Pain can be conditioned to voluntary movements through associative learning

Research Paper

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Pain can be conditioned to voluntary movements through associative learning: an experimental study in healthy participants

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Abstract:
Experimental data suggest that associative learning can influence defensive avoidance behavior and pain perception in humans. However, whether voluntary movements can become conditioned stimuli (CSs) and influence pain responses is yet to be evaluated. Forty healthy volunteers participated in this study. Electrocutaneous stimuli applied to the shoulder at pain threshold level (US^{test}) and at pain tolerance level (US) were determined before a movement-conditioning paradigm. First, reaching movements to visual cues shown on one side of a computer screen were associated with the US (CS+ movements) on 80% of trials, whereas reaching movements to visual stimuli shown on the other side were never associated with the nociceptive-US (CS− movements). Next, participants underwent a test phase in which movements to visual cues on both sides were paired with the US^{test} on 50% of trials. During the test phase, participants were asked to evaluate whether the movement was painful (yes/no) and to rate pain intensity after each trial. Movement onset and duration as well as skin conductance responses were collected. The US^{test} stimuli were more likely to be perceived as painful and were also rated as more painful during CS+ movements. Movement onset latency and skin conductance responses were significantly higher in anticipation of the CS+ movement as compared to the CS− movement. These findings suggest that pain can be conditioned to voluntary movements.

Keywords: Pain, Associative learning, Pain conditioning, Classic conditioning, Pavlovian conditioning

1. Introduction

Pain during voluntary movement is a common reason for seeking care in musculoskeletal clinical practice. Despite substantial scientific progress, it is still unclear why the spectrum of movement-related pain presentations is variable between individuals.[29,31,32]

Contemporary studies have investigated possible sources of variability about the relation between pain and movement through the lens of learning models.[24–26] The fear-avoidance model for chronic pain is one of the most known and studied learning models in the pain field, creating a theoretical framework for studies investigating the mechanisms that underpin pain persistence.[39–42] Recent work conducted by Meulders et al. have evaluated the acquisition of fear of movement-related pain[25] and of operant pain-related avoidance[24] using a movement-based paradigm. The results of these studies support the fear-avoidance model, indicating that fear of movement and avoidance can be acquired through learning during movement-related pain episodes.

It has been proposed that pain itself may be learned through Pavlovian conditioning.[27] Although the effects are moderate to small,[22] associative learning can influence an individual's pain perception, producing effects such as pain threshold modulation[9,21,37] and conditioned hyperalgesia.[10,12] Through associative learning, a person may learn the association between different stimuli.[33] For instance, when a neutral proprioceptive stimulus such as a voluntary arm movement (conditioned stimulus [CS]) is paired with a noxious and potentially harmful stimuli signaling nociceptive input (unconditioned stimuli [USs]) to which the person responds without the need of previous learning, the CSs (visual, tactile and proprioceptive stimuli associated with or preceding the US) might come to elicit defense responses such as fear and avoidance behavior in anticipation of a threat (conditioned responses [CRs]).[19,27]

Current evidence evaluating multisensory stimuli as CSs have used vibrotactile,[10,21,37] visceral,[11,48] or visual stimuli.[12,13] However, whether proprioceptive stimuli (during voluntary movements) can become conditioned and influence painful responses is yet to be evaluated. Because movements are complex behaviors that involve a chain of planning and anticipatory actions,[8] it is not possible to fully understand how pain conditioning takes place without observing how an individual changes the response to cues that predict painful movement execution.

This study aimed at examining whether proprioceptive inputs associated with arm movements can increase the chance of

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a stimuli at-pain-threshold to be perceived as painful after successive painful associations in healthy participants. We hypothesized that a Pavlovian conditioning procedure would lead to a higher percentage of stimuli at-pain-threshold level being perceived as painful during movements that were previously associated with an US-nociceptive stimulus (CS+ movements) in comparison with movements that were not previously associated with nociception (CS− movements). Furthermore, we hypothesized that pain conditioning would also lead to higher pain intensities during the CS+ in comparison with the CS− movements and higher defensive response including higher skin conductance responses (SCRs) (ie, measure of sympathetic arousal),[4] and higher movement onset latency (MOL) (ie, measure of avoidance tendency), before the CS+ movement initiation in comparison with the CS− movements.

2. Methods

2.1. Participants

Participants were recruited through flyers, social media, and word-of-mouth (on and off campus). This study included healthy participants aged 18 to 60 years old. The participants were excluded if they reported: (1) acute pain at the week of testing, (2) use of analgesic medication on the day of testing, (3) use of medications that could alter skin sensitivity, (4) a history of chronic pain (defined as pain every day for 3 months or longer), (5) use of an electronic implant (eg, a pacemaker), or (6) any serious reported psychological and neurological diagnosis.

Participants were informed that the purpose of the study was to investigate the relation between pain and movement, but were not informed about the experimental contingencies or the hypotheses. We conducted an a priori sample size calculation using G*POWER with the effect size obtained by Traxler et al. (2019), according to which 32 participants would need to be included for a power of 0.80% and 95% of statistical significance ($\alpha = 0.05$) to detect a 0.15 difference in primary outcome measure using Student *t* test.[37]

This study was conducted at the University of São Paulo. Once participants agreed with the study conditions, they were informed of the broad nature of the study and signed an informed consent. All procedures were approved by the Ethics Committee on Human Research of the Psychology Institute of the University of São Paulo (approval number 78039817.1.0000.5561) and followed the statements of the Declaration of Helsinki.

2.2. Stimulus material

Two white squares, measuring 5 cm × 5 cm, were presented on the right and the left upper corners of a 21.5″ computer screen (with gray background). Participants' reaching movements to these 2 visual stimuli were used as CSs. We considered the proprioceptive stimuli associated with reaching movement in addition to the appearance of the visual stimuli as CSs. CS+ movements were those towards the visual cue on one side of the screen, which were paired with the US, whereas CS− movements were those towards the visual cue on the other side of the screen, and which were never paired with the US. The assignment of which movement orientation served as CS+ or CS− was counterbalanced across participants. Moreover, movements were always performed with the dominant arm. Handedness was assessed using the Edinburgh Handedness Inventory,[30] and all participants were right-handed. The right arm was always used to perform the movements.

