Modulating pain thresholds through classical conditioning

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Background. Classical conditioning has frequently been shown to be capable of evoking fear of pain and avoidance behaviour in the context of chronic pain. However, whether pain itself can be conditioned has rarely been investigated and remains a matter of debate. Therefore, the present study investigated whether pain threshold ratings can be modified by the presence of conditioned non-nociceptive sensory stimuli in healthy participant.

Methods. In 51 healthy volunteers, pain threshold to electrocutaneous stimuli was determined prior to participation in a simultaneous conditioning paradigm. Participants underwent an acquisition phase in which one non-painful vibrotactile stimulus (CS^+) was repeatedly paired with a painful electrocutaneous stimulus, whereas a second vibrotactile stimulus of the same quality and intensity (CS^-) was paired with a non-painful electrocutaneous stimulus. Stimulation was provided on the lower back with close proximity between the CSs and the US. In the test phase, electrocutaneous stimuli at the individually-set threshold intensity were simultaneously delivered together with either a CS^+ or CS^-. Pain intensity ratings were obtained after each trial; expectancy ratings were obtained after each block. The primary outcome was the percentage of test stimuli that were rated as painful.

Results. Test stimuli were more likely to be rated as painful when they were paired with the CS^+ than when they were paired with the CS^-. This effect was not influenced by contingency awareness, nor by expectancies or mood states.

Discussion. The findings support the notion that the judgement of an event being painful or non-painful can be influenced by classical conditioning and corroborate the possible role of associative learning in the development and maintenance of chronic pain.

1 **Modulating Pain Thresholds through Classical Conditioning**

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17   **Abstract**

18   Classical conditioning has frequently been shown to be capable of evoking fear of pain and

19   avoidance behaviour in the context of chronic pain. However, whether pain itself can be

20   conditioned has rarely been investigated and remains a matter of debate. Therefore, the

21   present study investigated whether pain threshold ratings can be modified by the presence of

22   conditioned non-nociceptive sensory stimuli in healthy participants. In 51 healthy

23   volunteers, pain threshold to electrocutaneous stimuli was determined prior to participation

24   in a simultaneous conditioning paradigm. Participants underwent an acquisition phase in

25   which one non-painful vibrotactile stimulus (CS$^+$) was repeatedly paired with a painful

26   electrocutaneous stimulus, whereas a second vibrotactile stimulus of the same quality and

27   intensity (CS$^-$) was paired with a non-painful electrocutaneous stimulus. Stimulation was

28   provided on the lower back with close proximity between the CSs and the US. In the test

29   phase, electrocutaneous stimuli at the individually-set threshold intensity were

30   simultaneously delivered together with either a CS$^+$ or CS$^-$. Pain intensity ratings were

31   obtained after each trial; expectancy ratings were obtained after each block. The primary

32   outcome was the percentage of test stimuli that were rated as painful. Test stimuli were more

33   likely to be rated as painful when they were paired with the CS$^+$ than when they were paired

34   with the CS$^-$. This effect was not influenced by contingency awareness, nor by expectancies

35   or mood states. The findings support the notion that the judgement of an event being painful

36   or non-painful can be influenced by classical conditioning and corroborate the possible role

37   of associative learning in the development and maintenance of chronic pain.

Introduction

40   The development of chronic pain, i.e. the persistence of pain long after tissue healing, is

41   not well understood. A proposed mechanism in the transition from acute to chronic pain that seems

42   intuitive and is widely accepted among clinicians (Madden & Moseley 2016) is associative

43   learning in the form of classical conditioning. It is assumed that during acute pain, nociceptive

44   signalling serves as the unconditioned stimulus (US), and occurs alongside non-nociceptive

45   signalling such as touch, proprioception and haptic perception, which serves as the conditioned

46   stimulus (CS), such that the association of both stimuli is stored in memory. According to this

47   model, the CS then carries information about the occurrence of the US: a stimulus repeatedly

48   paired with pain (CS$^+$) indicates danger and elicits the expectancy of pain, while a stimulus paired

49   with non-painful sensations (CS$^-$) indicates safety (Domjan 2005). By virtue of the CS-US

50   association, CS's may then instil conditioned responses (CR) such as increases in muscle tension,

51   sympathetic activation, avoidance behaviour or fear in the absence of the US (Linton et al. 1984).

