Reproducibility of spatial summation of pain effect during COVID-19 pandemic

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Abstract

The ongoing COVID-19 pandemic has led to many restrictions affecting the research conduct. The purpose of this study was to reproduce the previously observed spatial summation of pain effect (SSp) using non-laboratory procedures and commercial equipment. An additional aim was to measure the association between expectation and SSp for the first time. The Cold Pressor Task (CPT) was used to induce SSp. Healthy participants (N=68) immersed their non-dominant hands (divided into 5 segments) into cold water (Cold Pressor Task). Two conditions were used 1) gradual hand immersion (ascending condition) and 2) gradual hand withdrawal (descending condition). Pain intensity was measured on a Visual Analogue Scale (VAS). The influence of psychological factors, such as the volunteer's expectation of pain intensity, on the actual perception of pain were also measured on a VAS. Results showed significant SSp ($\chi_{w(4)}$ = 116.9, p < 0.001), reproduced with non-laboratory equipment in a home-based set-up. Furthermore, two novel findings were observed: i) spatial summation increased with the increase in exposure to the noxious stimulus ($\chi_{w(2)}$ = 157.5, p < 0.001), ii) there was a significant correlation between expectation and perceived pain, indicating that pain expectations can contribute to SSp. Results showed that SSp is shaped by a mixture of excitatory and inhibitory mechanisms and is influenced by the sensitization of the nociceptive system. Moreover, spatial summation is influenced by expectation. This study proposes a new feasible way to induce SSp using a home-based set-up using the CPT during COVID-19.

Keywords: Spatial summation, noxious cold, cold pressor task, COVID-19, expectation, pain

1. Introduction

Spatial summation of pain (SSp) exists at least in three different contexts: from the Sherringtonian point of view, nociceptive neurons receiving multiple collateral stimuli from adjacent neurons can generate action potentials even though the stimuli are subthreshold in nature [37]. In a physiological context SSp can be observed when pain becomes more intense when body area [2,19,26] or distance [3,33] between stimulated areas are increasing – these phenomena are called area-based and distance-based SSp [35]. In a clinical context, SSp can be used as a framework to explain severe pain intensity reported by patients with widespread pain [41]. Several possible mechanisms have been proposed to contribute to the SSp effect, e.g., local stimulus integration [32], neural recruitment [32,33], lateral inhibition [34], or diffuse noxious inhibitory control [26]. An interesting observation, recently reported following two experiments, is that there is no linear increase in pain intensity during a linear increase in the stimulated body area [2] or in the distance between the stimuli [2,3].

The cold pressor task (CPT) is widely used in studies on nociception [15,17,19,20,27,46,47] and SSp [1,18,19,27,38,46,47]. In CPT a body part is irritated by noxious cold (water). The low temperature activates nociceptive fibers [9,30,47] through low temperature sensitive ion channels TRPM8 [29] leading to increasing pain of mild to moderate intensity [45]. CPT is a suitable method to study SSp; the stimulated bodily area can be adjusted in a controlled and standardized manner by immersion depth [18,19]. A similar method using warm water instead of cold, was also used by Marchand & Arsenault [26] to study SSp. Their results showed that SSp was observed only in the condition using a gradual decrease of the stimulated area but did not occur in a progressively increased stimulated area. Moreover, the perceived pain was less intense during the decreasing compared to the increasing condition. The authors [26] proposed an explanation that in the increasing condition, both facilitatory and inhibitory mechanisms were gradually being recruited at the same time, thus they were interfering with each other.

During the COVID-19 pandemic and its associated restrictions, conducting research was significantly hampered [31,39]. Taking this into account, the authors decided to empirically test if it is possible to conduct an experimental study on SSp outside the laboratory [38] and replicate the SSp effect using a similar yet adopted methodology from Marchand & Arsenault [26]. Furthermore, the current experiment aimed to investigate the potential association between pain-related expectancy and SSp. Indeed, there is an evidence that pain could be influenced by expectations [5,13]. Studies on stimulus expectancy in pain showed that even short-term expectancies could have effects on pain perception [4,22]. Previously studies shown association between pain-related expectancy and conditioned pain modulation (CPM) [7,14,16,21] or placebo effects [10,11]. The current experiment aims to investigate participants' predictions regarding pain within SSp paradigm. It was hypothesized that participants can

predict pain produced by noxious stimulation of different sizes.

2. Methods

2.1. General information

The study design was based on the experiment by Marchand & Arsenault [26]. The study was designed as a within-subject experiment. Each participant took part in two consecutive experimental conditions conducted in a random order during which they immersed their hand in a cold-water tank. The study was conducted in a home environment, i.e., each examiner performed an experiment within their households (see details below) following an intensive training for the precise data collection and screening procedures. The study was approved by the Bioethics Committee of Academy of Physical Education in Katowice (1-2021/02-25-21) and was conducted in accordance with the Declaration of Helsinki [48]. The study was pre-registered at the OSF platform (*https://osf.io/kjbdz*).