USs were electrocutaneous stimuli generated by an S48 stimulator (Grass Medical Instruments, West Warrick, RI). Specifically, we used a train of 100 electrocutaneous stimuli of 2-ms duration delivered through a rectangular bipolar surface electrode, placed on the shoulder region of the moving arm 1 cm above the acromion, with a delay of 10 ms. The anatomical location of the USs as well as its parameters was chosen because during 2 pilot studies with 15 participants each, other electrode placements and US characteristics were associated with strong muscle contractions that impaired the conditioning process. The final US parameters were perceived as a precise pinprick painful sensation during a qualitative pilot study. Using pulse trains also allowed for the production of more stable stimulus intensities.[28] Two different USs were calibrated before the experiment: US and US^{test}.

The USs intensities were individually calibrated to find the US and the pain threshold (US^{test}). The US^{test} was always calibrated before the calibration of the US. For the calibration of the US^{test}, a single ascending run of electrocutaneous stimuli was administered while the participant performed shoulder flexion movements to 90° in the scapular plane, until pain was perceived. The intensity that was considered painful was used as the central point of 5 different electrocutaneous stimulus intensities with a 0.4-mA difference between them, which were randomly varied 5 times each during arm flexion movements. The intensity of the central point was changed if all the 5 intensities were painful more than 3 times each. The US^{test} was determined using the method of constant stimuli as the intensity that produced a 50% probability of perceiving the stimulus as painful.[35] This method was adopted because response-dependent methods may be better suited to calibrate stimuli at-pain-threshold level that will be repeatedly delivered.[23]

The calibration of the US was also performed while the participant performed shoulder flexion movements to 90° in the scapular plane using a staircase method with a single ascending run. The stimulus intensity (mA) corresponding to the pain tolerance was used as the US. Participants were instructed to signal the highest electrocutaneous stimulation intensity they were able to tolerate, knowing that there could be a next higher intensity electrical stimulus. This stimulus intensity was chosen because, during a third pilot study with 10 participants, subjects experienced strong hypoalgesia induced by movement with lower or moderate stimulus intensities during the acquisition phase, which compromised the conditioning process.

Skin conductance responses were registered by means of 2 Ag-AgCl electrodes placed on the index and middle finger of participants' left hand connected to a GSR100C skin conductance module. Data were processed and analyzed offline using AcqKnowledge 4.2 software (BIOPAC Systems Inc, Galeta, CA).

The presentation of the visual cues and the delivery of the USs were controlled by a script developed with Matlab. The interface with which the subjects interacted was delivered using Psychtoolbox for Matlab (MATLAB 9.2 and Psychtoolbox 3.0.12., The MathWorks, Inc, Natick, MA).

2.3. Experimental design

The experiment consisted of 4 phases (calibration, baseline, acquisition, and test) conducted on a single day. During all phases of the experiment, participants remained seated on a chair with their arms resting on a table in front of them. The beginning of each phase was signaled by the experimenter, who gave participants the corresponding instructions (see below).

During the calibration phase, the US stimulation and SCR assessment electrodes were attached and US calibration

procedure was conducted (see Stimulus material). During the baseline phase, a general instruction was presented in the computer screen and participants received the opportunity to ask questions. They were instructed to keep the space key in the keyboard, spatially located in the midline, pressed with their right index finger and pay attention to the computer screen. A small cross, which was used as fixation stimulus, appeared in the middle of the screen. Visual cues were presented either on the right or left superior corners of the screen in a pseudorandom order with an interval between trials from 2.5 seconds to 3.5 (3 seconds in average). Participants were instructed to release the space key as soon as they saw the visual cue, reach to touch the visual cue with their index finger, and press the space key again. After the visual cue was touched, the subjects were instructed to quickly press the space key with the same hand, which turned the screen black and started the countdown to the next trial. This procedure was repeated 10 times for each side and the subjects were instructed to perform the movement in a comfortable speed. The trials were programed to run in a (semi)randomized order with the restriction of no more than 2 consecutive trials of the same CS type. Subjects were also reassured that no pain would be delivered during this phase. The basic temporal sequence of events during a trial is illustrated in **Figure 1**. During the acquisition phase, subjects were instructed that painful electrocutaneous stimuli may occur during some movements. In this phase, CS+ movements were paired with the US 80% of the time, whereas no US was paired with the CS− movements. The USs were delivered by means of a variable-interval (VI) schedule that was calculated using half of the mean time required for the subject to reach each of the visual stimulus during the baseline phase. This VI schedule was triggered by the release of the space key. This procedure was repeated 20 times for each side. Finally, during the test phase, subjects were instructed that this set of movements would be just like the previous. In this phase, 10 movements to each of the visual cues were paired 50% of the times with the US^{test}.

Participants were asked to rate pain intensity for each movement immediately after its execution, during the intertrial interval of the acquisition and test phases. After each movement, subjects were asked whether the movement was painful (yes/no) and to estimate their perceived pain intensity using a 0 to 10 visual analogue scale (VAS) if the answer was positive. This intertrial decision-making was used with the dual function of encouraging participants to stay vigilant during the movements, and to allow us to verify later the effect of the learning history upon the perceptual classification of nonpainful stimuli as painful.

After the acquisition and test phases, some questions for further exploratory analysis were added. The experimenter asked participants whether one movement was more painful than the other, and the participants could answer according to the following options: (1) Neither of the movements were painful, (2) The movement to the right was more painful than the other, (3) The movement to the left was more painful than the other, (4) Both movements were equally painful (pain decision-making measure). Participants were then asked to recall the overall pain felt for each side during the previous set of movements using a 0 to 10 VAS (pain recall [PR]). At the end of acquisition and test phases, subjects were also asked to do a US expectancy rating to each CS by answering the question "*To what extent do you expect movements for this side to be painful in the next phase?*" on a numerical rating scale ranging from 0 = "*I do not expect that movements to this side to be painful*" to 10 = "*I fully expect movements to this side to be painful*." Subjects were asked to make this judgement after the test phase without knowing that they would not pass through another phase.

