Ligato.

Study design

All volunteers underwent a total of 8 trials: 2 control-trials and 2 OA-trials for each stimulation modality (heat and electrical) applied to the dominant or non-dominant arm. The orders of the trial (control or OA), the modality (heat or electrical), and arm (dominant or non-dominant) were randomized. The test site was located and marked 3cm distal to the elbow joint on the volar side of each of the forearms. The OA experimental trials were

divided into three time intervals of painful stimuli: STIM1 (5 seconds), STIM2 (5 seconds), and STIM3 (20 seconds), in accordance with the original study (Grill & Coghill, 2002). The control-trial was a constant intensity stimulation with stimulus intensity equal to that of STIM1 and STIM3.

All the trials were separated by five minutes in an attempt to minimize the carry-over effects on the site of the stimulation (Pfau *et al.*, 2011), as primary afferents have adaptive behaviors (Tillman *et al.*, 1995; Derbyshire & Osborn, 2009).

Electrical trials

Electrical stimuli were applied as a train of rectangular pulses (frequency: 100Hz; pulse duration: 1ms) delivered by a constant-current stimulator (DS-5, Digitimer Ltd., Welwyn Garden City, UK). The electrical stimulation was delivered with a custom-built electrode consisting of 15 small cathodes (diameter: 0.2 mm; length: 1.6 mm) placed in a circle (diameter: 1cm) and a concentric anode (see figure1). The stimulation intensity was normalized to the Electrical Pain Perception (EPP) of each subject on each arm. The EPP was estimated as the average of three trials, where the current was increased from a baseline of 0.5mA in steps of 0.1mA with inter-pulse intervals of 5s until the participants reported the stimulation to be painful (Vo & Drummond, 2014).

Two OA-trials with the following intensities: STIM1=150% of EPP, STIM2=180% of EPP, and STIM3=150% of EPP, and two control-trials with a constant intensity of 150% of the individual EPP for 30s (i.e. the same duration as the OA-trial) were applied. The sequence of the electrical trials was randomized.

Thermal trials

Thermal stimuli were delivered with a 30×30mm square computer-controlled thermode attached to the forearm with a Velcro strap (ATS, Pathway system, Medoc ltd., Ramat Yishai, Israel). Baseline temperature of the thermode was set at 35°C and with a rise and fall rate of 6°C/s. Two OA-trials were conducted with the following temperatures: STIM1=48°C, STIM2=49°C, and STIM3=48°C, and two control-trials were conducted with a constant temperature of 48°C for 30s, according to the paradigm proposed by Grill and Coghill (Grill & Coghill, 2002). The sequence of the thermal trials was randomized.

Assessment of perceived pain intensity

The participants rated the experienced pain intensity on a continuous, electronic Visual Analog Scale (VAS; Aalborg University, Aalborg, Denmark), anchored at 0 (no pain) and 10 (worst imaginable pain).

Data analysis

The VAS scores were averaged across the two arms. The term 'OA-effect' was chosen to indicate the VAS difference of the OA-trial compared with the control-trial. The OA-effect is present after the decrease of the painful stimulus from STIM2 to STIM3; therefore, the mean VAS score from 1s to 5s after the onset of the STIM3 interval was used to analyze the OA-effect. This corresponds to 16s - 20s into the thermal trials (due to the delay of the

thermodes to reach the target temperatures and the latency of the response) and 13s - 17s into the electrical trials (figure 2).

Initially, a three-way rmANOVA was applied to investigate the difference in pain ratings during the OA-response with modalities (thermal, electrical) and trials (control, OA) as within-subject factors (SPSS 24.0, IBM Corporation, New York, USA). Bonferroni adjusted post-hoc was applied to adjust for multiple comparisons. Secondly, the OA-effect was calculated as the difference in the pain ratings between the control- and the OA- trials. Finally, Pearson's correlation was applied to investigate any potential relation between the thermal and electrical OA-effects and a frequency analysis were conducted to investigate thermal and electrical OA-effect above 0 using a chi-square test. P-values less than 0.05 were considered significant. In the text and figures, data is presented as mean ± standard error of mean (SEM).