2.2. Participants

A total number of 68 participants (29 males; 39 females; age: 18-57, mean: 31.08) completed the experiment. Examiners (n=13) consisted of members of the Laboratory of Pain Research at the Academy of Physical Education in Katowice or laboratory collaborators. Only healthy participants aged 19-65 years could take part in the study. A thorough interview was conducted using a screening questionnaire. Participants were excluded if they had a current trauma or wounds in the non-dominant hand, had COVID-19 disease (at the study date or in the past), suffered from acute pain on the study date or within 24 hours preceding the study, took psychoactive substances or medications on the study date, were diagnosed with a disease related to cold temperature intolerance (e.g., Raynaud syndrome, cryoglobulinemia, cold urticaria, etc.), had experienced in the past a pathological reaction to cold temperature (e.g., excessive edema or redness, blisters, etc.). Additionally, due to the fact, that the experiment was conducted outside the laboratory and to avoid any adverse events, rigorous exclusion criteria were applied (see **Appendix 4**). Apart from those derived from previous experiments on cold pressor task (see [23] for example) additional exclusion criteria were obtained from the literature regarding cryotherapy [24,42]. Lastly, if there was any concern about the health condition, the decision to participate was made by a medical doctor who was a member of the research team (AM).

2.3. Equipment for CPT

Because the experiment was designed to be performed in a non-laboratory setting, commercially available equipment and tools were used. Transparent plastic rectangular containers (36.5 × 27.5 × 22cm) filled with cold tap water (until 15cm height of the container) were used for the Cold Pressor Task (CPT). To obtain the desired water temperature of approximately 5°C, 6 foil ice-cube packs were used (a total of 144 ice cubes) for each experimental condition. An electronic (±0.1□) thermometer was attached to the plastic box [38] to monitor the temperature. This

non-laboratory version of the CPT was previously validated and led to comparable SSp induced via laboratory-based cold pressor with water circulation [38].

2.4. Experimental design

Before the first immersion of the hand, participants were instructed about the test procedure and prepared for the measurements. Subsequently, the participants were asked about their general fear of pain and fear of pain caused by the cold temperature by using a 0-10 Verbal Rating Scale, where 0 was defined as "no fear of pain" and 10 as "the greatest fear of pain you can imagine". The non-dominant hand was then divided into 5 segments using the anatomical points of the hand (**Figure 1**): (1) first segment - from the fingertips to the distal interphalangeal joint of the third finger; (2) second segment - from the distal interphalangeal joint to the proximal interphalangeal joint of the third finger; (3) third segment - from the distal interphalangeal joint of the third finger to the metacarpophalangeal joint; (4) fourth segment and (5) fifth segment - the distance between the flexion line of the metacarpophalangeal joint of the third finger and the beginning of the flexion line of the metacarpophalangeal joint of the thumb are divided into two parts. The size of areas exposed to cold stimulation is presented in **Figure 4.** Before and during the main phase of experiment, examiners recorded the water temperature at multiple time points as well as the room temperature before each condition. The amount of ice added during the experiment was also recorded. Experiment began when the water temperature had decreased to 4.5 - 5.5□. Two experimental conditions, ascending and descending, were used in the study for all participants. In the ascending condition (see **Figure 1**), participants started from immersion of just one segment and sequentially they immersed a greater number of segments finishing with all 5 segments immersed. In the descending condition the order was reversed, i.e., they first immersed all 5 segments and finished with immersion of just one. The order of starting from one of the two experimental conditions was pseudorandom.

Before and after each experimental condition, the cold pain threshold (PT_{COLD}) was tested on the examined limb: an ice cube was placed on the palmar surface of the participants' hand and the time (in seconds) until the first pain sensation was recorded [43]. This procedure was used to control for sensitivity and was an integral part of the screening procedure. The interval between each condition was one hour. During that break, participants were asked to complete the SEWL (subjective experience of workload score) questionnaire to measure physical activity [6,36]. At the end, subjects were asked to provide demographic information and guess the purpose of the study. No one of participants knew the correct purpose of the experiment. A detailed presentation of the study flow is presented in **Appendix 1**.

