Imprecise visual feedback about hand location increases a classically conditioned pain expectancy effect

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Short Running Title: Disrupted visual hand feedback increases pain expectancy

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Abstract: We tested the hypotheses that rendering sensory input about hand location imprecise increases a classically conditioned pain expectancy effect, increases generalization of the effect to novel locations and reduces extinction of the effect. Forty healthy volunteers performed movements with their right hand along predefined paths. Each path passed through two locations that were defined as either (i) the conditioned stimulus (CS+; paired with a painful unconditioned stimulus - UCS), and (ii) unpaired (CS-). During acquisition phase, participants watched their hand as they moved it. Participants were randomly allocated to an Imprecise group (IG), for whom visual feedback of the hand was offset 30-50mm from its true location, or a Precise group (PG), for whom vision was not disrupted. In the test phase, participants moved their hands to five locations – the CS+, CS- and three locations that lay between the two ('Generalisation stimuli'). Our first hypothesis was reported – pain expectancy was greater at the CS+ location in the IG than in the PG (6.9 [SD=1.9] vs 5.4 [SD=2.5], p=0.02). Pain expectancies generalised to novel locations similarly in both groups and there was no difference in extinction between groups. Our primary hypothesis was supported but our subsequent hypotheses were not.

Perspective: We conditioned pain expectancy at a certain location of one hand, even though most participants were unaware of the contingency. Conditioned pain expectancy was greater when sensory information about location was less precise. This adds support to the possibility that associative learning may play a role in the progression of an acute pain episode to a more generalized pain disorder.

Key words: Classical Conditioning, imprecise stimulus, illusion, pain expectancy, hand location

Introduction

Chronic musculoskeletal pain is a major health problem with a one-year prevalence of 25% to 36% in the general population[35]. Costs related to persistent pain in the United States of America are between $560 and $635 billion annually[18]. Many persistent pain states are not associated with ongoing tissue pathology, an originally perplexing observation that is now explained by functional changes in the nociceptive system and brain[79-81]. Broadly speaking, these functional changes may be considered learning; stimulus-response profiles change such that stimuli that are not normally painful come to evoke pain, a situation termed allodynia, and normally painful stimuli come to evoke more pain, a situation termed hyperalgesia[79].

The vast majority of research has considered this learning in the nociceptive system to reflect non-associative learning, whereby synaptic efficacy is enhanced by repeated signalling and the consequent long-term potentiation of the post-synaptic neurone results in 'central sensitisation'[80]. However, the vast majority of persistent pain states cannot be explained by this central sensitisation, a reality that led to a radical updating of the idea of central sensitisation to a clinical observation of allodynia and hyperalgesia in response to stimuli delivered outside the body area that was initially affected[81]. Even still, many pain states involve allodynia to a range of non-noxious somatosensory and, in fact, to non-somatosensory cues[1, 59, 24], pointing to the possibility that associative learning may also contribute to persistent pain.

The possibility that associative learning, or classical conditioning processes, might contribute to persistent pain, is widely endorsed clinically[45], even though supportive empirical data from humans has only emerged recently and inconsistent results between experiments point to a complex picture [3, 27, 32, 42]. It is critical to clearly differentiate this notion from that of associative learning of pain-related fear, for which there is a vast

literature [13, 75, 76]. In that work, pain is considered an unconditioned stimulus (US) and fear the conditioned response (CR). Here, however, nociception is considered the US and pain the CR[60].

That non-noxious signals might come to evoke or magnify pain would be predicted by contemporary theories of brain function[31, 78] and the biopsychosocial model[17] of pain[37, 38], the latter of which posits that pain emerges from the interplay between contributors from across biological, psychological and environmental or contextual domains [see Moseley and Butler[55, 56], for extensive reviews]. Empirical data are also supportive. For example, delivering a noxious cold stimulus with auditory or visual cues associated with heat evokes intense pain and often a feeling of intense heat, but delivering an identical noxious cold stimulus with auditory or visual cues associated with cool evokes less intense pain and usually a feeling of cold[2, 55]. Also, in people with neck pain, manipulating visual feedback during a head rotation task shifts the point at which they report the onset of pain, in a direction-specific manner[27].

These converging lines of discovery led to and support the proposal known as the 'Imprecision Hypothesis (IH) of chronic pain'[60]. It predicts that associative learning contributes to the progression of an episode of acute pain to a generalised pain disorder via over-generalisation of the conditioned response. Several studies have now supported that allodynia and hyperalgesia can be induced experimentally via classical conditioning procedures [3, 25, 26, 32, 46, 49, 71] as suggested by IH. Key to the IH is the notion that imprecise encoding of the multisensory CS that routinely coincides with the nociceptive input leads to over-generalisation of any classical conditioning effect[60]. Imprecise encoding might occur under situations in which sensory channels that dominate the multisensory CS are disrupted. Such disruptions have been documented in people with persistent pain[9, 58, 68]. Relevant here are contemporary ideas in predictive processing, wherein perception is

argued to result from the integration of incoming sensory data and top-down predictions based on internal, generative models[29]. In contexts of precise or ecologically important predictions and imprecise sensory inputs, perceptions can deviate from the actual state of the world[64]. That is, in contexts of predictions that are fundamentally important to protection – for example pain – alongside imprecise sensory inputs, perceptions can deviate toward increased probability of pain. We have previously shown pain expectancy – the likelihood that pain will occur - to be higher when a CS occurred in anatomical areas of low somatosensory precision (back) than when it occurred in areas of high somatosensory precision[26]. However, that result leaves open the possibility that differences in somatosensory precision between the two anatomical areas did not underpin the difference in pain expectancy.

Here we interrogated Pain Expectancy - the probability of pain – under different conditions of sensory precision, induced by the MIRAGE illusion system[66]. Our first hypothesis (H1) was that imprecise sensory input during conditioning would result in greater expectation of pain (the 'CR') at the CS+ location. Our secondary hypotheses were that (i) generalization of the conditioned pain expectancy would be greater, and (ii) extinction of the effect slower, when sensory input during acquisition was less precise.

Methods

Overview of the Procedure

Forty participants underwent a differential classical conditioning experiment. First, participants were submitted to a calibration procedure to determine the individual intensity of electrical aversive stimulation delivered. Afterwards, using the MIRAGE system, participants were trained on how to move their right hand along paths (without aversive stimulation) while they watched a real-time video of their hand beneath the

156 mirror (see Figure 1). The MIRAGE illusion system has a camera, a customized software
157 program, a monitor and mirrors to allow participants to watch a real-time video of their
158 hand beneath the mirror, from the same perspective and in the same spatial location as if
159 they were viewing the right hand directly.
160 During the *acquisition phase*, participants were randomly allocated to an Imprecise group
161 – vision was disrupted to right or left – or a Precise group - vision was not disrupted. To
162 manipulate the accuracy of visual input of hand location (imprecision), the MIRAGE
163 system applied a shift in the apparent (i.e. visually encoded) hand location of 30 to 50
164 mm (twice at each offset, order randomised), while the participants moved their right
165 hand on the paths randomly determined. The paths were nonlinear trajectories starting
166 and finishing always on the bottom right of the MIRAGE board and should include two
167 locations: one on the far upper left side and one on the far upper right side of the
168 movement path. One location was the conditioned stimulus (CS+), paired with an
169 electrical aversive unconditioned stimulus (UCS). Another location was the CS- and was
170 unpaired. The locations that lay between CS+ and CS- in the upper part of the MIRAGE
171 board, but outside the predefined movement paths, were the generalization stimuli (GSs).
172 In 50% of trials (n=6), when the hand crossed the CS+, the UCS was delivered to the
173 hand.
174 In *test phase*, participants reported how likely they expected pain if their hand reached
175 five specific locations (imagery task) marked with dots, including CSs and three novel
176 locations (GSs - not available during acquisition). The locations were projected on the
177 MIRAGE screen. The procedure was repeated randomly four times for each location (just
178 one location was visible on the screen per trial) and Pain Expectancy ratings were
179 obtained for each location.

The *extinction phase* required participants to move their hand along paths (12 times) that included the CS+ and CS- location, but no UCS was delivered. In each extinction trial, a movement path was shown on the screen, but this time the CS+ and CS- location were marked with dots (six trials for CS+ and six trials for CS-). Participants provided Pain Expectancy ratings as they had in the test phase, and then performed the movement as they had in the acquisition phase, but the UCS was never presented.

Participants

We calculated our sample size considering mean and standard deviations of Pain Expectancy for CS+ between groups, from the first 5 participants we enrolled in each group (n=10). We powered to detect a medium between-group effect size (Cohen's f=0.43); power of 90%, α=0.05, correlation between measures =0.40; repeated measures between factors (G*power, Faul, Erdfelder, Buchner & Lang; Institute of Psychology, University of Duesseldorf, Version 3.1, Germany). Accordingly, we required 17 participants per group. To allow for withdrawals and technical errors, we aimed to recruit 20 in each group.

The volunteers were recruited through flyers, social media and word of mouth at the University of South Australia. This study enrolled right-handed males and females (with normal or corrected-to-normal vision), aged between 18 and 50 years old. All eligible participants signed a consent form and received an honorarium of AUD $20 per hour. Participants were not aware of the real aim of the study. Specifically, they were unaware that: i) they could experience a visual illusion during the acquisition phase of the study, and ii) that it was a classical conditioning study. However, we provided them with the information that they would receive a noxious stimulation to investigate the relationship between learning about movement and pain. The experimental protocol described that it

was a deception study and was approved by the Human Research Ethics Committee from University of South Australia (HREC; protocol number: 200706).