RESULTS

Thermal and electrical trials

The stimulation intensities and the mean VAS scores during control- and OA- trials for electrical and thermal stimuli are shown in figure 2. Significantly lower STIM3 VAS scores (rmANOVA: $F(1,23) = 22.53$, $P<0.001$) were found for OA-trials compared with control-trials (thermal OA-effect: 1.81±0.54; electrical OA-effect: 2.12±0.42; figure 3). The STIM3 thermal stimuli were rated significantly higher than the electrical stimuli (rmANOVA: $F(1,23) = 10.78$, $P<0.01$), and there was no significant interaction between modality and trial (rmANOVA: $F(1,23) = 0.40$, $P>0.5$; figure 3). Therefore, heat and electrically evoked OA are generating the same degree of OA.

Correlation analysis

A significant and positive correlation was found between thermal and electrical OA-effect ($r = 0.48$, $P<0.02$), indicating that a large OA-effect to thermal stimuli was associated with a large OA-effect to electrical stimuli, see figure 4. A frequency analysis showed that 18 of 24 subjects demonstrated a thermal OA-effect larger than 0 and 19 of 24 subjects demonstrated an electrical OA effect larger than 0, which was not significantly different (chi-square: 0.12, $P<0.73$).

DISCUSSION

The current study is the first to demonstrate that electrical stimuli can evoke OA, indicating that the OA-phenomenon is independent of peripheral receptor transduction mechanisms. Further, this study demonstrated that the effect size of OA is similar between the two modalities.

Central or peripheral mechanisms underlying OA?

The underlying mechanisms of OA are unknown, and it has been debated to which extent the mechanisms are mainly central or peripheral. Early studies demonstrated that activities in the brain areas related to pain processing are modulated during OA (Derbyshire & Osborn, 2009; Yelle *et al.*, 2009). OA appears to rely on other mechanisms than conditioned pain modulation mechanisms, as the analgesic effects add to each other (Honigman *et al.*, 2013) and evoke activity in the brain stem differently (Naugle *et al.*, 2013). The OA-effect is

most likely not dependent on NMDA (Niesters, Dahan, *et al.*, 2011; Niesters, Hoitsma, *et al.*, 2011) or opioid-receptors (Martucci, Eisenach, *et al.*, 2012; Suzan *et al.*, 2015). Capsaicin-induced sensitization does not significantly alter the OA-response (Martucci, Yelle, *et al.*, 2012). However, OA is decreased in neuropathic pain patients (Niesters, Dahan, *et al.*, 2011), while chronic pain patients showed impairment of OA (Kobinata *et al.*, 2017). Recently, resting heart rate variability (HRV) was related to OA-responses (Van Den Houte *et al.*, 2017) and clonidine-induced increases of HRV have an effect on OA but not conditioned pain modulation (Nahman-Averbuch *et al.*, 2016). In addition, the pain inhibitory effect from OA might be age-dependent, as younger adults demonstrate an increased OA-effect compared with older and middle-aged adults (Naugle *et al.*, 2017).

Furthermore, it was shown that the OA-response can be evoked when the three stimuli (STIM1, STIM2, and STIM3) are applied at three different areas of the same or the contralateral arm (Ligato *et al.*, 2018). Although the OA-effect was less when the areas were separated, the results indicated that the temporal variation of the heat stimulation was centrally modulated and not only at the periphery (Ligato *et al.*, 2018). The present results further demonstrated that OA could be evoked when the peripheral receptors are bypassed.

Thermal and electrical stimulation paradigms

The current study applied the traditional heat evoked OA-paradigm (Grill & Coghill, 2002) with temperatures of 48-49-48°C. The traditional OA-paradigm has also been used in other studies, but applied at different temperatures ranging from 41-42-41°C (Derbyshire & Osborn, 2009) to 49-50-49°C (Grill & Coghill, 2002; Yelle *et al.*, 2009; Martucci, Eisenach, *et al.*, 2012; Nahman-Averbuch *et al.*, 2014). The electrical OA-paradigm used similar timing as

the thermal OA-paradigm, but since the thermode increased and decreased the temperature by 6°C/s, the stimulus durations (STIM1, STIM2, and STIM3) were longer as compared with the square wave electrical stimuli. The selected electrical stimulation intensities of 150%-180%-150% of the EPP resulted in lower VAS scores when compared with the thermal trials, but the OA-effect sizes were similar between the two modalities. Higher pain intensities during the electrical stimuli would be ideal for comparison between the stimuli modalities, but the electrode design and the high frequent electrical stimuli tend to produce a mild to moderate pain intensity (Klein *et al.*, 2004, 2008; Lelic *et al.*, 2012), which is not comparable to the pain intensities evoked during the thermal OA-paradigm proposed by Grill and Coghill (Grill & Coghill, 2002).