2.4. Measures and outcomes

2.4.1. Exit questions

At the end of the experiment, participants answered a number of questions to assess (1) whether they were naive to the purpose of the experiment (ie, blinding check), (2) how they perceived the timing of the electrocutaneous stimuli relative to the movements, and (3) whether they noticed a relationship between the movements CSs and the intensity of discomfort of the electrocutaneous USs (ie, contingency awareness).

2.4.2. Primary outcome

The primary outcome of this study was the percentage of trials during the test phase in which at-pain-threshold electrocutaneous stimuli (US^{test}) were experienced as painful during CS+

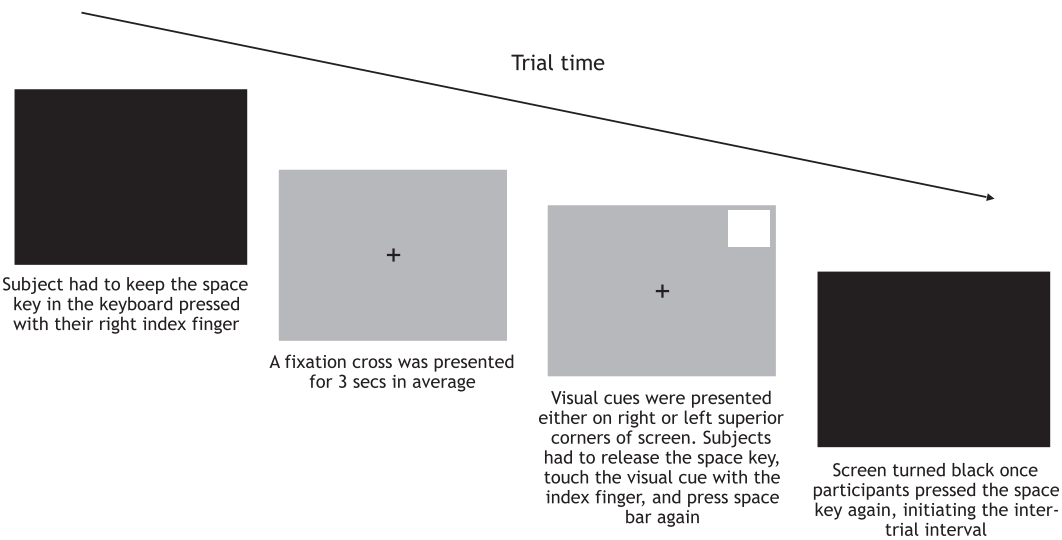

Figure 1. Illustration of the basic temporal sequence of a trial during all phases of the experiment.

movements in comparison with CS− movements. For that purpose, the intratrial answers to the question "*Was this movement painful (yes/no)?*" were used.

2.4.3. Secondary outcomes

Two motor behavior measures were assessed: (1) MOL, calculated as the delay between the presentation of the visual CS cue and the beginning of the CS movement (ie, the release of the space key); and (2) CS movement duration (MD), calculated by the delay between the release of the space key and the moment in which the space key was pressed again. The screen used in this experiment did not enable recording of the moment in which it was touched.

Skin conductance response was measured after the appearance of the visual CS cues during baseline, acquisition, and test phases. The SCR amplitudes were determined by manually selecting the difference between the base and the peak of the largest waveform (measured in microsiemens [μS]) in which the onset occurred in a range between 0.5 and 4.5 seconds after the presentation of each visual cue. Waveform was defined as an increase in skin conductance level to a peak followed by a slower recovery period. The minimum response criterion was 0.02 μS. Responses below 0.02 μS were scored as zero and were included in the analysis. Raw SCR values were square-root transformed before the analyses.[4,31]

The pain decision-making measures for both the acquisition and test phases (see Experimental design) were computed as 0 (neither of the movements were perceived as painful during the previous phase), 1 (the CS+ movements were perceived as more painful during the previous phase), 2 (the CS− movements were perceived as more painful during the previous phase), and 3 (both movements were equally perceived as painful during the previous phase).

3. Statistical analysis

Data were analysed using SPSS 25.0 (IBM SPSS Statistics for Windows, Version 25.0; Armonk, NY). Plots were generated in R v3.4.3 (R Core Team, 2017), in RStudio v1.1.414 (RStudio Team, 2018).

Before analysis, data were checked for normality, outliers, and missing values. When appropriate, nonparametric tests were applied. Post hoc comparisons with Bonferroni adjustment were applied to analyses of variance (ANOVAs) with significant results.

To test whether conditioning was successful, expectancy ratings were analysed by means of 2 separate 2 (Condition: CS+ vs CS−) × 2 (Phase: acquisition vs test) repeated-measures ANOVAs. The SCRs for each CS during the first and second halves of the acquisition phase were analysed by means of 2 Wilcoxon signed rank tests. These measures were used as an index of learning. In addition, blinding for the study goal and contingency awareness for the CS-US pairings were checked.

To test the main hypothesis that a Pavlovian conditioning procedure would lead to a higher percentage of stimuli at-pain-threshold level being perceived as painful during movements that were previously associated with an US-nociceptive stimulus (CS+ movements) in comparison with movements that were not previously associated with nociception (CS− movements), a binary variable reflecting nonpainful (0) and painful (1) at-pain-threshold trials was computed, based on the intratrial decision-making task. The percentage of US^{test} rated as painful was compared across the conditions $CS+/US^{test}$ and $CS-/US^{test}$ by means of a Wilcoxon signed rank test.