2.5. Trial design

Each immersion/trial lasted 60 seconds (regardless of the number of segments involved) and inter-trial intervals were set at 5 minutes [18,26]. Each single trial started with the question about expected pain intensity for this trial. Participants were told and shown a figure demonstrating the area of the hand which will be exposed to water immersion. Participants were asked to mark a point on the Visual Analogue Scale (VAS) which best reflected their expected pain intensity. The scale for expectations had anchors of 0 (no pain) to 100 (worst pain imaginable). First measurement of expectations (prior to any immersion) started from descending condition segments 5/5 (segments 1-5). Next, participants were instructed to immerse their hand until the line which separated a given number of segments (**Figure 1**). They were explained and shown to stabilize their thumb to avoid its accidental stimulation. Participants were prompted to rate their pain intensity on the VAS scale (same as for expectancy) on the following time points: after 10, 30 and 50s. Participants were blinded towards their previous ratings as these were covered and remained inaccessible during the study. While participants provided ratings, examiners recorded the temperature of the water in a room where assessments were conduct. After hand withdrawal, participants were asked to place their hand in their axilla for about 4:30 minutes so that the skin temperature of the hand would reach the baseline level prior to the next immersion.

2.6. Data extraction and analysis

The main analyses were conducted in the following stages: In the first stage, the effect of stimulation area on pain intensity was investigated using a General Estimated Equations (GEE) model with three within-subject factors: “condition” (ascending, descending), “segment” (1, 2, 3, 4, 5 segments) “time” (10s, 30s, 50s).

In addition, the GEE was repeated with the variable “temperature” to investigate if this variable was systematically different within specified factors. The same analysis was repeated with pooled temperature set as a covariate. In the second stage, polynomial contrast analysis was performed to check the pattern (nonlinear, linear) of pain increase between immersions. Furthermore, in case of significant main and/or interaction effects, Bonferroni corrected t-tests contrasts were performed to describe reported effects. In the third stage, the effect of stimulation area on pain expectation was tested using a general linear model (GLM) with two within-subject factors: “condition” (ascending, descending) and “segment” (1, 2, 3, 4, 5 segments). Lastly, the correlation between pain intensity and pain expectancy was conducted using Spearman rank coefficients. Correlation between SSp (difference between pain in immersion of 5 segments versus only 1 segment) and physical activity (subjective experience of workload [SEWL] score), age, Δarea (difference in size between all five segments and one segment), and temperature of the water was

performed using Pearson or Spearman rank coefficients (according to distribution of the data). All statistical analyses were performed using SPSS software (version 25, Armonk, NY, USA). The α level was set as 0.05.

3. RESULTS

A total of 68 healthy volunteers were included in the study (57% females, mean age 31.1 years (± 12.17). Further descriptive characteristics are shown in **Table 1.** All participants completed the study. No complications were described. Mean temperature of the cold water was 5.12°C (± 0.47). During the ascending condition ($t_{[66]}$ = -0.5, p = 0.63) as well as during the descending condition ($t_{[66]}$ =1.2, p = 0.22) no significant differences (before condition vs. after condition) were shown for the pain thresholds, indicating that pain sensitivity was stable over the course of experiment **(Appendix 2)**.

3.1. Primary analyses

3.1.1. Spatial summation of pain

The GEE showed a significant main effect for the factor "segment" (Wald $\chi^2(4)$ = 116.90, p < 0.001), indicating a significant SSp effect. Pairwise post-hoc Bonferroni-corrected comparisons showed significant differences in pain between the immersions of different number hand segments. SSp occurred between segment 1 and segments 1+2 (Mean difference (MD): -4.1; 95% CI -6.84, -1.36), segment 1 and segments 1+2+3 (MD: -9.88; 95% CI -13.66, -6.09), segment 1 and segments 1+2+3+4 (MD: -17.17, 95% CI -22.01, -12.33) as well as between segment 1 and segments 1+2+3+4+5 (MD: -23.72, 95% CI -29.95, -17.49). Furthermore, a linear relationship of these areas could be reported as indicated by the polynomial contrast (p < 0.001).

Furthermore, a significant main effect was found for the factor "time" (Wald $\chi^2(2)$ = 157.45, p < 0.001), indicating that sensitization occurred during immersion. Pairwise comparisons showed a significant difference between the first (10s) and second (30s) as well as between the first and third (50s) (last) pain measurement (p < 0.001). No significant effect of "condition" (Wald $\chi^2(1)$ = 1.07, p = 0.30) was found.

Significant two-way interactions were found between the factors "condition" × "segment" (Wald $\chi^2(4)$ = 18.57, p = 0.001), "condition" × "time" (Wald $\chi^2(2)$ = 8.31, p = 0.02) and "segment" × "time" (Wald $\chi^2(8)$ = 80.80, p < 0.001). Pairwise comparisons following these effects revealed that there was a significant difference in pain between the ascending and descending condition but only for the immersion of segment 1 **(Figure 2)** (p = 0.016). Likewise, for the two-way "segment" × "time" interaction, pairwise comparisons showed that spatial summation was significant regardless of the timepoint of measurement however, largest effects were observed for the last pain measurement (50s) (p < 0.001).