The following exclusion criteria were applied: i) Neurological diseases (Cerebral paralysis, cerebrovascular accident or sequelae, epilepsy, Multiple Sclerosis, Parkinson's Disease, post-Herpetic neuralgia) or any history of trauma in the hand; ii) Chronic disorders (diabetes), vascular problems or chronic pain (pain which has lasted longer than 12 weeks and is present on most days); iii) History of chronic pain within the last 6 months, iv) Pregnancy, v) Acute pain (at any site); vi) Hand pain or recent hand injury within the previous 12 weeks; vii) diagnosed psychiatric disorder; viii) any skin sensitivity (dermatitis, psoriasis and eczema); ix) heart problems or a pace maker; and x) surgical pins or plates or metal-based tattoos in the hands.

Classical conditioning: stimuli and manipulation check

This experiment used a classical conditioning procedure designed to create an association between the participant's hand being at a certain location (CS+) and the occurrence of a aversive stimulus (US).

Conditioning and generalisation stimuli

A set of three different hand movement paths was created such that each path passed through two defined locations: one on the far upper left side and one on the far upper right side of the movement path (Figure 1A). One of the locations was defined as CS+ and was paired with an aversive electrical stimulus to the hand; the other was defined as CS- and was unpaired. The allocation of location (left/right) to CS was counterbalanced between participants within each group. Three distinct locations that lay between the two CSs, but outside the predefined movement paths, were defined as generalisation stimuli (GSs). The

GSs were numbered consecutively, with the numbers increasing with distance from the CS+. A picture illustrating the board with the five locations (marked with dots) and one of the paths adopted in the study can be found in the (Figure 1B).

Unconditioned stimulus

The UCS was an electrocutaneous stimulation (square wave pulse of duration 100ms, 400V) applied to the back of the hand in the space between the first and second metacarpal bones. A pushpin-type electrode resembling that of Inui et al.[30] , comprising a concentric anode and a blunt pin-type cathode in the center, was manufactured in house and attached to the skin with a circular adhesive sticker. Electrical current was passed across the electrode using a Digitimer device (DS7AH, SDR Scientific, Power: 12 Va, Freq: 47-63 Hz, Hyde Way Welwyn, Garden City, UK) which was manually controlled by a researcher.

Contingency awareness

Immediately after the end of the experiment, participants responded to four questions that aimed to investigate associations formed between locations, movement paths and painful stimulation or visual distortion during the experiment: (i) "*in which position did you feel a painful stimulation?"*, (ii) "*in which position(s) did you perceive visual distortion?",* (iii) "*in which path(s) did you feel a painful stimulation?*" and (iv) "*in which path(s) did you perceive a visual distortion?".* To help participants to answer the questions, the pictures of the locations and paths were projected on the screen.

The Precise and Imprecise conditions

Participants were randomly allocated to one of two groups – Precise or Imprecise. The Precise group undertook the classical conditioning procedure with normal visual input. The Imprecise group undertook the classical conditioning procedure with modified visual input that shifted the seen position of the hand (Figure 1B). To manipulate the visual input for the Imprecise group, we used the MIRAGE illusion system (Figure 1A), which has a camera, a customized Labview software program (2010 National Instruments®), a monitor and mirrors to allow participants to watch a real-time video of their hand beneath the mirror, from the same perspective and in the same spatial location as if they were viewing the right hand directly (Figure 1B). The MIRAGE system was used for all participants, but the illusion was applied for the Imprecise group only. Participants sat on a chair and placed their hand into the lower level of the MIRAGE box, to lie on a horizontal board. Thus, when a participant looked down towards their hand, they saw a live footage of their right hand. A partition and bib secured around participants' necks prevented the participant from seeing their real hand. The participant's limb appeared in the same spatial location and from the same perspective as it would if they were viewing the limb directly (Figure 1B).

To manipulate the accuracy of visual input of hand location, the MIRAGE system applied a shift in the apparent (i.e. visually encoded) hand location of 30mm, 40mm or 50mm to the right or left (twice at each offset, order randomised), while the participant performed movements on paths during the acquisition phase (See Figure 1). The magnitude of the shift was determined through pilot testing (Supplementary Method S1 and Figure S2).

It was necessary to prevent the participant from updating or correcting the hand position to match visual estimates [proprioceptive recalibration[12]] as they performed the movements during the acquisition and extinction phases. To achieve this, a black rectangle obscured 70% of the board on the screen, so that the participant only saw his/her

279 hand once it reached the CS+ or CS- location. This limited the opportunity to adjust the
280 movement path on the basis of visual input.
281
282 *Visual illusion manipulation check*
283 In order to verify that the actual and seen hand locations during painful stimulation had
284 differed within the Imprecise group, we measured the seen and actual positions of the
285 hand in the space at the moment when the painful stimulation was delivered
286 (Supplementary methods - Figure S3). The tracking system (Labview customized
287 software) was used to calculate the distance between the tip of the middle finger and the
288 lateral border of the screen for the Precise group. For the Imprecise group, both the actual
289 and the seen distances between the tip of the middle finger in relation to the lateral border
290 of the screen were considered (Supplementary methods - Figure S3). The difference
291 between the actual and seen distance was used to identify the "real visual shift" achieved
292 for the Imprecise group.
293 The program ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health,
294 Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2016) was used to obtain the
295 values in millimeters. To clarify – the true hand location at which the noxious stimulus
296 was delivered was intended to consistently vary from the visually encoded location of the
297 hand in the Imprecise Group via illusion. The above measures were used to confirm
298 whether this occurred.
299
300 *Awareness of manipulation of visual input*
301 At the end of the study, before disclosing the real nature of the study, participants
302 indicated how much they agreed (or not) with four statements: "*I felt as if I was looking*
303 *at my own hand*", "*I felt as if I was causing the movement I saw*"; "*Sometimes, I felt there*

was something wrong during some movements; "Sometimes, I felt like an incongruence between my hand position and the visual feedback about my hand". The response options were Likert-type scale ranging from: strongly agree, agree, neither agree or disagree, disagree and strongly disagree. Although only the Imprecise group had received the visual manipulation, all participants responded to these questions.

Outcomes and Questionnaires

Primary hypothesis outcome: Pain expectancy ratings for the CS+ location during test phase. Secondary hypothesis outcomes: Pain expectancy ratings at the remaining locations during test phase, and Pain Expectancy ratings at each location during extinction phase. The question and anchors in both cases were: '*How likely do you think it is that you will receive a painful stimulation*?', with 0 meaning "*not at all likely*", and 10 meaning "*extremely likely"*.

Sensation and pain intensity

To assess discomfort and pain during the calibration procedure, participants rated all stimuli using the Sensation and Pain Rating Scale (SPARS)[43] which has anchors of ''no sensation'' (-50), ''the exact point at which what you feel transitions to pain'' (0), and ''most intense pain you can imagine'' (+50). The SPARS performs well in the experimental context and overcomes limitations in scale range inherent to conventional pain rating scales[43]. We asked participants to rate their experience on the appropriate side of the scale, with -50 meaning '*no sensation*', +50 meaning '*the most intense pain you can imagine*' and 0 meaning 'th*e exact point at which what you feel transitions to pain*'. The written explanation of the SPARS emphasizes that ratings between -50 and 0 reflect a non-painful experience and ratings between 0 and 50 reflect a painful one.

Questionnaires administered at the baseline

To characterize the sample, participants completed the Positive and Negative Affect Schedule (PANAS)[11] and the State-Trait Anxiety Inventory (STAI)[67]. Both have acceptable internal consistency (α>.85), construct validity and structural validity[11, 67]. Low positive affect and high trait anxiety have been linked to reduced extinction in fear conditioning experiments[52].

Questionnaire administered at the end of the experiment

The extent to which participants engaged in catastrophic thinking during the application of individually calibrated painful stimuli[16] was assessed using the Catastrophizing Questionnaire. There are no widely accepted measures of catastrophizing in response to experimental pain stimulation. This questionnaire is a modified version of the Pain Catastrophizing Scale[70]. The wording of 6 items were modified to represent the three primary dimensions of catastrophizing in the context of laboratory procedures: rumination, magnification, and helplessness. Immediately after undergoing the experimental procedure, participants rated the degree of catastrophizing (during the painful stimulation) using the 6-item scale. The response options were: 0 (not at all), 1 (to a slight degree), 2 (to a moderate degree), 3 (to a great degree) and 4 (all the time). Catastrophizing scores were obtained by summing the scores on the 6 items (maximum possible score: 24). Cronbach's alpha for the scale was 0.87, suggesting a high degree of internal consistency.

Procedure

In the first contact with the volunteers (email or phone call), they were screened for right hand dominance (using the Edinburgh Handedness Inventory[63] and exclusion criteria were applied. Upon arrival at the laboratory, participants completed the informed consent form, filled in the baseline questionnaires and we obtained anthropometric data (weight, height and age). The skin on the dominant hand was exfoliated and cleaned with alcohol, and the electrode was taped to the skin. Micropore hypoallergenic medical tape fixed the cable to the dorsal surface of the wrist.

Participants were then allocated to one of the groups (Precise or Imprecise) via concealed randomisation. Also, the CS+ location and the sequence of administration of the CS+ and CS- were determined via a concealed simple randomization from five pre-defined possible sequences. This procedure guaranteed that half the participants would receive the US on each side of the board in each group. The randomization order was predefined using study randomizer online (https://studyrandomizer.com/).

Calibration procedure

We used an established calibration procedure to determine the intensity of electrical stimulation needed to elicit a self-report of moderate pain, defined as 'painful and requires effort to tolerate' and corresponding to a SPARS rating of between +25 and +35[71]. First, participants were submitted to the following electrical stimuli with a 30-second inter-stimulus interval: 1mA (presented twice), 2mA (twice), 4mA (twice), 6mA (once) and 8mA (once). During this time, if any impedance occurred, then the electrode was repositioned until the impedance no longer occurred and the procedure was restarted. Next, a series of electrocutaneous stimuli of increasing intensity was administered to the participant's hand, starting with 2mA[71]. The final higher electrical stimulus, in which the participant rated four in six trials as painful and greater than +25 using the SPARS, was

used as the US during Acquisition phase. After three minutes of rest, the acquisition phase has started.