The secondary hypothesis that the conditioning would also lead to higher skin conductance responses to the appearance of the visual CS+ cue in comparison with the visual CS− cue, and higher response onset latency times to the CS+ movement initiation in comparison with the CS− movements was verified by means of Wilcoxon signed rank tests and a 2 (Condition: CS+ vs CS−) × 3 (Phases: baseline, acquisition, test) RM ANOVA, respectively. A similar ANOVA test was used to examine differential MD. Besides that, a Wilcoxon signed rank test was conducted to compare the pain intensity ratings for the $CS+/US^{test}$ and $CS-/US^{test}$ trials.

The relationship between the primary outcome, SCR, blinding, expectancy, and contingency awareness was explored by means of analyses of covariance (ANCOVAs), with the mean difference of the expectancy ratings and of the SCR at test phase.

4. Results

A total of 40 participants (22 females) were included. Six participants were excluded during the calibration phase because they failed to report the electrocutaneous stimuli within the intensity safety limits as being painful. Therefore, the analyses were conducted with 34 participants (19 females) aged 18 to 58 years (mean = 25.69 ± 9.66).

4.1. Blinding and contingency awareness

After the experiment, 31/34 (91%) participants were unaware of the actual objective of the study (ie, blinding check), and 19 (55.9%) reported to have noticed that both movements were equally painful during the acquisition phase in the final decision-making task. All participants noticed that the electrocutaneous stimuli occurred during the movements, and 5 (14.7%) reported to perceive some level of causality between pressing the screen and receiving the painful stimulus.

4.2. Manipulation checks

4.2.1. Behavior measures

At baseline, no significant differences were found between the mean MOL ($F(1,66) = 2.12$; $P = 0.14$; CS+ = 0.55; CS− = 0.66) and MD ($F(1,66) = 2.88$; $P = 0.094$; CS+ = 1.21; CS− = 1.37) for both CS movements. Similarly, SCRs did not differ significantly between the CS movements at baseline (CS+ = 0.81; CS− = 0.76; Wilcoxon signed rank test: $Z = -1{,}274$, $r = 0.15$; $P = 0.2$).

4.2.2. Expectancy ratings

To check whether the conditioning procedure was successful, a 2 (Condition: CS+ vs CS−) × 2 (Phase: Acquisition vs Test) RM ANOVA was performed on expectancy ratings. This analysis revealed both a significant condition ($F(1,136) = 18.04$; $P < 0.001$; $\eta^2_P = 0.98$) and phase effect ($F(1,136) = 14.45$; $P < 0.001$; $\eta^2_P = 0.96$), without a significant condition × phase interaction.

4.2.3. Skin conductance response

Mean SCR after the appearance of the CS+ movement cue was significantly higher in comparison to the mean SCR after the CS− cue appearance at the first half of the acquisition phase (Wilcoxon signed rank test: $Z = -2.293$, $r = 0.27$; $P = 0.02$), and this difference remained during the second half of the acquisition phase (Wilcoxon signed rank test: $Z = -2.701$, $r = 0.32$; $P = 0.007$).

4.3. Main hypothesis

We tested whether there was a difference between the number of stimuli at-pain-threshold level (US^{test}) being perceived as painful vs nonpainful during CS+ movements in comparison with CS− movements. The Wilcoxon signed rank test revealed a statistically significant CS+/CS− difference (Wilcoxon signed rank test: $Z = -2.65$, $r = 0.32$; $P = 0.008$). Participants rated more US^{test} trials as painful during CS+ movements (85%, SD: 25) than during CS− movements (73%, SD: 32).

4.4. Secondary hypotheses

Participants rated the US^{test} as significantly more painful when it occurred during CS+ movements (M = 2.41; SD: 1.62) than during CS− movements (M = 1.96; SD: 1.49; Wilcoxon signed rank test: $Z = -4.29$, $r = 0.52$; $P < 0.0001$).

The test of the response onset latency times revealed a significant Condition × Phase interaction ($F(2,198) = 51.217$; $P < 0.001$; $\eta_P^2 = 1$). Follow-up pairwise comparisons revealed that latency times for both CS movements directed increased significantly from baseline to acquisition as well as from baseline to test (both $P < 0.001$), with higher times to initiate CS+ movements in comparison to CS− movements during the acquisition and test phases (both $P < 0.001$). SCR to both CSs increased significantly from baseline to acquisition ($P < 0.001$) and to test ($P < 0.001$), and decreased from acquisition to test ($P = 0.001$), whereas higher SCR were observed to the appearance of the CS+ cue in comparison to the CS− cue both in acquisition (Wilcoxon signed rank test: $Z = -4.67$, $r = 0.29$; $P < 0.0001$) and test (Wilcoxon signed rank test: $Z = -2.419$, $r = 0.29$; $P = 0.016$) phases. No statistically significant difference was found between CS+ and CS− movements duration ($F(2,198) = 1.3$; $P = 0.27$; $\eta_P^2 = 0.28$). None of these results changed when contingency awareness was added to the analysis as a covariate. The values of these variables across phases are depicted in **Figure 2**.

The ANCOVAs revealed no effect of blinding status, expectancy ratings, and contingency awareness on the percentage of US^{test} trials rated as painful during CS+ movements in comparison to movements to the CS− movements ($P > 0.05$). This means that the main results were not influenced by the participants' knowledge about the purpose of the experiment, conscious expectancy, or by contingency awareness.

4.5. Exploratory analysis

An exploratory analysis was conducted between the first half and second half of the test phase to verify whether extinction of the differences in MOL, SCR, and VAS ratings between CSs occurred. There were no statistically significant differences in these outcomes between the first half and second half of the test phase, although MOL and SCR visually appeared lower in the second half of the test phase (**Fig. 3**).