A significant three-way, i.e., "condition" × "area" × "time" interaction was shown (Wald $\chi^2(8) = 17.49$, $p = 0.03$), indicating that different spatial summation trajectories were observed across the two conditions (ascending vs. descending) in respect to timepoint of measurement (10, 30, 50s). Exploration of this interaction with the pairwise comparisons showed that significant differences between the ascending and descending condition occurred for the immersion of just segment 1 only during the third (50s) timepoint of measurement ($p = 0.017$), but not for the second (30s) ($p = 1.00$) and first (10s) ($p = 0.23$). Adding "temperature" as a covariate had only a marginal effect on the three-way interaction (Wald $\chi^2(8) = 15.3$, $p = 0.054$) and had no influence on other statistical results.

3.1.2. Expected spatial summation

Descriptive statistics for expectation are presented in **Figure 2** and **Table 2**. No difference between expectancy measured before the first immersion (unbiased) versus expectancy measured on a trial-by-trial basis were found (Wald $\chi^2(1) = 1.52$, $p = 0.22$), thus it was decided to perform main expectancy analysis on the pooled dataset. GEE on expectations data did not show significant main effect for the factor "condition" (Wald $\chi^2(1) = 2.16$, $p = 0.14$), but for factor "segment" (Wald $\chi^2(4) = 109.133$, $p < 0.001$). Pairwise comparisons showed significant differences in expected pain between immersions of different numbers of hand segments (all p values < 0.001). Namely, the expected pain level was lower for a single segment compared to two (MD): -4.01; 95% CI -5.32, -2.69), three (MD: -9.56; 95% CI -11.93, -7.18), four (MD: -16.11, 95% CI -19.25, -12.98) as well as five segments (MD: -20.62, 95% CI -24.65, -16.58). The "segment" × "condition" interaction was also significant (Wald $\chi^2(4) = 31.89$, $p < 0.001$), indicating that the pattern of increase in expected pain level was not different across conditions **(Figure 2)**. Spearman rank coefficients ($r = 0.53$ - 0.81) were statistically significant for all correlations between expected pain and actual pain **(Table 3)**.

3.2. Exploratory analyses

Exploratory correlations revealed no significant relationship between the magnitude of SSp and physical activity ($r = -0.13$, $p = 0.30$), maximal increment in the stimulated area (total stimulation are for five segments minus only one segment, $r = -0.13$, $p = 0.29$), or mean water temperature ($r = -0.18$, $p = 0.14$), age ($r = -0.04$, $p = 0.77$). Interestingly, the repeated measures ANOVA of the size of immersed segments showed a significant effect for "size", indicating that the area of stimulation increased from trial to trial ($F_{(1,67)} = 135.17$, $p < 0.001$, $\eta^2_p = 0.70$). Polynomial contrasts showed that the increase in size of the stimulated area could be explained by both a linear and an exponential function ($F_{(1,67)} = 35.47$, $p < 0.001$, $\eta^2_p = 0.35$).

4. Discussion

The main aim of this study was to introduce a novel methodology to study SSp during the COVID-19 pandemic and to reproduce a SSp effect, reported previously using an ascending/descending paradigm [18,19,26] and noxious cold stimulation [18,19]. The second aim was to investigate the associations between expectancy and SSp and to test if the effect is robust when controlling for temperature variability. Current results confirmed that SSp, as previously shown with noxious cold [18], and heat [26] stimulation of the whole upper extremity, can be reproduced in a home-based setting. This finding implies that the proposed "adapted" paradigm is feasible for conducting bedside testing in clinical and non-laboratory environments. Furthermore, our results contribute to the mechanistic understanding of SSp by showing that spatial summation i) can be inferred from subjects' pain expectations and ii) is strongly influenced by sensitization, iii) is likely shaped by descending pain inhibition.

4.1. Spatial summation during pandemic

Assessment of spatial summation outside the laboratory has never been investigated. The proposed methodology was inspired by difficulties with the data collection during the COVID-19 pandemic. In fact, experimental pain research often requires expensive equipment allowing for e.g. a control of the temperature of a stimulus during the experiment [45]. It can be discussed that the current study is a step forward by moving the laboratory-based pain research into field studies. The methodology employed here was recently proposed in a preliminary validation study, conducted on 9 volunteers. This previous publication validated home-based CPT against laboratory-based equipment with a constant temperature and circulation of the water [38]. The pilot study showed that the SSp trajectory was comparable in the laboratory vs. the non-laboratory paradigm, although the average temperature of the water might have been higher and more variable in the home set-up. In another study by McIntyre et al. [28] healthy participants were trained (online) to self-administer CPT and showed that 97% of participants did not report issues with the test procedure of CPT. These findings together with these current results suggest, that the assessment of pain modulation can be used safely in the home environment.