Training phase

After calibration, participants sat at the MIRAGE and placed their right hand inside the lower level of the MIRAGE box (Figure 1A), such that they could see the real-time footage of their hand. First, each pre-defined movement path was projected onto the MIRAGE screen (mirror reflecting the monitor), and the participant memorized the path, including the direction of movement, as cued by visible arrows. Participants practised performing a 15-second movement of their hand along the path in a clockwise or counter-clockwise direction, 2 or 3 times per path, with feedback given to improve accuracy. The time was controlled by a chronometer. To maintain the accuracy of the movement tracking system, the prescribed position of the hand was with the fingers pointing forward, the hand held flat, the thumb in adduction and the fingers held together. Participants were also instructed to begin the movement after verbal cuing, to keep the hand within the visible area of the screen, and to return to the start position after the end of each movement. During training phase, the participants on both groups were not submitted to any illusion.

Acquisition phase

The Acquisition phase consisted of 12 trials. Each trial involved one cued movement along a prescribed movement path. There were three different possible movement paths (three in clockwise and three in counter-clockwise direction), each presented twice in a variable counterbalanced fashion. Every prescribed movement path passed through the

CS+ and CS- locations but did not pass through any of the GSs locations (Figure 2 and Supplementary files - Figures S4 and S5).

In 50% of Acquisition trials, the US was delivered at the moment when the participant's hand crossed the CS+ location. Participants received CS-UCS parings just 50% of the trials (6 times), because in all the trials participants had to move on paths crossing the CS+ position. This was based on pilot trials. If we administered the aversive stimulus every time the participant crossed the CS+ position, there was a risk that participants would pair 'movement' and 'pain', rather than a particular location and pain, thus confounding our intended manipulation .

Participants were requested to move the hand along each path spending approximately 15-seconds as they were trained. This time interval was determined during the pilot study as a comfortable speed. Feedback was provided at the end of each trial, with instructions to move fast or slow. We controlled the time spent and gave instructions to improve accuracy. The time was controlled by a chronometer.

For the Imprecise group, the visual image of the hand was offset by 30mm, 40mm or 50mm to the right or left (two trials of each, order randomised). A snapshot was captured on the researcher's screen and stored at each moment when the hand reached the CS locations mandatory in that trial (i.e.: if the trial was related to CS+, the snapshot was obtained just for such hand position) so as to later confirm the visual shift in the hand position when the electrocutaneous stimulus was administered (Supplementary methods Figure S6).

To avoid proprioceptive recalibration (for further details see the section "The Precise and Imprecise conditions") the participants were required to move their hand along the paths without visual information until they reached the position of the CS+. A rectangular zone (10 cm wide, 16 cm long) at the location of each CS was designated as a 'target zone' in

427 the customized Labview tracking system. During the acquisition phase, as soon as the
428 tracking system detected that a participant's hand had entered the CS+ "target zone", the
429 UCS was delivered (Supplementary methods Figure S6). After three minutes of rest, we
430 started the test phase.
431
432 *Test phase*
433 The test phase involved no presentation of CS or UCS, was identical for both groups, and
434 involved no visual illusions. In each of 20 trials, the participant was shown one of the five
435 locations (marked with dots) corresponding to Stimulus type: both CSs and three
436 intermediate positions between CS+ and CS- (GSs positions). Then, they were invited to
437 report "*... how likely do you think is that you will receive a painful stimulation?*" if they
438 move their hand to a specific location illustrated on the screen (Figure 2).
439 Each location was presented in 4 trials, and the order of trials was chosen by lot (five
440 sequences pre-defined) (Supplementary file S2).
441
442 *Extinction phase*
443 The extinction phase started just after the test phase (2 minutes of rest). The extinction
444 phase consisted of 12 trials, 6 trials for CS+ location and 6 trials for CS- location, was
445 identical for both groups, and involved no visual illusion. In each extinction trial, a
446 movement path was shown on the screen, but this time the CS+ and CS- locations were
447 marked with dots. The volunteers were invited to move on the same paths performed
448 during acquisition. Participants provided Pain Expectancy ratings as they had in the test
449 phase, and then performed the movement as they had in the acquisition phase, but the US
450 was never presented (Figure 2).
451

Post-experiment questions and debriefing

Finally, participants completed the catastrophizing questionnaire, responded to the conditioned stimuli recognition test, were asked about contingency awareness and visual manipulation awareness, and, finally, the real nature of the study was explained.

Statistical Analysis

The primary analysis for this study used Pain Expectancy ratings provided during the test phase. We used Mixed-Design ANOVAs to compare expectancy ratings across locations and between groups. Our primary aim was to compare Pain Expectancy for CS+ between groups (H1). Our secondary aim was to compare pain expectation generalization (H2) and delay in extinction (H3), within and between-groups.

To test the primary hypothesis, we ran a Mixed-Design ANOVA considering Pain Expectancy to CS+ and CS- (4 levels) as repeated measures and Groups (2 levels: Precise or Imprecise) as between-subjects factor. Considering test phase results, we ran another Mixed-Design ANOVA to test our secondary hypothesis (H2), in which GS1, GS2 and GS3 (4 levels) were the repeated measures and Groups (2 levels: Precise or Imprecise) as the between-subjects factor. With the aim to test our secondary hypothesis (H3), we also performed a Mixed-Design ANOVA with Pain Expectancy to CS+ and CS- (6 levels) as repeated measures and Groups (2 levels: Precise or Imprecise) as between-subjects factor. In addition, separate repeated measures ANOVAS on both the Precise and Imprecise groups were conducted to test H2, considering the mean Pain Expectancy on test phase trials (average of Pain Expectancy across trials) for each stimulus (CS, GS1, GS2, GS3, CS-, five levels). And two separate repeated measures ANOVAs on both Precise and Imprecise groups to investigate within-subject effect of trials on Pain Expectancy during extinction phase.

The Kolmogorov-Smirnov test and the M Box test were applied to assess the normality of the distribution and homogeneity of variance of our data, respectively. The Mauchly's test was used to verify the assumption of sphericity. To compare the means between the different levels of independent variables (pairwise comparisons), Bonferroni test (correction) was used to control for type I error. The comparison between the Precise and Imprecise groups for anthropometric data and psychosocial variables was carried out using a simple multivariate ANOVA ($p<0.05$) considering Group as the between-subjects factor. Also, the chi-square test was used to analyze percentage values. Data were analyzed using Statistical Package for the Social Sciences (SPSS, Chicago, IL) 22 for Macbook and were expressed as estimated mean and standard deviation (SD).

Results

Fifty participants volunteered. Four were excluded: one participant reported isopropyl alcohol allergy, one participant reported a rare skin disease (*Dermatographic urticaria),* one participant reported a heart disorder (prolapsed mitral valve) and one participant reported Raynaud's syndrome. Additional four volunteers were excluded after changes to the experiment (pilot study) and two participants were excluded from analysis after the procedure because they did not report moderate pain on the SPARS (score of at least 25/50) even when exposed to a high intensity noxious stimulation (more than 60 mA) – suggesting a possible nerve accommodation/habituation phenomenon[28]. Finally, 40 participants (n=20/group) were included in the study. Baseline questionnaire results are provided in Table 1. No differences were observed between groups for age. The Precise group had a higher body mass index (BMI) than the Imprecise group, however, both groups showed a BMI score of normal weight (18.5–24.9). Nevertheless, we investigated the effect of the BMI as a confounding variable on the Pain Expectancy ratings.

Actual and seen hand measures

We calculated the actual and seen hand distances (mm) from the top middle finger to the closest border of the picture (screen) in the exact moment the participants received the painful stimulation. The mean difference in distance [actual $_{hand}$ – seen $_{hand}$] was similar to the shift intended, except when the leftwards shift of 50mm was administered to the group with CS+ on the right side of the board, when the actual shift was -42mm (Supplementary results - Table 1S). Regardless, these findings confirm that in the Imprecise condition, a difference between seen and actual hand locations was always introduced, reflecting a consistent imprecision in sensory input as intended.

General statistics

The assumption of normality was not met for all variables: the Kolmogorov-Smirnov test showed a significant deviation from normality ($p<0.05$) for two ratings of two locations (GS2, CS-) during test phase. However, the literature suggests that the F-test is robust, in terms of power, tolerating violations of normality even with very small sample sizes[33]. Also, because the assumption of sphericity was violated (significant Mauchly's test), we adopted the results as recommended according to the epsilon boundary of 0.75[19]. Huynh-Feldt-corrected results were adopted for both test and extinction phases ($\varepsilon>0.75$).

Pain expectancy during test phase

To test our first hypothesis (H1), we ran a mixed model ANOVA which showed a main effect of Group on Pain Expectancy at the CS+ ($F_{(1,692, 64,278)}=4,95$, $p<0.01$, $\eta_p^2= 0.12$). We ran the analysis twice, with and without adjustment for seven predictors: positive affect, negative affect, anxiety, catastrophising, pain rating obtained during calibration, sex and

BMI. None of the predictors showed a significant effect on Pain Expectancy and did not change the main effect of Group on Pain Expectancy, hence we considered the results of the analysis with no adjustment. In pairwise comparison, the Imprecise group showed a higher Pain Expectancy for CS+ than the Precise group did (6.9 vs. 5.4, p=0.03) which upheld our first hypothesis (H1) (Table 2).