Given the importance of explicit memory on individual's expectancy ratings, an exploratory analysis was conducted to verify the effect of the conditioning process upon PRs. Pain recalls were significantly higher during the CS+, in comparison to CS− movements both in acquisition and test phases (ANOVA: $F(1,66) = 6.21$; $P = 0.015$; $\eta_g^2 = 0.7$ and Wilcoxon signed rank test: $Z = -2.65$, $r = 0.32$; $P = 0.008$, respectively). A 2 (Condition: CS+ vs CS−) × 2 (Phase: Acquisition vs Test) RM ANOVA was performed on pain memory recall ratings. It revealed a significant Condition ($F(1,132) = 7.19$; $P = 0.008$; $\eta_P^2 = 0.759$) and Phase ($F(1,132) = 39.87$; $P < 0.001$); $\eta_P^2 = 1$) main effects, with no Condition × Phase interaction. None of these results changed when contingency awareness was added to the analysis as a covariate ($P > 0.05$), meaning that they were not influenced by the participants' contingency awareness.

To verify the relation between PRs, expectancy, blinding, contingency awareness, and VAS, ANCOVAs were conducted. They revealed a significant effect of VAS at test phase on the PR only ($F(1,59) = 84.92$; $P < 0.0001$; $\eta_P^2 = 1$).

5. Discussion

In this study, we examined whether proprioceptive inputs associated with arm voluntary movements can increase the chance of a stimuli at-pain-threshold to be perceived as painful after successive painful associations in healthy participants. We hypothesized that a Pavlovian conditioning procedure would lead to a higher percentage of stimuli at-pain-threshold level being perceived as painful during movements directed to an external visual cue that were previously associated with an US-nociceptive stimulus (CS+) in comparison with movements directed to an external visual cue that were never previously associated with nociception (CS−).

This hypothesis was confirmed because US^{test} stimuli (ie, electrocutaneous stimulus at-pain-threshold) were significantly more likely to be perceived as painful and were also rated as more painful during movements directed to the CS+. These findings are consistent with the data of other studies that investigated the effect of Pavlovian conditioning using vibrotactile and visual stimuli as CSs, and found that painful associations can decrease pain threshold[21,37,47] and increase its perceived intensity.[10,12] Together, these studies suggest that pain experience may be modulated through associative learning.

During our study, the association of a specific movement (ie, reaching to touch) and an initial nociceptive stimulus (ie, electrocutaneous stimuli) modulated the pain experience even when the nociceptive stimulus was reduced. This potentially suggests that during movement-related pain episodes, individuals may learn to continuously associate pain to contexts and/or multisensory stimulus that was associated with the initial painful event. In turn, the pain experience remains.

The other secondary hypotheses were that conditioning would lead to higher SCR to the appearance of the visual CS+ cue in comparison with the visual CS− cue (ie, higher arousal), and lead to higher MOLs to the CS+ movement's initiation in comparison with the CS− movements (ie, higher avoidance tendency). These hypotheses were also confirmed because SCRs were significantly higher after the appearance of the visual CS+ cue during the test phase in comparison to the visual CS− cue, and movement onset latencies were significantly higher to initiate the CS+ movements in comparison to the CS− movements during the test phase as well. This increased arousal to the visual CS+ cue, that was also a cue to start the movement, was associated with higher MOL to initiate the CS+ movements in comparison with the CS− movements both in acquisition and test phases, which can be interpreted as a sign of attempted avoidance in a forced choice paradigm. These results are in line with several studies that verified the mechanisms beyond fear of movement-related pain conditioning, in which increased CRs (eg, eye blink response) are observed in predictable movement conditions to the visual cues that predict painful interactions.[4,15,25,26]

Regarding the secondary hypotheses, one interesting finding of our data, also shown in **Figure 3**, is that although not statistically significant, the visually observed reduction of the SCR