52   Some theorists have espoused the idea that pain itself can occur as a CR which continues to

53   maintain itself after the initial source of nociception has subsided (Flor & Birbaumer 1994; Linton

54   et al. 1984; Moseley & Vlaeyen 2015). Accordingly, classical conditioning might evoke and

55   maintain chronic pain.

56   While it has repeatedly been shown that various pain-related constructs including

57   expectancies, pain-related fear, fear of movement and avoidance behaviour can be classically

58   conditioned (Claes et al. 2016; Gramsch et al. 2014; Jepma & Wager 2015; Meulders et al. 2011;

59   Peuterl et al. 2011; Zaman et al. 2015), less is known about whether pain itself can be conditioned

60   as well. Only a few attempts have been made to elicit pain as a classically conditioned response

61   (for a review, see Madden et al. 2015). So far, most studies evaluated *conditioned hyperalgesia*

62   (increased painful response), and provided strong support that pain can indeed be amplified

63   through associative learning, as identical nociceptive stimuli were found to evoke different

64   perceptions merely depending on established, frequently unconscious associations (Harvie et al.

65   2016a; Jensen et al. 2015). In contrast, only three studies indirectly investigated *conditioned*

66   *allodynia* (a painful response upon non-nociceptive stimuli) and reported contradictory findings

67   (Bräscher et al. 2017; Klinger et al. 2010; Williams & Rhudy 2007). Examining this topic further,

68   Madden and colleagues conducted two targeted experiments using laser-stimuli (Madden et al.

69   2016a) or thermal stimuli (Madden et al. 2016b), both of which activate nociceptive Aδ- and C-

70   fibres, as US and vibrotactile stimuli activating non-nociceptive Aβ-fibres (Abraira & Ginty 2013)

71   as CSs to specifically test the effect of classical conditioning on pain threshold ratings. At-pain-

72   threshold stimuli are considered to be arbitrary, meaning they are perceived as painful or non-

73   painful at chance level. It was hypothesized that the classical conditioning procedure would lead

74   to a higher percentage of these at-pain-threshold stimuli being perceived as painful, signifying a

75   decrease in pain threshold. However, only the former study (Madden et al. 2016a) demonstrated a

76   decrease in pain thresholds on CS^{+} trials whereas the latter (Madden et al. 2016b) did not. It was

77   speculated that these inconsistent findings could be due to the different types of USs used, as laser

78   stimuli produce more discrete sensations than contact heat stimuli, allow for faster temperature

79   increases, and selectively activate Aδ- and C-fibre nociceptors whereas thermodes used for thermal

80   stimulation concomitantly activate low threshold mechanoreceptors (Plaghki & Mouraux 2003).

81   Consequently, studies on the conditioning of allodynia and pain thresholds are scarce and their

82   findings are mixed at best. In order to better understand and extend previous findings, further

83   research is required.

84   The present study aimed at examining whether the judgment of stimuli as painful or non-

85   painful can be a classically conditioned response in healthy participants by replicating and

86   extending the studies by Madden et al. (Madden et al. 2016a; Madden et al. 2016b). Similar to

87   these studies, in the present experiment two neutral vibrotactile stimuli (CS^+, CS^-) were paired

88   with a painful (US_P) and a non-painful (US_{NP}) electrocutaneous stimulus, respectively, all of which

89   were applied to the lower back. The USs evoked an unconditioned response (URs), namely

90   reported pain. Subsequently, both CS^+ and CS^- were presented simultaneously with at-pain-

91   threshold electrocutaneous stimuli (US_{PT}). It was expected that these US_{PT}'s would be experienced

92   and rated as painful as opposed to non-painful (CR) on more of the CS^+ trials than the CS^- trials,

93   confirming that the judgment of stimuli as painful or non-painful can be modulated through

94   classical conditioning. The main difference with the previous studies was the type of US used,

95   namely an electrocutaneous stimulus instead of laser stimulation (Madden et al. 2016a) or thermal

96   stimulation (Madden et al. 2016b). This type of stimulation was chosen as it shares some of the

97   features of laser stimuli, namely the discreteness and speed with which activation of nociceptors

98   is achieved, while co-activating mechanoreceptors like thermal stimuli. Thus, although not directly

99   comparing the different types of USs, the present study may help to interpret previous

100   contradictory findings.