4.2. Reproducibility of spatial-related effects

The current study aimed to reproduce the effect previously shown by Marchand & Arsenault [26], yet using a modified methodology. In the mentioned experiment, participants' upper extremities were divided into 8 segments, such that the first segment included only the fingertips and the last segment included the entire arm (from fingertips to axilla). Authors not only observed a significant SSp effect, as pain was on average higher when a larger area was stimulated, but also an interaction between the sequence of immersions and the size of the stimulated area. This interaction indicated that the same size of stimulated body area (segments) was perceived differently in the ascending (immersion from fingertip to the axilla) compared to the descending (from axilla to fingertips) condition. In our study,

this interaction was reproduced, although it was prominent when only the immersion of the first segment (fingertips) was compared. In line with the previous observation, this interaction can be a manifestation of a robust activation of the descending pain inhibitory system in the descending condition.

Interestingly, SSp was reproduced in our experiment in a cohort of 68 healthy individuals, despite introduced changes to original methodology [18,26,40]. Firstly, only the hand was used and divided into 5 segments. Secondly, cold stimulation was used. Thirdly, the study was conducted with home-based equipment outside of the laboratory. The latter aspect supports the robustness of observed findings: Despite larger variability and random noise cause by different assessors and temperatures within the individual households, SSp was stable.

4.3. Spatial summation using cold pain

Additionally to the topical administration of cold stimuli using a thermode [12,25], SSp was previously reproduced by CPT in 6 experiments [27]; five of these showed significantly higher pain during the immersion of a larger area of the body [18,27,38,46]. A first attempt by Wolf & Hardy [47], was not successful. The authors compared pain provoked by the immersion of one finger to pain provoked by whole hand immersion and did not observe SSp. However, the study sample was small (n=2), and results could be explained by individual differences in SSp which are known to be large: lateral inhibition [34] may paradoxically lead to lower pain in larger stimulated area [1,34]. In a study by Westcott et al. [46], SSp was confirmed in 40 individuals by demonstrating more intense pain during full-hand immersion compared to the immersion of one finger into water of 0°C. In one study, SSp was provoked with a temperature comparable to that used in the current experiment (4.7°C) [27]. Participants withdrew their hand faster during stimulation of the whole hand compared to partial immersion. Julien et al. [18] divided the upper extremity into 8 segments and performed ascending and descending immersions with a water temperature of 12°C, which was necessary to allow patients with chronic pain to tolerate the stimuli. They provoked robust SSp and its disruption in the group of patients suffering from chronic pain. Two recent studies from our group confirmed the existence of SSp during hand immersion by dividing the hand into 5 segments [38], or two halves (ulnar and radial side) [1].

4.4. Physiological mechanisms of SSp

The mechanisms of SSp are not fully understood, however, it seems that both facilitatory and inhibitory processes interact during summation. This can be inferred from an interaction between the sequence and the stimulated body area (segments). It was observed that when the stimulation started from the largest area, pain provoked by immersion of segment 1 was slightly higher than the analogue stimulation in the ascending sequence. That discrepancy can be explained by the fact that in the descending sequence, inhibitory mechanisms are activated to

their maximum and persist during subsequent immersions, thereby resulting in lower pain during the immersion of segment 1. This is in line with Marchand & Arsenault's [26] study and a study on rats which showed that an increase in stimulation area from 1.9 to 18cm^2 gradually decreased the frequency of convergent neurons discharge in intact yet not spinally transected animals [8]. Furthermore, the increase in pain in either sequence was disproportional. Simply, a 5 times larger area does not multiply the reported pain intensity by the same value, which is in line with previous SSp studies [2,18,26]. It seems that the duration of the stimulus strongly affects the summation pattern. The curve representing the summation trajectory was steeper when the last measurements were considered (50s), indicating that SSp can be partially mediated by the sensitization within the neuroaxis. Another feature of SSp is the pattern of pain increase which was explained by a linear and a non-linear equation to the same extent. In a recent experiment with electrical stimuli, the pain increase followed a logarithmic curve when the size of the stimulated area increased in a linear fashion [2]. It can be hypothesized that in the current study, the increase in stimulus area was exponential (**Figure 4**), which could result in less efficient inhibition and thus a more linear increase in pain. However, this requires further research as a linear fit was negligibly no fitting model for the current data.

4.5. Expectations and spatial summation

Expectations have not yet been considered in SSp, in contrast to conditioned pain modulation (CPM), a pain-inhibiting-pain modulation paradigm. In a typical CPM experiment, participants are exposed to a test stimulus after the pre-exposure to a conditioning stimulus (CS). In a study by Traxler et al. [44], a high level of expected pain hampered the magnitude of the CPM effect. However, in a study by France et al. [14], expectations were matching the level of pain after application of the CS. Namely, those participants who expected lower reductions in CPM, experienced a more robust inhibitory effect. In another study, expectations were manipulated showing that expectations -if influenced by verbal suggestion- lead to a reduced CPM effect - but only in females [7]. As both CPM and SSp are pain modulation paradigms investigating the spatial aspects of pain, it is reasonable to assume that expectations also shape SSp. Correlations reported in the current study were positive and significant in 9/10 cases, indicating that participants expected more pain before immersion of the larger area. This can explain the anecdotal observations that humans prefer to gradually enter cold water instead of immediate full-body exposure.