Between-group comparison on Pain Expectancy at each of the generalization stimuli was conducted with the aim to test our secondary hypothesis (H2). There was no difference between-groups for GS1 ($F_{(1, 38)}$=0.005, p<0.92, η_p^2= 0), GS2 ($F_{(1, 38)}$=0.36, p<0.54, η_p^2= 0) and GS3 ($F_{(1, 38)}$=0.23, p<0.63, η_p^2= 0) (Table 2). We also ran separate repeated measures ANOVA for Precise and Imprecise groups to investigate within-subject differences in Pain Expectancy between different stimuli (across locations), particularly differences between CS+ and the GSs. We showed a significant effect of stimulus location (conditioned or generalization stimulus) on Pain Expectancy for both the Precise ($F_{(1,68\ 36,750)}$=3.8, p=0.01, η_p^2= 0.23) and the Imprecise groups ($F_{(3,190,\ 41,472\ 36,750)}$=3.77, p=0.01, η_p^2= 0.22). Pairwise comparisons (Bonferroni correction) showed a lower Pain Expectancy at GS3 and CS- than at CS+ for both groups (Table 3). The pain expectancies for each group and each location showed a gradual decrease in Pain Expectancy as the distance from the CS+ increased (Table 3).

Pain expectancy during extinction phase

To investigate our third hypothesis, we ran a Mixed Model ANOVA on Pain Expectancy ratings during extinction phase. This ANOVA showed no main effect of Group on Pain Expectancy at CS+ ($F_{(3,107,\ 118,05)}$=0.91, p=0.44, η_p^2= 0.02) and CS- ($F_{(3,56,\ 118,05\ )}$=0.20, p=0.67, η_p^2= 0.01). We ran also the analysis twice with and without adjustment for the same seven predictors as adopted for the test phase: positive affect, negative affect,

552 anxiety, catastrophising, pain rating obtained during calibration, sex and BMI. We found
553 a significant main effect of anxiety for CS+ ($F_{(1, 24)}$=4.35, p=0.05, η_p^2= 0.15), however,
554 this effect did not change the mean effect of Group on mean Pain Expectancy for CS+
555 during extinction.
556 We also ran separate repeated measures ANOVA for the Precise and the Imprecise group
557 data. These analyses demonstrated extinction of Pain Expectancy ratings for the Precise
558 group at CS+ ($F_{(2,500, 47,497)}$=8.92, p<0.01, η_p^2= 0.32) and CS- ($F_{(3,545, 67,532)}$=4.63, p<0.01,
559 η_p^2= 0.20) and for the Imprecise group at CS+ ($F_{(3,532, 67,104)}$=3.92, p<0.01, η_p^2= 0.18).
560 Pairwise comparisons showed that for the Precise group, Pain Expectancy at the CS+ had
561 lowered by the 5th extinction trial and for the Imprecise group, Pain Expectancy had not
562 lowered by the final extinction trial (Table 4). The Pain Expectancy across trials for each
563 group during extinction phase shows a gradual decrease with subsequent trial (Table 4).
564
565 *Manipulation check – evidence of classical conditioning*
566 As a confirmation of the classical conditioning effect, we expected a main effect of
567 Stimulus type (higher expectation for CS+ than CS-) during test phase. Higher Pain
568 Expectancy for CS+ than CS- was observed on both groups (Precise group - $F_{(1, 19)}$ = 6.31,
569 p=0.02, η_p^2 = 0.12, Imprecise Group - $F_{(1, 19)}$ = 25.92, p<0.01, η_p^2 = 0.57). Pairwise
570 comparisons showed a significant mean difference between CS+ and CS- of 3.9 (95%CI:
571 1.8-6.0, p<0.01) for the Imprecise group and 2.15 (95%CI: 0.2-4.0, p=0.02) for the
572 Precise group.
573
574 *CS-US contingency awareness and the visual illusion*
575 For both groups, we classified a participant as having been contingency aware if they
576 identified either CS+ or GS1 as the location in which they had received painful

stimulation. In the Precise group, 8 participants (40%) accurately reported the CS+ as the location in which they had received noxious stimulation. Of these 8 participants, four responded to this question by reporting two locations, one of which was either the CS+ or GS1. In the Imprecise group, 3 participants (15%) accurately reported the CS+ as the location in which they had received painful stimulation.

The assessment of awareness of the visual manipulation showed that only two participants (one from each group) disagreed with the statement "*I felt as if I was looking at my own hand*" during the procedure. However, 21% (n=4) from the Precise group and 40% (n=8) from the Imprecise group agreed that *"…something was wrong during some movements*", and 11% of the participants from the Precise group and 20% of the Imprecise group agreed that there had been some "*incongruence between visual information and the actual position of the hand*" during some movements.

Discussion

The main objective of this study was to investigate whether imprecise sensory feedback increases conditioned pain expectancy, and generalization and extinction of that conditioned effect, when a specific location is used as the CS+. Our results supported our primary hypothesis that imprecise sensory input during conditioning would result in greater pain expectancy at the CS+ location. That is, pain expectancy was significant increased when the hand was at a location previously associated with pain when visual feedback had been disrupted via illusion than it was when visual feedback had not been disrupted. Our results also showed generalization of pain expectancy but, contrary to the first of our secondary hypotheses, generalization was not affected by rendering sensory input imprecise during conditioning. Our final hypothesis was not supported either – we detected no difference between groups in the rate at which extinction of the elevated pain

602 expectancies occurred. Post-hoc separate analysis on group-specific data raise the
603 possibility that an effect was present and we were not powered to detect it.
604 The main finding of the current study was that rendering sensory input imprecise
605 increased pain expectancy at the CS+ location. To put this finding into context, it is
606 important to consider it in light of the broader context of the IH[60]. The IH appreciates
607 that, just as visual stimuli are encoded as meaningful singular percepts, not as an array of
608 features or simply a retinal 'impression'[41] (which allows us to be tricked by illusions such
609 as the Necker cube[61]), so too painful movements and events are encoded as meaningful
610 singular multisensory events, not as an array of nociceptive and non-nociceptive sensory
611 features or simply a nociceptive 'impression'. Such high-order integrated percepts present
612 an excellent situation for associative learning, which permits rapid protective responses.
613 That the current experiment induced elevated pain expectancies associated with a given
614 location supports that principle. A mean difference of 1.47 on an 11-point pain
615 expectancy rating should be considered a small effect, although it is comparable in
616 magnitude with previously reported differences between patients and healthy controls[48]
617 and may well be clinically important – further work is clearly required to determine if
618 this is the case.
619 That imprecise sensory input increased pain expectancies extends a previous result from
620 our group in which differential learning between CS+ vs. GSs and CS- locations in a skin
621 area with low tactile precision (the lower back), was poorer than it was in a skin area with
622 high tactile precision (the hand)[26]. Notably however, the current research question was
623 different in two critical ways. First, we aimed to define a specific *location* of the hand in
624 space as the CS+. That spatial data can influence the learning of associations was
625 established in early studies[72, 73], and associating certain environments with aversive
626 stimuli is a well-used paradigm to study fear conditioning and learned helplessness in

rats[34]. Moreover, the notion that spatial cues might sufficiently signal threat so as to modulate pain expectancies is well recognized clinically[45].

An important consideration is whether or not our manipulation to induce sensory imprecision may have had other pain-relevant effects unrelated to the conditioning procedure. Sensorimotor conflicts may deflagrate sensory disturbances in chronic pain patients[7] or exacerbate symptoms[14]. Also, experimentally induced pain has been associated with increased report of sensory disturbances in healthy volunteers, but the increase in sensory disturbances was not explained by an interaction between stimulation and sensorimotor incongruence[7]. Another consideration relates to previous findings that showed that lower stimulus predictability of pain is associated with higher reports of pain, fear and greater physiological arousal[8, 62]. We cannot exclude the possibility that processes similar to those interrogated in those studies were also at play here.

This approach (combining sensorimotor conflict + unpredictability) clearly has ecological limitations – there is no suggestion that the reliability of visual input is compromised during painful events outside of the laboratory. However, we selected this approach because we wanted to disrupt the final encoding of *location* and we can be sure, based on our previous work using the MIRAGE system, that we can achieve this aim[4, 5, 20]. The evaluation of participants' awareness of the visual input manipulation also confirmed that there was approximately a two-fold increase in reports of perceived incongruence between real and seen hand position in the Imprecise versus the Precise group, supporting the idea that participants would likely be less certain and/or accurate in localizing their hand under imprecise feedback.

In addition, we investigated the effect of several confounding variables on the pain expectancy. Our results showed just an effect of anxiety on pain expectancy during extinction phase, although, no interaction between anxiety and Group was observed,

652 suggesting anxiety, prior to the experiment, influenced both groups equally. On the other
653 hand, we did not gather data regarding anxiety or arousal during the experiment. Future
654 studies may investigate the pain anxiety evoked by contexts with different levels of
655 predictability and sensorimotor conflict.
656 Regardless of the sensorimotor conflict and unpredictability, our paradigm was effective
657 in inducing location specific expectancies - differential learning with higher pain
658 expectancy at CS+ than at CS-. However, we did not reliably induce contingency
659 awareness. Just 40% of participants from the Precise group and 15% from the Imprecise
660 group, could accurately recall the location at which they received painful stimulation
661 during acquisition phase. That is, more of the Precise group showed contingency
662 awareness in post-experiment questioning, but 60% of that group still remained unaware.
663 The 50% reinforcement rate of the association between CS+UCS during acquisition phase
664 may explain low contingency awareness, but not the between-group difference. In fact,
665 such a low rate of contingency awareness with such a clear conditioning effect suggests
666 that conditioning occurred outside of awareness. Whether or not this is possible is a long-
667 standing debate – the first studies reporting fear conditioning outside of awareness
668 emerged over 75 years ago [e.g. Diven[15], Haggard[23]] and studies reporting otherwise
669 emerged a decade later, clearly linking both conditioning and generalization to both
670 contingency awareness and ability to articulate it [e.g. Chatterjee and Eriksen[10]].
671 The current experiment demonstrated that once a pain expectancy was established during
672 'acquisition', the expectancy was elicited also by events that shared some features with
673 it, a process called generalization. In conditioning experiments, generalisation is inversely
674 related to the degree to which a stimulus can be differentiated from other functionally
675 distinct stimuli, which allows optimisation of behavioural specificity[22, 65]. Contrary to our
676 prediction, which was based on the IH, generalization of the conditioned response did not

677 increase, nor did the speed of extinction, when acquisition occurred under imprecise
678 sensory input. Perhaps the alterations in the task during test phase limited differential
679 generalization. That is, generalization was investigated using locations (GSs) outside of
680 the learned movement paths, which may reduce generalization. In addition, CS-UCS
681 reinforcement of 50% during acquisition phase, (i.e. 50% of the occasions on which
682 participants crossed the CS+ location they did not receive a stimulus) may have reduced
683 contingency awareness and perhaps conditioning effects and between group differences.
684 However, a previous study showed that despite continuous CS–UCS pairings during
685 acquisition showed stronger conditioned responses, it speeds the rate of extinction of
686 differential UCS expectancies in a study of human fear conditioning[21].