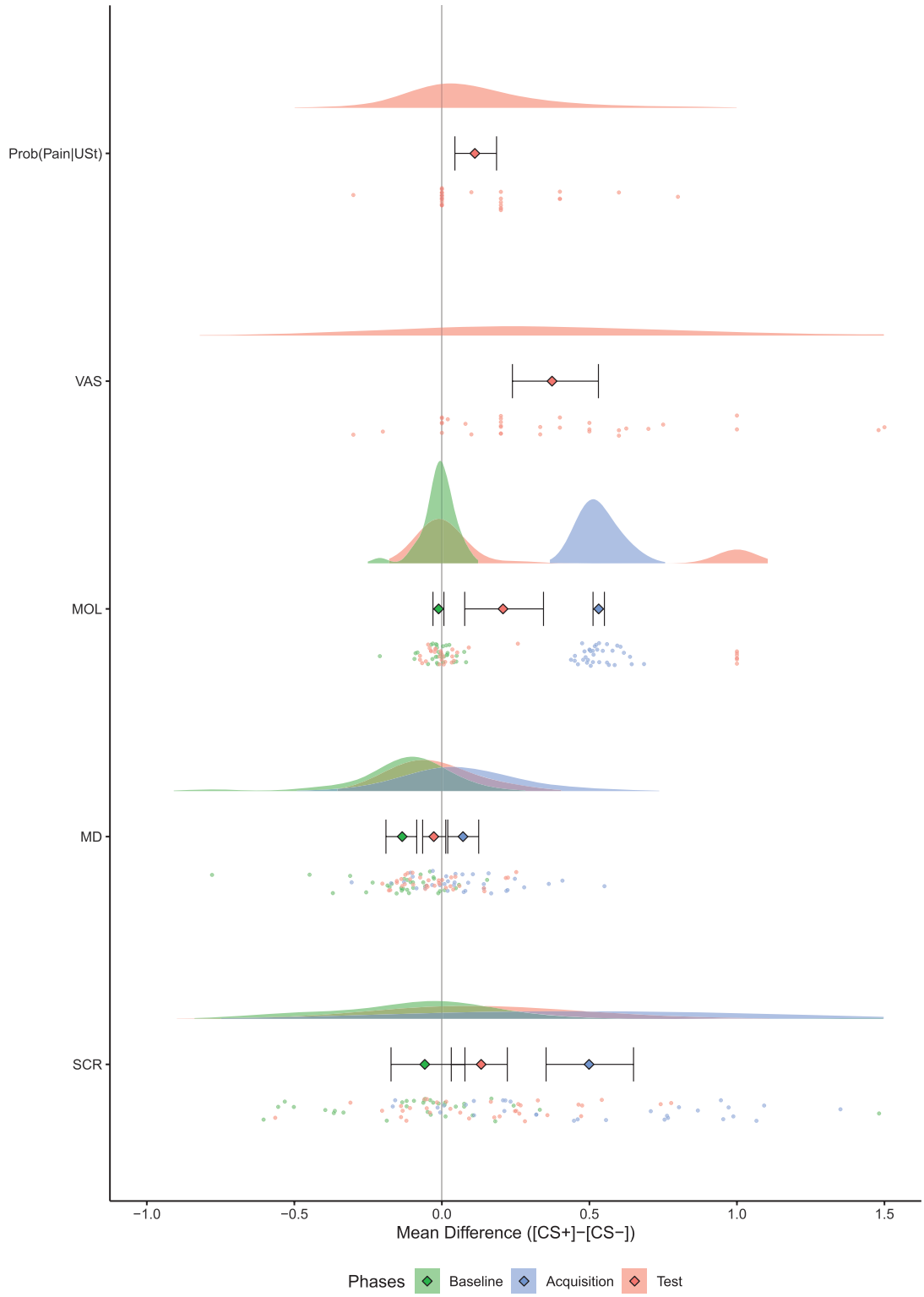

Figure 2. Raincloud plots of the mean difference ([CS+] − [CS−]) and 95% confidence intervals of behavioral measures through phases. MOL, movement onset latency (in sec); MD, movement duration (in sec); SCR, mean skin conductance response (in μS); VAS, visual analogue scale; Prob(Pain|US^t), probability of perceive the US^{test} as painful.

and movement onset latencies to the CS+ through the test phase was not accompanied by a full extinction of the perceived pain intensity differences between CS+ and CS−. It seems that behavioral and psychophysiological responses do not always covary with verbal reports, as shown in other experimental studies.[24–26] Given these findings, future studies could explore the possible contributions of verbal vs physiological responses in the maintenance of conditioned pain because this information might be relevant for planning clinical interventions that target chronic pain.

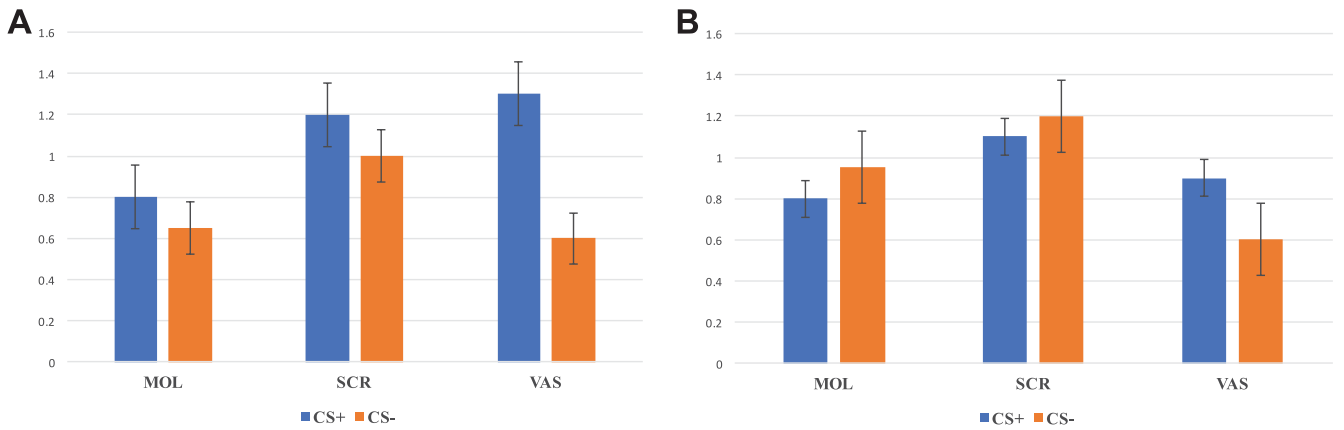

Figure 3. Mean movement onset latencies (in sec), skin conductance responses (in μS), and pain ratings (VAS) from the first half (A) and second half (B) of the test phase. MOL, movement onset latency; SCR, skin conductance response; VAS, visual analogue scale.

The exploratory analysis showed that PR was higher for the CS+ movements during the test phase in comparison to the CS− movements, although both CSs were associated with the same intensity of electrocutaneous stimuli (US^{test}). This finding is consistent with research on memory for bodily symptoms, in which memory recall is relatively inaccurate, and pain is usually overestimated retrospectively.[1,7,20,43] An interesting finding was that most subjects reported to remember feeling pain during movements toward the CS− after the acquisition phase, although this stimulus was never associated with the US. Given the unpredictability of the acquisition phase, this could be a form of stimulus generalization influencing memory retrieval.[17,36,38]

Notably, although our experimental design was not designed to exclude the effect of expectancy on pain perception, our findings were not influenced by the participants' blinding status, conscious expectancy, or contingency awareness. Other studies reported similar findings, suggesting that expectations or conscious awareness of learned associations might not be necessary for Pavlovian conditioning to occur.[10,12,13,37] Furthermore, movement-induced hypoalgesia[3,18] was observed during the first pilot studies and impaired the conditioning process. However, its effect was reduced with higher US intensities,[6] which suggests that pain intensity may play a role in conditioned movement-related pain.

5.1. Implications

We speculate that the clinical implications of the present findings are related to the knowledge of the mechanisms by which movement-related pain and associated responses, such as the arousal increase in the presence of a visual cue that predicts painful interactions, could become CRs and modulate pain occurrence and behavior selection to similar motor contexts or future executions of the same movement.

Voluntary movements involve a chain of planning and anticipatory actions that are triggered based on context,[8] including internal and/or external cues. As such, sensory stimuli that were present during the planning of a given movement may acquire a CS function after the exposure to painful movements, by its capacity to predict pain. In other words, during a movement-related pain episode (eg, shoulder pain during overhead reaching for an object), every stimulus that has a predictive value about the painful experience (eg, height of the object) can become a CS and produce CRs—such as attentional bias to the region of pain,[2,34] movement-related fear,[4,25] protective behavioral responses (eg, bracing of shoulder and neck muscles)[16] or, as found in this study, an increase in arousal and modulation of the pain threshold. The CRs that occur before movement can continue to be evoked during future executions of the same movement.