4.6. Conclusions

This study proposes a new way to induce SSp using a home-based CPT. Three novel aspects were revealed in this study. Firstly, spatial summation of pain can be assessed outside of the laboratory, providing a new tool for experiments outside of the laboratory in e.g., clinical settings. Secondly, SSp can be shaped by a mixture of excitatory and inhibitory mechanisms and is influenced by the sensitization of the nociceptive system. Lastly, spatial summation

may be strongly influenced by expectation, but future studies with expectancy manipulation must confirmed this hypothesis.

5. ACKNOWLEDGEMENTS

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7. Figures, tables, appendixes

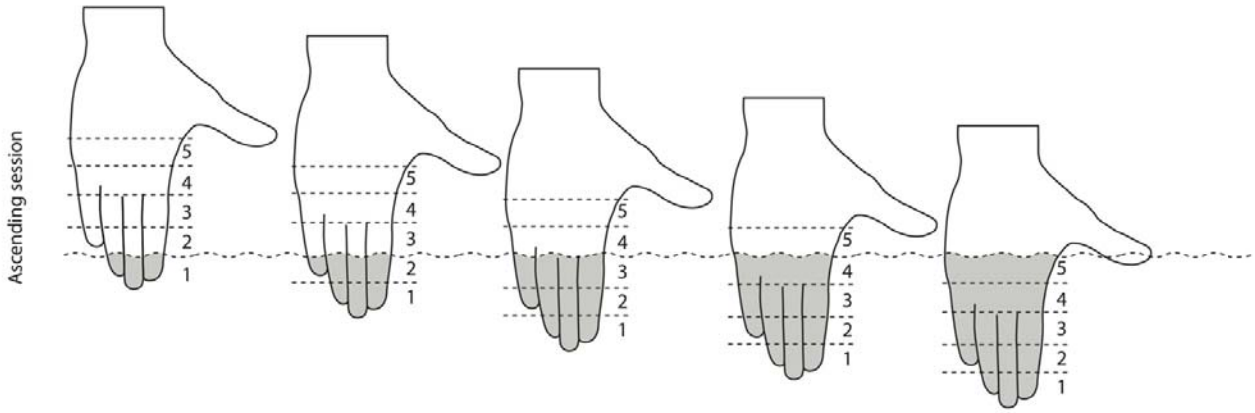


Figure 1. Experimental procedure. Example of the "ascending" condition with the hand divided into 5 segments: In the ascending condition, nociceptive stimulation started from a small area of the hand (fingertips) and increased in subsequent immersions. In the descending condition, the order was reversed: nociceptive stimulation started with the whole hand (segments 1-5) and decreased in subsequent immersions.

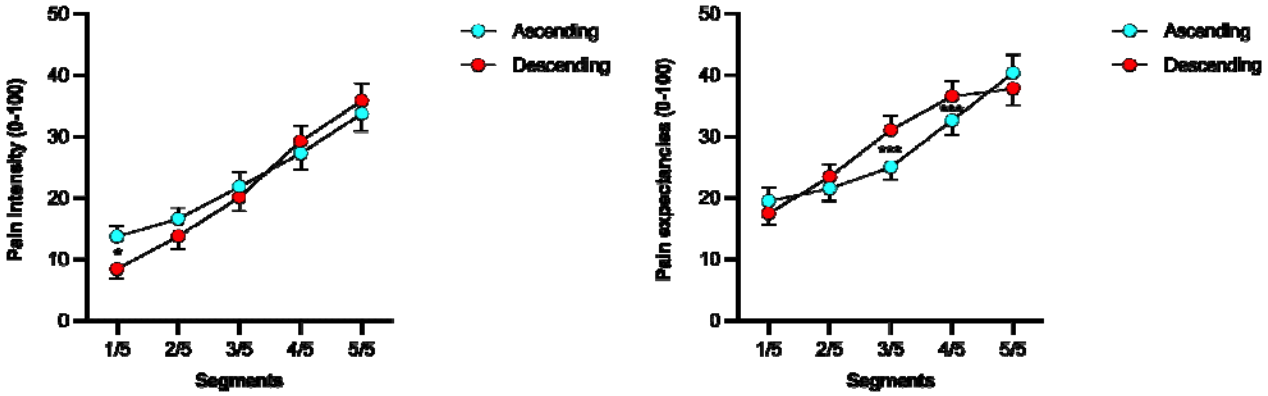


Figure 2. Significant interactions between segment(s) immersions and condition. Left: Mean pain ratings during immersion of each number of segments for the two conditions (ascending, descending). Right: Mean expectancy ratings prior to the first immersion of each number of segments for the two conditions. *Significant difference between the ascending and descending condition of the segment 1 (p = 0.016). *Significant difference in expected pain level ($p<0.001$) between the ascending and descending condition.