687 The current study has several limitations. First, we cannot disregard that we were
688 underpowered for analysing all three hypotheses – that our secondary hypotheses were
689 unsupported does not exclude the possibility that an effect exists. Observation of the data
690 and planned pairwise comparisons raise that possibility particularly for our second
691 secondary hypothesis – pain expectancies during extinction were lower after the 4th trial
692 in the Precise group but were still not lower after the 6th trial in the Imprecise group. A
693 second limitation was that the researchers in the current study were not blinded to group
694 allocation because they had to administer the shock and the illusion. That our secondary
695 hypotheses were not upheld suggests this did not impact results, but nonetheless it
696 represents a shortcoming of the study. Third, only healthy subjects were enrolled in the
697 current study, so results are not generalizable to clinical populations and it seems possible
698 that differences may exist in contingency learning and generalization gradients between
699 those with and without chronic pain[27, 49, 51]. Fourth, we were interested in pain expectancy,
700 but we are unable to exclude the possibility that we inadvertently modulated fear of pain.
701 We considered that within the current design, any change in fear of pain would be

secondary to changes in the expected likelihood of pain and saw no theoretical justification for why either condition would be associated with more or less fear of pain aside from that mediated by expectation. Future studies would be well served to evaluate fear of pain in order to verify these assumptions. Fifth, the CS-UCS reinforcement of 50% adopted in our study may not have been ideal - continuous and partial CS-UCS pairings can result in different outcomes. Finally, we did not lodge and lock our protocol and statistical analysis plan prior to data collection. When we commenced this study, such practice was uncommon in our field, but now it is recommended, and our group is among those at the forefront of this push[36]. Failure to do this clearly represents a shortcoming in transparency and reporting.

Conclusion

Our results supported our primary hypothesis that imprecise sensory input during conditioning would result in a small but increased pain expectancy at the CS+ location, even though most participants, particularly those in the Imprecise group, remained contingency unaware. Such a result adds to a growing body of literature centered around the Imprecision Hypothesis and to evidence for contingency unaware conditioning in pain conditioning studies. We also showed generalization of pain expectancy but, contrary to our hypothesis, neither generalization nor extinction were affected by rendering sensory input imprecise during conditioning. That a post-hoc analysis raised the possibility that an effect of imprecise sensory input on extinction may have gone undetected suggests appropriately powered studies to thoroughly test that hypothesis might be warranted. Future studies should also determine whether the results hold when participants are contingency aware and whether pain modulation is affected in a similar way to pain expectancy modulation.

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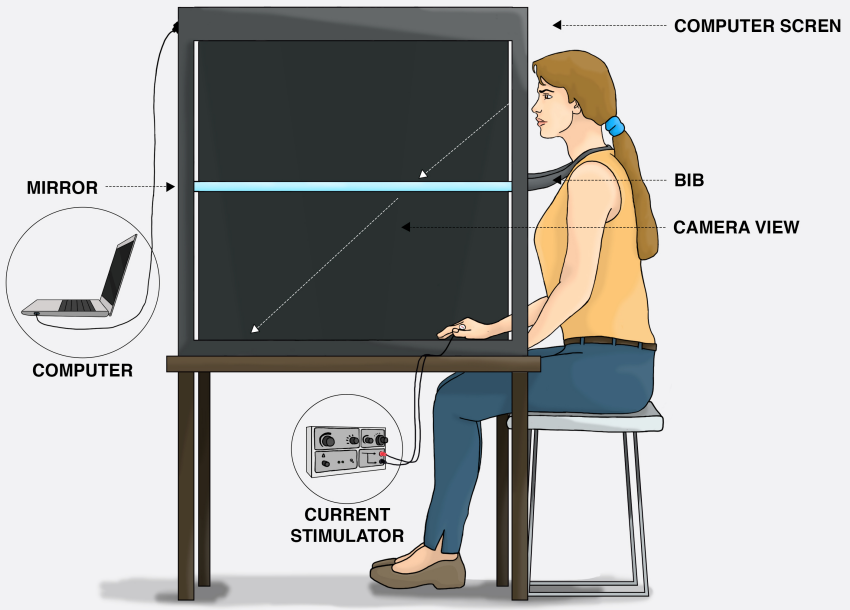
Figures

1A



1B

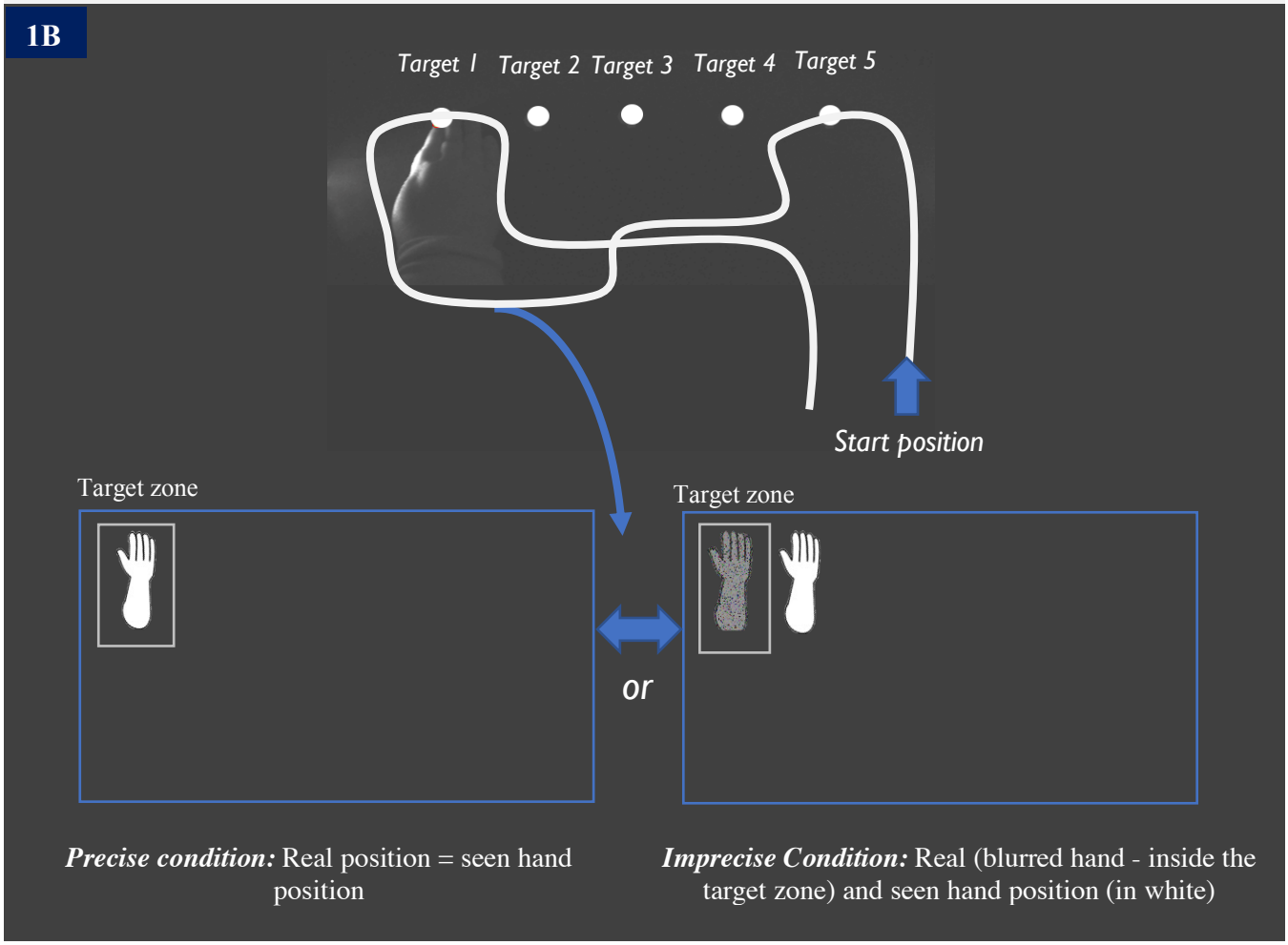


Figure 1A. Schematic representation of the MIRAGE multisensory system. The angles of the camera and mirrors were adjusted to enable real-time video capture of the participants actual hand to be presented in the same spatial position and same visual perspective as if viewing the hand directly.

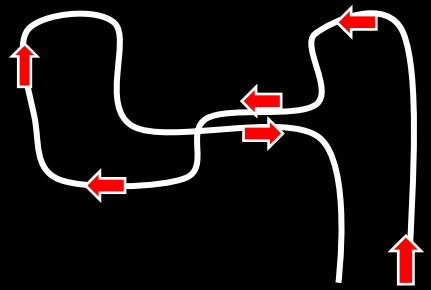
Figure 1B. (top) Path showing the movement executed during acquisition and extinction phases of the experiment. Participant's hand crossed just the positions related to target 1 and target 5 (conditioned stimulus - CS). This picture is merely illustrative. In other words, no target or path was visible during the acquisition phase, however during test phase the locations were marked with dots [one per trial] and no path was presented. Ultimately, at the beginning of each trial of the extinction phase the same paths performed during acquisition phase, marked with one dot over CS+ or CS- locations were shown to volunteers. The middle positions (Targets 1-3), represent the generalization stimuli locations. **Figure 1B. (bottom)** The schemas are depicting the real and seen hand locations in the precise condition (right) and imprecise conditions (left) when the participant crossed the target zone (the hand is on the location volunteers received the aversive stimulation). In the example, target 1 was the CS associated with Unconditioned Stimulus (UCS).