A moderate correlation between electrical and thermal evoked OA were found and a frequency analysis demonstrated that both paradigms have similar responders (OA-effect above 0), indicating that these are associated.

Activation of sensory afferents by thermal and electrical stimulation

When heat energy is deposited at the surface of the skin, it is passively transported to the epidermal layer where the nociceptors terminate. However, this passive heat transport only delays temperature changes occurring at the skin surface for some milliseconds (Frahm *et al.*, 2010). Subsequently, the heat sensitive ion channels, e.g. the Transient Receptor Potential Vanilloid 1 (TRPV1), opens and generate action potentials (Caterina *et al.*, 1997). Applying 50°C heat stimulation to a culture cell expressing TRPV1 indicated a slowly increasing current over several seconds, whereas the current rapidly reduced as the heat

stimulation was removed (Caterina *et al.*, 1997). This heat transduction of the TRPV1 receptor could at least partially explain the OA-phenomenon.

In contrast to heat stimulation, the electrical stimulation bypasses any modality-specific ion channels by depolarizing the nerve membrane directly (Palmer *et al.*, 2004). Therefore, temporal delay caused by heat transduction is not present when using electrical stimulation.

An electrode consisting of an array of small cathodes was used in the present study in order to preferentially activate the nociceptive epidermal nerve fibers (Inui *et al.*, 2002; Mouraux *et al.*, 2010; Mørch *et al.*, 2011). Originally, Grill and Coghill 2001(Grill & Coghill, 2002) argued that OA was pain intensity driven. The current electrical methodology was exploratory and was standardized according to pain thresholds and the data on the electrical intensities was not recorded and therefore cannot be reported. Future studies are encouraged to collect and report electrical intensities, since higher intensities will active different fibers. Electrical and thermal stimulation activated different, but also overlapping populations of primary afferents, and therefore, differences in the perception responses may be attributed to differences in the temporal behavior of primary afferents and signal pathways.

Adaptive behavior of heat sensitive primary afferents

Heat-sensitive Aδ- and C- fibers are categorized as slowly adapting and rapidly adapting (Meyer & Campbell, 1981; Treede, 1995; Schepers & Ringkamp, 2010). The former, slowly adapting, gradually respond to a thermal stimulus, and could less easily adapt when a

thermal nociceptive stimulus is maintained over time (Meyer & Campbell, 1981; Treede *et al.*, 1995; Schepers & Ringkamp, 2010). In contrast, the latter responds virtually immediately after the onset of a thermally induced nociceptive stimulation, and, consequently, rapidly adapting when the heat stimulus is maintained over time (Colon *et al.*, 2017). Previous microneurography studies have suggested that thermal stimuli activate both slowly and rapidly adapting thermo-nociceptors (Meyer & Campbell, 1981; Treede *et al.*, 1995). Therefore, thermal-OA could be partly explained by the differences in the responses of slowly-adapting Aδ- and C- fibers, which both respond to sustained (about 30s), high (49°C) thermal stimuli on the skin (Treede, 1995; Treede *et al.*, 1998; Schepers & Ringkamp, 2010). The current interpretation is that rapidly adapting C-fiber nociceptors are located more superficially in the skin, while the slowly adapting nociceptors are located less superficially (Wooten *et al.*, 2014). However, depth alone cannot entirely explain this different behavior of the two different fibers; moreover, slowly adapting C-fibers have confirmed poor adaptation when the thermal stimulation is maintained over time (Meyer & Campbell, 1981; Schepers & Ringkamp, 2010; Wooten *et al.*, 2014). This could indicate that the fibers may differ regarding heat transduction and/or sensitization mechanisms (Wooten *et al.*, 2014).

Nevertheless, the variation of the slowly and the rapidly adapting fibers could be related to a different expression of the transduction channels and, therefore, lead to a different response in pain intensities. The electrical stimuli bypass this transduction mechanism (Men & Matsui, 1994; Hitoto *et al.*, 1998), and are yet able to evoke an OA-effect. The different behaviors seen in figure 2 (in particular, referring to the last part of the pain intensity curves in STIM3) indicate that while the OA-effect is similar in size, processing of the nociceptive information differs between thermal input and electrical stimulation.