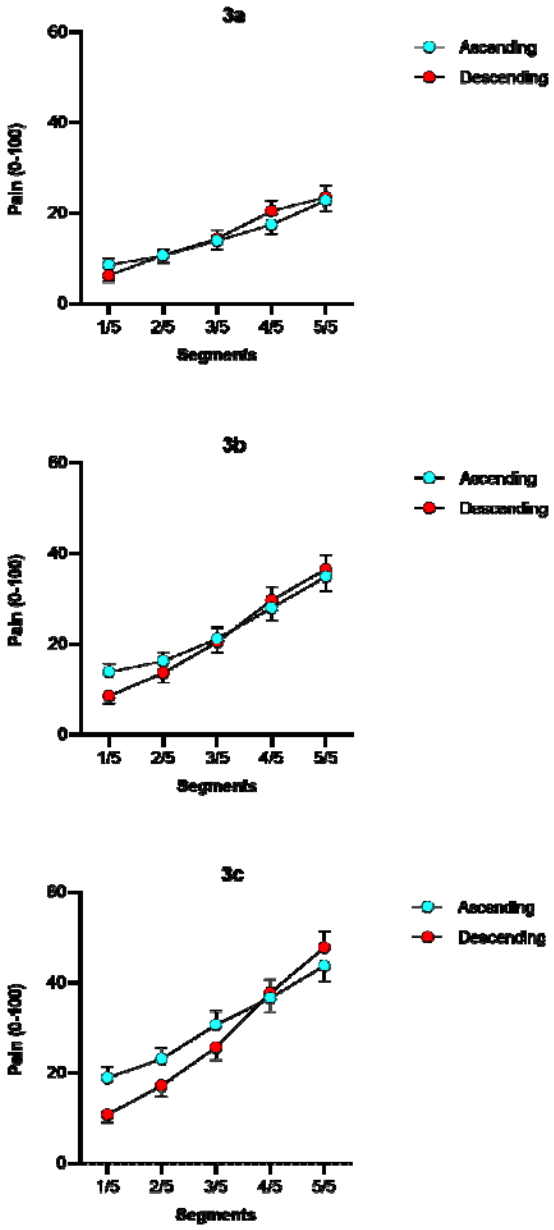


Figure 3. Sensitization drives the interaction between condition (ascending, descending) and spatial summation of pain. Top - mean pain ratings collected at 10s (**3a**), at 30s (**3b**), at 50s (**3c**). Note that the difference of pain intensity during immersion of the segment 1 becomes significant ($p = 0.017$) after 50s of immersion.

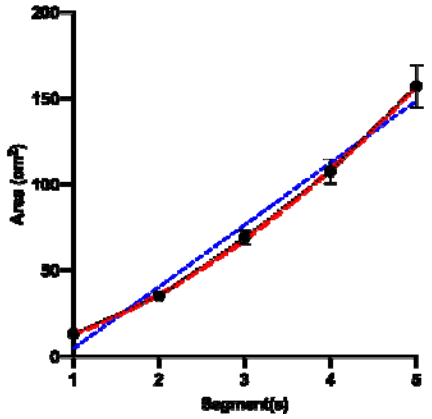


Figure 4. Hand areas exposed to noxious cold stimulation. Exponential (red) growth negligibly better ($R^2 = 0.48$ vs. 0.47) than

Table 1. Descriptive statistics

Variable	Mean (SD)
Age (years)	31.08 (12.17)
Height (cm)	173.83 (9.50)
Body mass (kg)	68.23 (14.75)
Fear of Pain General (NRS 0-10)	4.38 (2.15)
Fear of Pain Cold (NRS 0-10)	3.36 (2.19)
SEWL	10.31 (1.27)
	N
Sex	F = 39; M = 29
Handedness	R = 67; L = 1

F- Female, M -Male, R - right, L - left. NRS - numeric rating scale, SEWL - subjective experience of workload questionnaire.