Screen 1

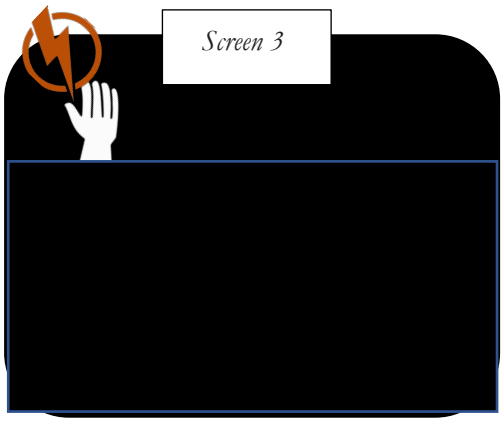
Please, move your hand according to the path and come back to the start position

Pay attention to the arrows that are showing the direction

Screen 2



Screen 3



A

If move your hand to the location ... how likely do you think is that you will receive a painful stimulation?

0 1 2 3 4 5 6 7 8 9 10

Not at all likely — *Extremely likely*

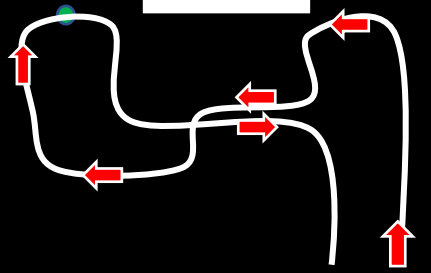
If move your hand to the location ... how likely do you think is that you will receive a painful stimulation?

0 1 2 3 4 5 6 7 8 9 10

Not at all likely — *Extremely likely*

B

Screen 1



We want you to perform this path. Note the location of the dot.

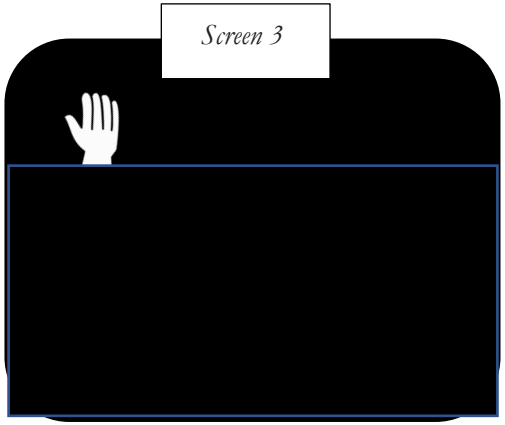
Screen 2

0 1 2 3 4 5 6 7 8 9 10

Not at all likely — *Extremel y likely*

How likely do you think it is, that you will receive a painful stimulation, if you move your hand to that location?

Screen 3



C

Figure 2B. Flowchart depicting the acquisition phase of the group not submitted to the illusion (precise group). This block is describing the delivery of the conditioned stimulus with noxious stimulation (CS+, target 1). **Figure 2B.** Flowchart depicting the test phase from the classical conditioning experiment. The screen 1 is describing a trial related to target 1 position and the screen 2 is describing a trial related to target 3. **Figure 2C**. Flowchart depicting the extinction phase. Screen 1: Screen illustrating the path and the dot related to conditioned stimulus on target 1. Screen 2: Instructions on how to assess pain related expectancy. Screen 3:articipant moving on the path – no noxious stimulation provided.

Table 1. Descriptive data (mean and standard deviation [SD]) for precise and imprecise groups.

Anthropometric and clinical variables	**Precise group (n=20)**	**Imprecise group (n=20)**	**p-value**
Age (years)	30 (12)	26 (6)	$F_{(1,38)}$=1.80, p=0.19
Body Mass Index (BMI)	25 (3)	22 (3)	$F_{(1,38)}$=5.16, p=0.03*
Handedness (0-100%)	80 (21)	80 (27)	$F_{(1,38)}$=0.03, p=0.86
Gender **	11F/9M	15F/5M	X^2= 1.75, p=0.32
Noxious stimulus intensity (mA)	21 (19)	16 (14)	$F_{(1,38)}$=1.03, p=0.31
SPARS (-50 to +50)	28.8 (3)	29 (3.1)	$F_{(1,38)}$=0.22, p=0.87
Anxiety (20-80 points)	37.8 (9.3)	36.2 (8.3)	$F_{(1,38)}$=0.32, p=0.56
Positive affect (10-50 points)	31 (7)	30 (8)	$F_{(1,38)}$=0.05, p=0.80
Negative effect (10-50 points)	17 (7)	15 (6)	$F_{(1,38)}$=0.06, p=0.41
Catastrophising (0-24 points)	6 (5)	5 (4)	$F_{(1,38)}$=0.41, p=0.53

* $p<0.05$ (ANOVA)

** Chi-square, fisher correction

Handedness - Edinburgh Handedness Inventory; Anxiety – State Trait anxiety Inventory; The Catastrophizing Questionnaire; The Positive and Negative Affect Scale (PANAS); SPARS (Sensation and Pain Rating Scale)

Table 2. Pain expectancies at each location for each group. Estimated means for pain related expectancy (mean along the trials) and Standard Deviations (SD) for the precise (P) and imprecise (IMP) groups during test phase

	Estimated mean* (SD) **Precise group**	**Estimated mean* (SD)** **Imprecise group**	**Mean difference (IMP-P)**
CS+	5.4 (2.48)	6.87 (1.93)	1.47**
GS1	4.87 (1.48)	5.6 (1.84)	0.72
GS2	4.04 (2.12)	4.75 (1.37)	0.71
GS3	3.48 (2.02)	3.58 (1.51)	0.1
CS-	3.27 (2.69)	2.97 (2.1)	-0.31

*Estimated marginal mean (model precise and imprecise groups vs. conditioned stimuli)

** $p<0.05$, ANOVA, Bonferroni correction

CS+: conditioned stimulus associated with noxious stimulation – right or left extreme position, GS1: Generalization stimuli closest to CS+; GS2: middle position; GS3: Generalization stimuli closest to CS- and CS-: the opposite position regarding CS+.

Table 3. Pain Expectancies at each location for each group during test phase. Estimated mean difference for pairwise comparisons between conditioned and/or generalization stimuli for the Imprecise group (top panel) and Precise group (bottom panel) during test phase.

	CS+	**GS1**	**GS2**	**GS3**	**CS-**
Imprecise group (estimated mean difference)					
CS+	NA	—	—	—	—
GS1	-1.27	NA	—	—	—
GS2	-2.13*	-0.84	NA	—	—
GS3	-3.28*	-2	-1.15	NA	—
CS-	-3.9*	-2.63*	-1.77	-0.62	NA
Precise group (estimated mean difference)					
CS+	NA	—	—	—	—
GS1	-0.53	NA	—	—	—
GS2	-1.35	-0.84	NA	—	—
GS3	-2.01*	-1.39	-0.56	NA	—
CS-	-2.15*	-1.6	-0.75	-0.2	NA

*Significant difference (ANOVA, Bonferroni correction, $p<0.05$)

NA= Not applicable, CS+ = conditioned stimulus associated with the noxious stimulus; GS = Generalisation stimuli, with GS1 closest to CS+; GS2; CS- unpaired location in the opposite position (and furthest from) the CS+.

Gray cells = repeated comparisons

Table 4. Pain expectancies during extinction phase. Estimated mean pain expectancies (standard deviations) at the CS+ and CS- locations for the Precise group (left) and Imprecise group (right). Mean values adjusted by anxiety score

	CS-	CS+	CS-	CS+
	Precise group (SD)		**Imprecise group (SD)**	
	n=20		**n=20**	
Trial 1	4.05 (2.21)	6.25 (2.22)	3.6 (2.21)	6.4 (1.82)
Trial 2	3.9 (2.36)	5.9 (1.62)	3.65 (2.66)	6.3 (1.81)
Trial 3	3.5 (1.99)	5.4 (1.9)	3.05 (2.46)	5.45 (2.16)
Trial 4	2.9 (2.47)	4.9 (2.45)	2.65 (2.58)	5.2 (2.86)
Trial 5	2.40 (1.96)	3.6*#& (2.46)	2.6 (2.39)	4.95 (3.35)
Trial 6	2.25 (1.89)	4.0 # (2.71)	2.8 (2.65)	4.7 (2.9)

* Different from Trial 1 in pairwise comparisons ($p<0.05$, Bonferroni)

Different from Trial 2 in pairwise comparisons ($p<0.05$, Bonferroni)

&Different from Trial 3 in pairwise comparisons ($p<0.05$, Bonferroni)

CS+ = location that was paired with noxious stimulus during acquisition; CS- = the location that was unpaired during acquisition and furthest from the CS+.

Supplementary results Table S8. Description of the mean and standard deviations (SD) of the seen and actual hand position (mm) obtained from pictures captured during acquisition phase of classical conditioning for precise and imprecise groups.