In the present study, a further fundamental difference between thermal and electrical stimuli was that thermal stimulation was applied as a tonic stimulation, whereas electrical stimulation was applied as tetanic stimuli. In consequence, thermal stimulation provides a de-synchronous stochastic input to the central nervous system, whereas electrical stimulation provides synchronous, almost discretized input to the central nervous system.

It remains unclear to which extent the human nociceptive system utilizes such 'pattern coding'; however, there are some indications that bursting information is processed differently than regular tetanic information, and possibly even in different brain areas (De Ridder & Vanneste, 2016).

Pain modulation by repeated electrical stimulation

LTP is considered an important feature that contributes to the pain amplification in the spinal nociceptive pathways (Sandkühler, 2009; Pfau *et al.*, 2011). In order not to involve the absolute and the relative refractory periods with a maximum discharge frequency of the C-fibers up to 190Hz, the frequency of the stimulation was set to 100Hz in the current study since a higher frequency could result in less efficiency of the afferent input to the spinal neurons (Weidner *et al.*, 2002). It has been demonstrated that high frequency (100Hz) noxious electrical stimuli can induce LTP (Liu & Sandkühler, 1997; Klein *et al.*, 2004). In contrast, the low frequency (1Hz) noxious electrical stimuli are reported to induce a LTD (Klein *et al.*, 2004; Rottmann *et al.*, 2010). Currently, no studies have confirmed an association between facilitated or decelerated activation of wide dynamic range neurons in the dorsal horn and OA-effects, why this remains speculative.

The present study showed a slightly decreased VAS score during constant intensity of 100Hz electrical stimulation (figure 2B, control-trial), which is assumed to reflect the balance between facilitatory and inhibitory mechanisms possibly related to the generations of LTP and LTD. However, the slight increase in stimulation intensity during STIM2 caused a significant decrease in the VAS scores during STIM3, similar to the thermally induced OA. However, the electrically evoked VAS score did not increase again as seen for thermal stimulation, which could suggest a facilitated adaptation. Additional research regarding the stimulus paradigm is needed to confirm if this is electrically evoked OA or facilitated adaptation. The current study applied a novel electrical stimuli protocol to evoke Offset Analgesia, which could be improved by investigating if e.g. higher electrical evoked pain intensities or different duration of stimuli would yield a larger OA-effect.

CONCLUSION

For the first time, it was shown that electrical stimuli can evoke OA to the same degree as heat evoked OA. This indicates that OA is independent of the temporal filtering caused by the peripheral transduction mechanisms. Further, the study demonstrated that the OA-responses to heat and electrical stimuli were associated. The electrical OA-paradigm could be used to progress the knowledge on OA, and possibly be translated into animal research to investigate the contribution of peripheral and central pain mechanisms, and how OA is pharmacologically modulated.

AUTHOR CONTRIBUTION

All authors designed the study. Davide Ligato collected and analyzed the data. All authors discussed the results and commented on the manuscript. All authors have carefully read and reviewed the manuscript.

ACKNOWLEDGEMENT

Shionogi Science Program and TaNeDS Europe were acknowledged for their support.

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Figure legends

Figure 1: Custom-built electrode consisting of 15 small cathodes (diameter: 0.2mm; length: 1.6mm) placed in a circle (diameter: 1cm) and a concentric anode.

Figure 2: Mean of the intensities of the VAS scores of thermal (red) and electrical (blue) stimuli. The control-trials are indicated with dashed lines and OA-trials with continuous lines. The gray window represents the time window chosen for the statistical analysis. VAS: Visual Analogue Scale. EPP: Electrical Pain Perception.

Figure 3: Pain intensity scores (±SEM) for thermal and electrical control and offset analgesia trials. "*" indicates $P<0.05$. Abbreviations: VAS: Visual Analogue Scale, SEM: Standard Error of the Mean.

Figure 4: The correlation between thermal OA-effect (x-axis) and electrical OA-effect (y-axis). Pearson's correlation: $r = 0.48$ (continuous black line), $P < 0.02$. The dashed lines represent the cut-off chosen (at VAS 2). The OA-effect indicates the VAS difference of the OA-trial compared with the control-trial. VAS: Visual Analogue Scale.