Table 2. Expected pain levels

Segment	Mean (SD)	
	Ascending	Descending
1/5	20.77 (21.91)	22.45 (21.44)
2/5	23.58 (19.99)	25.70 (19.38)
3/5	25.85 (19.70)	29.67 (19.23)
4/5	34.74 (23.60)	34.27 (21.09)
5/5	42.04 (27.11)	37.05 (23.58)

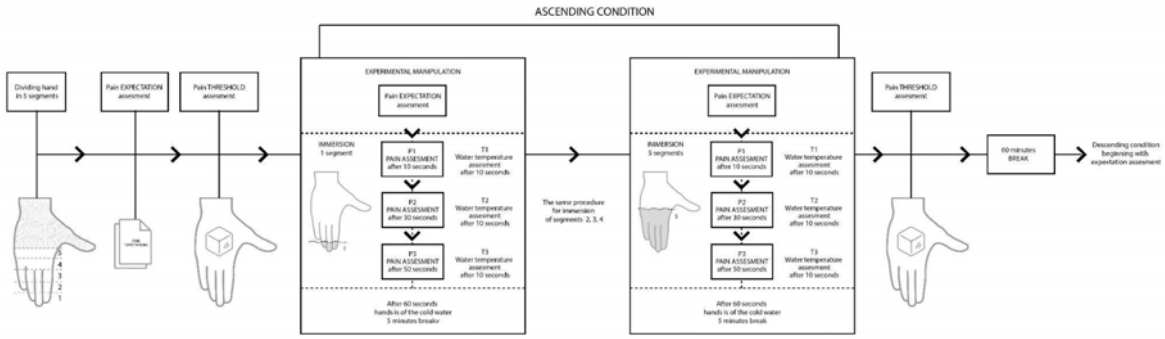
1/5 – Segment 1, 2/5 – Segments 1 to 2, 3/5- Segments 1 to 3, 4/5 – Segments 1 to 4, 5/5- Segments 1 to 5.

Table 3. Correlations between expectation and perceived pain

Condition	Immersed segments				
	1/5	2/5	3/5	4/5	5/5
Descending	0.53^{***}	0.70^{***}	0.72^{***}	0.77^{***}	0.60^{***}
Ascending	0.66^{***}	0.65^{***}	0.78^{***}	0.75^{***}	0.81^{***}

Spearman`s rank correlation coefficient: $^{*}p < 0.05$, $^{***}p < 0.001$

Appendix 1. Study procedures: Sequence of conditions was counterbalanced, ascending condition is presented as an example.



Appendix 2. Measurement of pain thresholds.

Pain threshold (seconds)	Mean (SD)
Before Ascending	73.28 (134.88)
After Ascending	76.53 (125.87)
Before Descending	74.10 (139.38)
After Descending	55.73 (85.08)

Appendix 3. Pain response during immersions

Condition	Time epoch	Segment(s)				
		1/5	2/5	3/5	4/5	5/5
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Ascending	10 s	8.54 (11.31)	10.60 (12.27)	13.85 (15.18)	17.44 (17.45)	22.77 (20.39)
	30 s	13.82 (15.42)	16.23 (16.39)	21.13 (20.53)	27.97 (23.35)	34.89 (25.43)
	50 s	18.92 (20.09)	23.07 (20.40)	30.63 (25.09)	36.57 (26.53)	43.19 (29.24)
Descending	10 s	6.17 (11.49)	10.64 (14.35)	14.30 (14.59)	20.42 (18.63)	23.48 (21.95)
	30 s	8.50 (13.32)	13.61 (17.60)	20.45 (19.84)	29.73 (24.07)	36.50 (25.62)
	50 s	10.79 (15.44)	17.19 (20.62)	35.63 (23.16)	37.80 (25.56)	47.73 (29.10)

Appendix 4. Exclusion criteria

Specific exclusion criteria: Raynaud's phenomenon (cold fading of the fingers, followed by blush numbness and redness), cold allergy (cold urticaria), cold intolerance, cryoglobulinemia, paroxysmal cold hemoglobinuria, rheumatic diseases (e.g. osteoarthritis, RA, fibromyalgia, systemic lupus erythematosus, etc.), pheochromocytoma, skin sensitivity disorders, sympathetic neuropathies, cardiovascular diseases (coronary artery disease, chronic heart failure, cardiac insufficiency), neuropathy (e.g. cardiovascular disorders), adrenal pheochromocytoma, sensory skin disorders, sympathetic neuropathies, cardiovascular diseases (coronary artery disease, chronic heart failure class III and IV according to NYCHA), hypothyroidism, purulent gangrenous skin lesions, local blood flow disorders.

Appendix 5. Protocol deviations

The following deviations from the pre-registered protocol must be acknowledged: i) the main outcome (pain) was not normally distributed, hence the GLM was replaced by a GEE, ii) the declared sample of N=90 was not reached due to

rigorous inclusion criteria and the peak of incidence rates of COVID-19 during recruitment for this experiment, iii) the type of correlation coefficients was applied according to normal distribution of analyzed variables (e.g., Pearson if normally distributed), iv) apart from polynomial contrast results, pairwise comparisons are reported.