	Imprecise Group						**Precise group**
	Shift to left (SD)			**Shift to right (SD)**			
Target 1 – left side (n=10)	**50**	**40**	**30**	**30**	**40**	**50**	**Mean of 6 pictures**
seen hand (mm)	118.7 (23.9)	108.6 (44.8)	98.6 (14.5)	136.3 (28.9)	145.6 (31.3)	163.8 (52)	115.1 (15.8)
actual hand (mm)	158.8 (32.5)	149.3 (44.8)	132.1 (24)	106.4 (29.4)	106.5 (30.3)	113.3 (52.1)	115.1 (15.8)
Actual $_{hand}$ – seen $_{hand}$*	**47.5**	**40.7**	**33.5**	**-29.9**	**-39.1**	**-45.4**	
Target 5 – right side (n=9)**							
seen hand (mm)	160.9 (34.2)	133.2 (37.7)	145 (37.9)	108.8 (36.8)	123.1 (41)	86.6 (41.4)	128.4 (14.2)
actual hand (mm)	112.7 (36.4)	93.7 (37.4)	111.3 (42.6)	133 (35.4)	163.7 (40.4)	124.3 (26.2)	128.4 (14.2)
Actual $_{hand}$ – seen $_{hand}$*	**-42.3**	**-39.6**	**-33.7**	**30.9**	**40.6**	**49.3**	

* Mean value obtained after use the formula for each participant (not the subtraction between mean actual hand - mean seen hand positions)

** During the experiment we missed the pictures from one participant

SUPPLEMENTARY FILES

Supplementary Methods

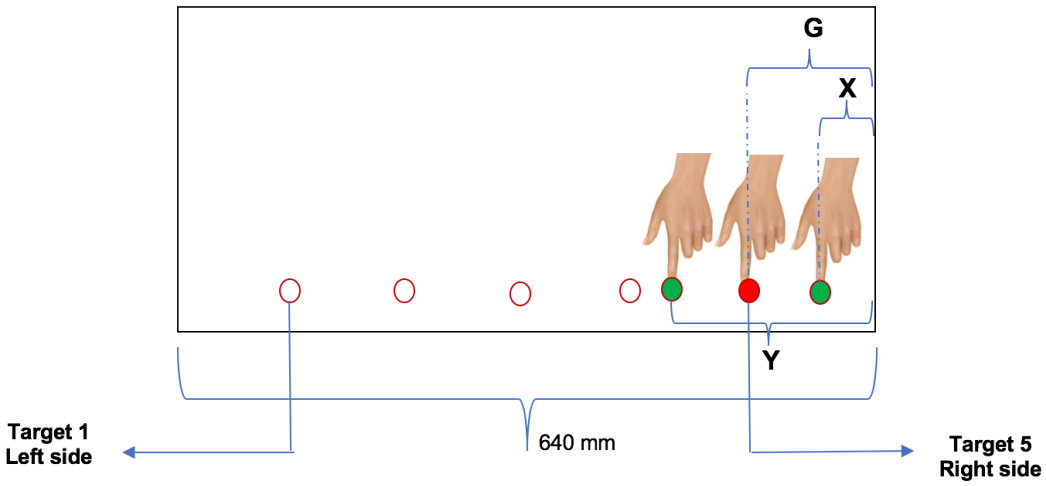
Supplementary Methods file S1 - Determining the image shift used in the imprecise procedure

The amount of shift for the imprecise group was determined during a pilot study (n=3) in which we calculated the position of the tip of the index finger on the right hand (in millimeters - mm) with and without the shift. During the pilot testing, participants were asked to reach dots projected virtually on the board of the MIRAGE box in five positions (Figure S1). Afterwards the dot flashed, participants were instructed to move and reach the position and "to freeze the hand" in the final position. The participants repeated the trials in each position and at different shift ranges and directions (no shift: precise situation, imprecise: shift of 10-50mm to right and left) at least five times. For each final position of the hand, the researcher calculated the coordinates using the tip of index finger as a reference with Labview software (Figure S2). The mean value obtained for the coordinate (position on the board in mm) for imprecise condition was subtracted from the precise condition, to establish the "real shift", or in other words, the final level of deviation from the correct position.

The standard error of the measurement (SEM) was obtained in an attempt to control the level of "real shift" we provided to participants. To calculate the standard error of the measurement (SEM), we adopted the formula described by Bland and Altman[6]. For the extreme positions, the SEM obtained in the pilot study in the precise condition were: extreme end right position (target 5) 9.71mm and extreme end left position (target 1) was 10.32mm. In order to guarantee that the shifts would surpass the SEM obtained, we adopted a minimum shift of 20 mm for the study. Values higher than 50 mm were not considered since the displacement of the screen would be too gross that was possible to

identify a dark rectangular area on the boundary which may provide cues regarding the manipulation of the image showed and introduce bias.

Supplementary Methods file S2



G = position where the dot flashed - without shift (119 mm)

X (shift to right) = 70mm and Y (shift to left) = 167mm

$Final\ Shifted_{position} - Final\ non\text{-}shifted_{position} = Real\ Shift$

50mm shift to the right: 167 - 119 = **48mm of real shift**

50mm shift to the left: 70 - 119 = **49 mm of real shift**

Figure S2. The picture is showing the calculation to obtain the mean difference for the index finger tip position from imprecise – precise position (Formula: Final Shifted $_{position}$ – Final non-shifted $_{position}$ = Real shift). The illustration depicts position shifted by 5 cm (50 mm) to left and right for target 5 (right).

Figure S3a. Illustration depicting the measure of the distance between the target 1 (extreme end - left) and the left border of the screen on the picture. In this picture the seen hand and actual hand are the same (precise context). The distance "x" is 137mm.

Figure S3b. Illustration depicting the measurement of the distance between the target 5 (extreme end - right) and the right border of the screen on the picture. In this picture the seen hand is the green one and the actual hand is the hand marked with a red dot (imprecise context). It is possible to see the image shifted to the right (40 mm), considering the participant perspective. The distance "y" is 126mm and the distance x is 83 mm.

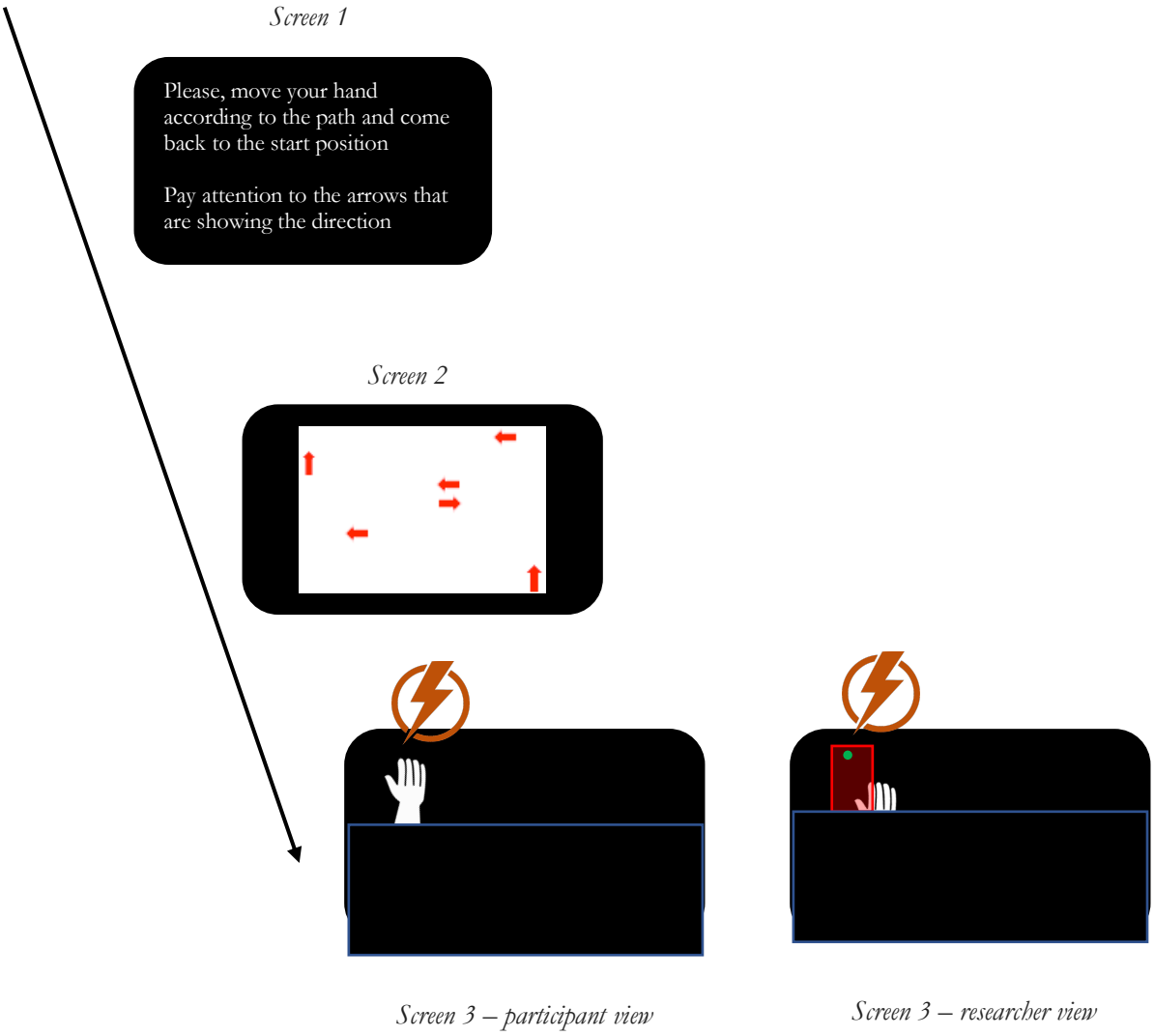


Figure S4. Flowchart depicting the acquisition phase of the group not submitted to the illusion (precise group) showing participant and researcher views. This block is describing the delivery of the conditioned stimulus with high noxious stimulation (CS+, target 1). The blue rectangle was used during the experiment to avoid the participant to update/correct the hand position to match the visual estimate (proprioceptive recalibration).