These initial CRs elicited by the CSs cues that precede motor behavior (eg, height of the object), such as increased arousal and fear, could initially lead the individual to avoid such contexts in a more or less generalized way,[38] and for those subjects whose operant contingencies are sufficient to make them not engage in avoidance behaviors (eg, urge to thirst reduction), these same CRs could leave them more primed to feel pain.[5] The interplay between these CRs (eg, contextual pain threshold modulation during overhead reaching for an object) and operant learning (eg, reinforcement of an maladaptive strategy of movement that fulfill the motor task), in combination with other conscious and nonconscious processes associated to threat processing and movement planning and execution, may enhance and even self-reinforce these processes, which may ultimately play a role in the pathway to pain chronicity.[5,14,17,44–46]

Knowledge of these mechanisms suggests that for those with movement-related pain, movement may be a key link between body and perception. Thus, although movement is a common and effective trigger for pain experience and persistence, it also offers the most powerful way to access and modify the contextual and behavioral learning processes related to pain chronicity.

5.2. Limitations

The main limitation of this study was the calibration of the US^{test} intensity for the test phase. Assuming that the threshold is a point of uncertainty, we expected that the US^{test} stimuli would be perceived as painful around 50% of the times with differences according to the previous history of learning to each movement. Despite careful calibration, the elevated percentage of US^{test} perceived as painful suggests that, besides the differences found between the $CS+/US^{test}$ and $CS-/US^{test}$ in the test phase, a global modulation of the pain thresholds occurred across the experiment. However, this should not have affected the data.

Furthermore, this study used a mechanism-based approach. Its results should only be used to raise hypotheses for further clinical and experimental studies, and not for clinical reasoning and decision-making.

6. Conclusion

This study provides evidence that in healthy subjects, pain can be conditioned to voluntary arm movements. After an acquisition

phase, the percentage of stimuli at-pain-threshold level that were perceived as painful during movements directed to an external visual cue that were previously associated with painful movements in comparison with movements directed to an external visual cue without a history of pain learning was significantly higher, and the stimuli were also perceived as more intense. Furthermore, defensive behavioral responses (ie, SCRs and movement onset latencies) were more pronounced after the appearance of the visual cue that predicted possible painful interactions.

The present data offer support for the current scientific literature suggesting that pain modulation through associative learning may be one of the mechanisms by which persistent pain may occur. The present data also suggest that during a movement-related pain episode, a complex interplay between contextual and behavioral learning takes place, which could be related to clinical processes of avoidance and pain persistence during movement execution.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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References

[1] Broderick JE, Schwartz JE, Schneider S, Stone AA. Can end-of-day reports replace momentary assessment of pain and fatigue? J Pain 2009; 10:274–81.
[2] Crombez G, Viane I, Eccleston C, Devulder J, Goubert L. Attention to pain and fear of pain in patients with chronic pain. J Behav Med 2013;36: 371–8.
[3] Crombie KM, Brellenthin AG, Hillard CJ, Koltyn KF. Endocannabinoid and opioid system interactions in exercise-induced hypoalgesia. Pain Med 2018;19:118–23.
[4] den Hollander M, Meulders A, Jakobs M, Vlaeyen JW. The effect of threat information on acquisition, extinction, and reinstatement of experimentally conditioned fear of movement-related pain. Pain Med 2015;16:2302–15.
[5] Forkmann K, Wiech K, Sommer T, Bingel U. Reinstatement of pain-related brain activation during the recognition of neutral images previously paired with nociceptive stimuli. PAIN 2015;156: 1501–10.
[6] Gajsar H, Nahrwold K, Titze C, Hasenbring MI, Vaegter HB. Exercise does not produce hypoalgesia when performed immediately after a painful stimulus. Scand J Pain 2018;18:311–20.
[7] Giske L, Sandvik L, Roe C. Comparison of daily and weekly retrospectively reported pain intensity in patients with localized and generalized musculoskeletal pain. Eur J Pain 2010;14:959–65.
[8] Haggard P. Human volition: towards a neuroscience of will. Nat Rev Neurosci 2008;9:934.
[9] Harvie DS, Broecker M, Smith RT, Meulders A, Madden VJ, Moseley GL. Bogus visual feedback alters onset of movement-evoked pain in people with neck pain. Psychol Sci 2015;26:385–92.
[10] Harvie DS, Meulders A, Madden VJ, Hillier SL, Peto DK, Brinkworth R, Moseley GL. When touch predicts pain: predictive tactile cues modulate perceived intensity of painful stimulation independent of expectancy. Scand J Pain 2016;11:11–18.
[11] Icenhour A, Labrenz F, Ritter C, Theysohn N, Forsting M, Bingel U, Elsenbruch S. Learning by experience? Visceral pain-related neural and behavioral responses in a classical conditioning paradigm. Neurogastroenterol Motil 2017;29.
[12] Jensen K, Kirsch I, Odmalm S, Kaptchuk TJ, Ingvar M. Classical conditioning of analgesic and hyperalgesic pain responses without conscious awareness. Proc Natl Acad Sci U S A 2015;112:7863–7.
[13] Jensen KB, Kaptchuk TJ, Kirsch I, Raicek J, Lindstrom KM, Berna C, Gollub RL, Ingvar M, Kong J. Nonconscious activation of placebo and nocebo pain responses. Proc Natl Acad Sci U S A 2012;109:15959–64.
[14] Jepma M, Koban L, van Doorn J, Jones M, Wager TD. Behavioural and neural evidence for self-reinforcing expectancy effects on pain. Nat Hum Behav 2018;2:838–55.
[15] Kilintari M, Bufacchi RJ, Novembre G, Guo Y, Haggard P, Iannetti GD. High-precision voluntary movements are largely independent of preceding vertex potentials elicited by sudden sensory events. J Physiol 2018;596:3655–73.
[16] Klinger R, Soost S, Flor H, Worm M. Classical conditioning and expectancy in placebo hypoalgesia: a randomized controlled study in patients with atopic dermatitis and persons with healthy skin. PAIN 2007; 128:31–9.
[17] Koban L, Kusko D, Wager TD. Generalization of learned pain modulation depends on explicit learning. Acta Psychol 2018;184:75–84.
[18] Koltyn KF, Brellenthin AG, Cook DB, Sehgal N, Hillard C. Mechanisms of exercise-induced hypoalgesia. J Pain 2014;15:1294–304.
[19] LeDoux JE. Coming to terms with fear. Proc Natl Acad Sci U S A 2014; 111:2871–8.
[20] Linton SJ, Melin L. The accuracy of remembering chronic pain. PAIN 1982;13:281–5.
[21] Madden VJ, Bellan V, Russek LN, Camfferman D, Vlaeyen JWS, Moseley GL. Pain by association? Experimental modulation of human pain thresholds using classical conditioning. J Pain 2016;17: 1105–15.
[22] Madden VJ, Harvie DS, Parker R, Jensen KB, Vlaeyen JW, Moseley GL, Stanton TR. Can pain or hyperalgesia be a classically conditioned response in humans? A systematic review and meta-analysis. Pain Med 2016;17:1094–111.
[23] Madden VJ, Kamerman PR, Catley MJ, Bellan V, Russek LN, Camfferman D, Moseley GL. Rethinking pain threshold as a zone of uncertainty. bioRxiv 2019:521302.
[24] Meulders A, Franssen M, Fonteyne R, Vlaeyen JW. Acquisition and extinction of operant pain-related avoidance behavior using a 3 degrees-of-freedom robotic arm. PAIN 2016;157:1094–104.
[25] Meulders A, Vansteenwegen D, Vlaeyen JW. The acquisition of fear of movement-related pain and associative learning: a novel pain-relevant human fear conditioning paradigm. PAIN 2011;152:2460–9.
[26] Meulders A, Vlaeyen JW. The acquisition and generalization of cued and contextual pain-related fear: an experimental study using a voluntary movement paradigm. PAIN 2013;154:272–82.
[27] Moseley GL, Vlaeyen JW. Beyond nociception: the imprecision hypothesis of chronic pain. PAIN 2015;156:35–8.
[28] Mouraux A, De Paepe AL, Marot E, Plaghki L, Iannetti GD, Legrain V. Unmasking the obligatory components of nociceptive event-related brain potentials. J Neurophysiol 2013;110:2312–24.
[29] Mun CJ, Suk HW, Davis MC, Karoly P, Finan P, Tennen H, Jensen MP. Investigating intraindividual pain variability: methods, applications, issues, and directions. PAIN 2019;160:2415–29.
[30] Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971;9:97–113.
[31] Rabey M, Beales D, Slater H, O'Sullivan P. Multidimensional pain profiles in four cases of chronic non-specific axial low back pain: an examination of the limitations of contemporary classification systems. Man Ther 2015; 20:138–47.
[32] Rabey M, Smith A, Kent P, Beales D, Slater H, O'Sullivan P. Chronic low back pain is highly individualised: patterns of classification across three unidimensional subgrouping analyses. Scand J Pain 2019;9:743–53.
[33] Rescorla RA. Pavlovian conditioning: it's not what you think it is. Am Psychol 1988;43:151.

[34] Schmidt K, Gamer M, Forkmann K, Bingel U. Pain affects visual orientation: an eye-tracking study. J Pain 2018;19:135–45.
[35] Simpson W. The method of constant stimuli is efficient. Percept Psychophys 1988;44:3.
[36] Tenenbaum JB, Griffiths TL. Generalization, similarity, and Bayesian inference. Behav Brain Sci 2001;24:629–40.
[37] Traxler J, Madden VJ, Moseley GL, Vlaeyen JWS. Modulating pain thresholds through classical conditioning. PeerJ 2019;7:e6486.
[38] Vervliet B, Iberico C, Vervoort E, Baeyens F. Generalization gradients in human predictive learning: effects of discrimination training and within-subjects testing. Learn Motiv 2011;42:210–20.
[39] Vlaeyen JW. Learning to predict and control harmful events: chronic pain and conditioning. PAIN 2015;156(suppl 1):S86–93.
[40] Vlaeyen JW, Crombez G, Linton SJ. The fear-avoidance model of pain. PAIN 2016;157:1588–9.
[41] Vlaeyen JW, Linton SJ. Fear-avoidance and its consequences in chronic musculoskeletal pain: a state of the art. PAIN 2000;85:317–32.
[42] Vlaeyen JW, Linton SJ. Fear-avoidance model of chronic musculoskeletal pain: 12 years on. PAIN 2012;153:1144–7.
[43] Walentynowicz M, Bogaerts K, Van Diest I, Raes F, Van den Bergh O. Was it so bad? The role of retrospective memory in symptom reporting. Health Psychol 2015;34:1166–74.
[44] Wiech K. Deconstructing the sensation of pain: the influence of cognitive processes on pain perception. Science 2016;354:584–7.
[45] Wiech K, Ploner M, Tracey I. Neurocognitive aspects of pain perception. Trends Cogn Sci 2008;12:306–13.
[46] Wiech K, Shriver A. Cognition doesn't only modulate pain perception; it's a central component of it. AJOB Neurosci 2018;9:196–8.
[47] Williams AE, Rhudy JL. The influence of conditioned fear on human pain thresholds: does preparedness play a role? J Pain 2007;8:598–606.
[48] Zaman J, Madden VJ, Iven J, Wiech K, Weltens N, Ly HG, Vlaeyen JWS, Van Oudenhove L, Van Diest I. Biased intensity judgements of visceral sensations after learning to fear visceral stimuli: a drift diffusion approach. J Pain 2017;18:1197–208.