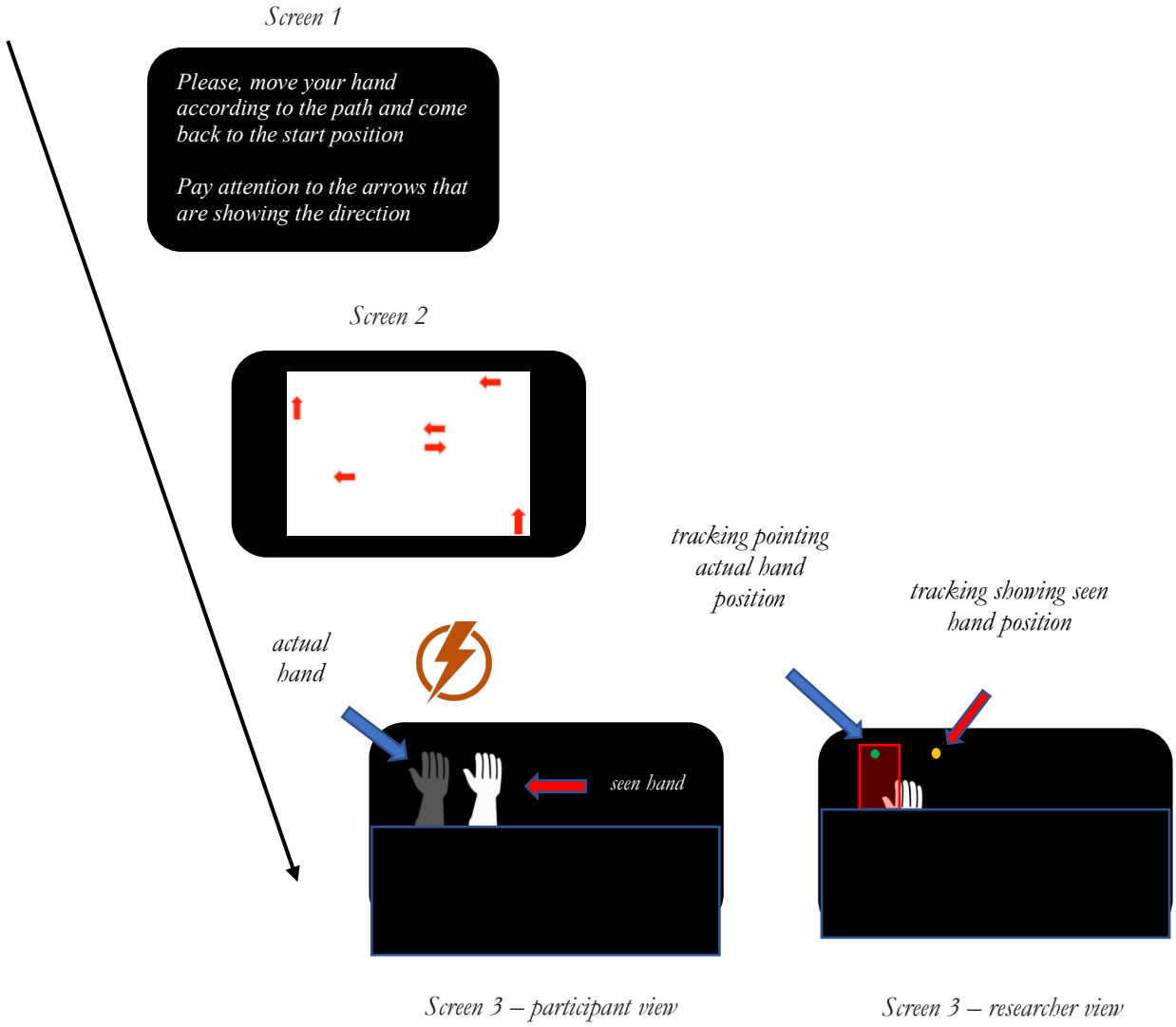


Figure S5. Flowchart depicting the acquisition phase of the group submitted to the illusion (imprecise groups) showing participant and researcher views. This block is describing the delivery of the conditioned stimulus with noxious stimulation (CS+, target 1). The blue rectangle was used during the experiment to avoid the participant to update/correct the hand position to match the visual estimate (proprioceptive recalibration).

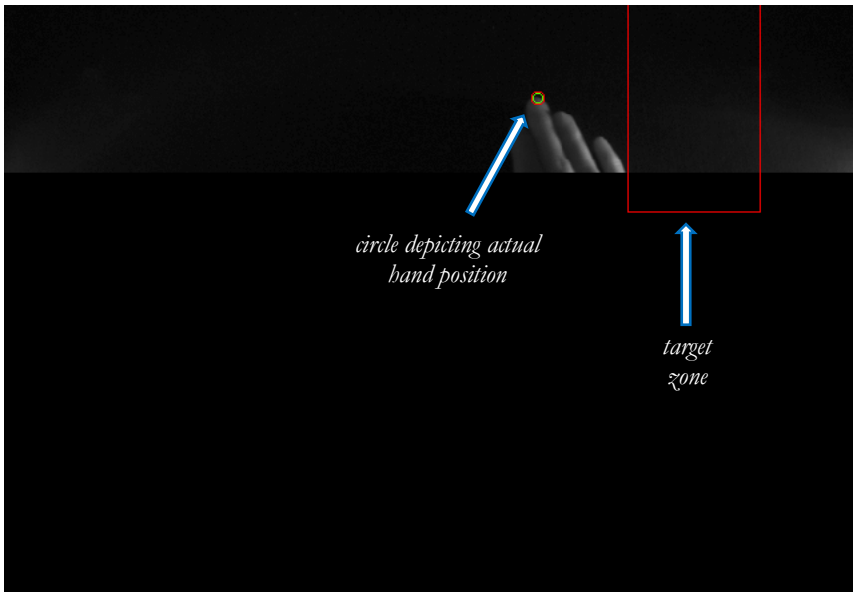


Figure S6a. Picture of the participant moving on the path (left to right) and showing the target zone deactivated, as the hand did not reach the perimeter of the target zone. This view was not available for the participants.

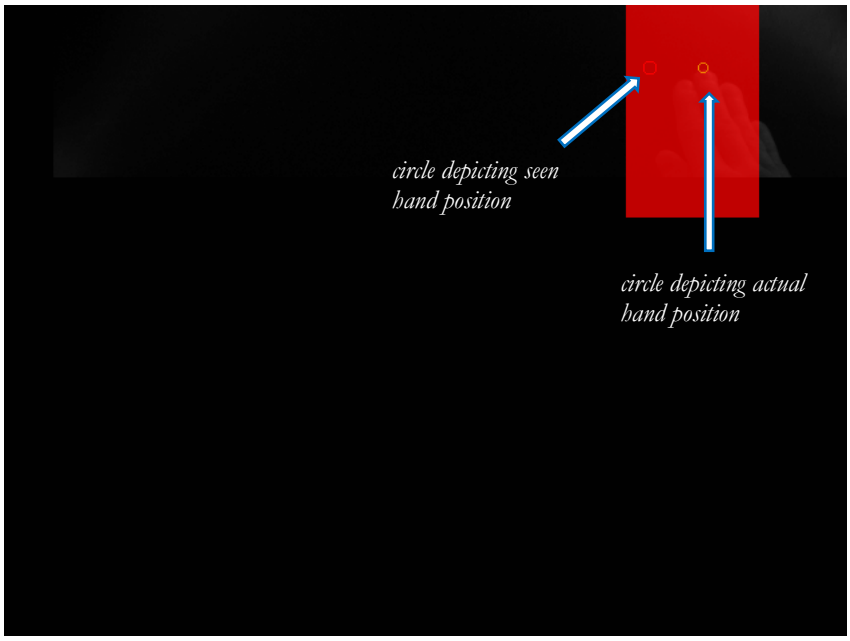


Figure S6b. Picture of the participant moving on the path (left to right) when crossing the position related to conditioned stimulus (in this case target 5) and triggering the target zone. This view was not available for the participants.

Supplementary Methods file S7.

Five different sequences - Test phase (chosen by lot)

Trial order	Sequence A	Sequence B	Sequence C	Sequence D	Sequence E
1	TARGET 1	TARGET 3	TARGET 5	TARGET 4	TARGET 1
2	TARGET 2	TARGET 1	TARGET 1	TARGET 1	TARGET 3
3	TARGET 5	TARGET 5	TARGET 3	TARGET 3	TARGET 5
4	TARGET 4	TARGET 4	TARGET 4	TARGET 5	TARGET 2
5	TARGET 3	TARGET 2	TARGET 2	TARGET 2	TARGET 4
6	TARGET 2	TARGET 1	TARGET 2	TARGET 5	TARGET 3
7	TARGET 5	TARGET 4	TARGET 5	TARGET 3	TARGET 1
8	TARGET 4	TARGET 3	TARGET 4	TARGET 2	TARGET 2
9	TARGET 1	TARGET 2	TARGET 1	TARGET 4	TARGET 4
10	TARGET 3	TARGET 5	TARGET 3	TARGET 1	TARGET 5
11	TARGET 1	TARGET 4	TARGET 1	TARGET 1	TARGET 5
12	TARGET 4	TARGET 1	TARGET 3	TARGET 4	TARGET 3
13	TARGET 3	TARGET 2	TARGET 4	TARGET 2	TARGET 2
14	TARGET 2	TARGET 5	TARGET 5	TARGET 5	TARGET 4
15	TARGET 5	TARGET 3	TARGET 2	TARGET 3	TARGET 1
16	TARGET 4	TARGET 5	TARGET 3	TARGET 3	TARGET 5
17	TARGET 1	TARGET 1	TARGET 1	TARGET 1	TARGET 1
18	TARGET 5	TARGET 3	TARGET 5	TARGET 5	TARGET 3
19	TARGET 2	TARGET 2	TARGET 4	TARGET 4	TARGET 2
20	TARGET 3	TARGET 4	TARGET 2	TARGET 2	TARGET 4