101

102   **Materials & Methods**

103   **Participants**

104   As the present experiment closely resembled the study by Madden, Bellan, et al. (2016a),

105   an *a priori* sample size calculation using G*Power [8] was conducted with the effect size obtained

106   by Madden, Bellan, et al. (2016a) (ES = 0.58), according to which 34 participants would need to

107   be included for a power of .95 ($\alpha = 0.05$). In order to deal with a potentially overestimated effect

108   size of the original study (Brandt et al. 2014), the study aimed at including 50 participants in total.

109   Participants were recruited at the University of Leuven, Belgium, using flyers, posters, word of

110   mouth, and through an online platform for participant recruitment.

111   Participants were eligible for participation if they were older than 18 years, proficient in

112   the Dutch language and able to consent autonomously. Exclusion criteria based on self-report were

113   (a) a history of chronic pain (defined as pain on most days for 3 months or longer), (b) acute pain

114   at the time of testing, (c) use of analgesic medication on the day of testing, (d) use of medication

115   that could alter skin sensitivity or healing, (e) a skin condition that would not allow to tolerate

116   electrode application without damage (e.g., a past operation at the location where the electrodes

117   would be placed), (f) sensation problems, (g) a serious medical condition (e.g., diabetes mellitus,

118   peripheral vascular disease, cardiovascular disease, neurological problems), (h) pregnancy, (i) use

119   of an electronic implant (e.g., a pacemaker) or (j) a previous or current psychiatric diagnosis.

120   Furthermore, participants were excluded mid-procedure if they (1) were unable to discriminate the

121   locations of the vibrotactile stimuli during baseline (<50% correct), and/or (2) reported a pain

122   threshold that was too high or too low for the equipment's technical and safety settings.

123   Written informed consent was obtained from each participant prior to testing, and

124   participation was rewarded with course credit or with the chance of winning a weekend trip worth

125   250,- Euros. The procedures conformed to the Helsinki Declaration and were approved by the

126   institutional ethics committee (Social and Societal Ethics Committee; approval reference number:

127   G-2016 11 663).

128

129   **Stimuli**

130   Electrocutaneous stimuli were used for the unconditioned stimuli. They were delivered by

131   means of two reusable Coulbourn electrodes (4 mm) that were attached 5 mm apart from each

132   other on participants' lower back and were controlled by a constant-current stimulator (DS7,

133   Digitimer Limited, Hertfordshire, UK). Single pulses as well as 3-pulse, 5-pulse and 7-pulse trains

134   were used, with a duration of 0.5 ms each, and an inter-stimulus interval of 5 ms. Using pulse

135   trains allows for the production of more stable stimulus intensities (Mouraux et al. 2013; Mouraux

136   et al. 2014). Due to this effect, a lower electric current was necessary to evoke a painful sensation,

137   which improved the signal-to-noise ratio and reduced the risk of causing burns to the skin (Inui et

138   al. 2002). Stimulus intensities were determined by first determining individual pain thresholds

139   using a 5-pulse train. This was called the pain-threshold intensity. The non-painful stimuli (US_{NP})

140   were single pulse stimuli at the pain-threshold intensity and the painful stimuli (US_P) were 7-pulse

141   trains at the pain-threshold intensity. During the test phase, 3-pulse trains served as at-pain-

142   threshold stimuli (US_{PT}) instead of 5-pulse trains in order to account for sensitization and for

143   perceived control during the calibration phase as participants calibrated the pain threshold intensity

144   themselves by means of clicking on "more" or "less" buttons displayed on the computer screen.

145   The output of this was visible for the experimenter who adjusted the stimulus intensity on the DS7

146   accordingly. All choices about pulse-trains and calibration procedure were based on a pilot study

147   that is described in the section *Procedure*.

148   Vibrotactile stimuli were used as CSs and were delivered by three tactors taped to the skin

149   on the left side of the lower back. One tactor (cephalad/"upper") was placed 40 mm above the

150   electrodes, one (caudad/"lower") was placed 40 mm below the electrodes, and one was attached

151   15 mm lateral to the electrodes (see Fig. 1). The intensity of vibrations was set to a clearly

152   perceptible but non-painful level, which remained the same for all participants, with a duration of

153   17 ms. Either the caudad or cephalad tactor was conditioned to be CS$^+$ by repeatedly being paired

154   with a painful electrocutaneous stimulus (US$_P$), while the other became the CS$^-$ by being paired

155   with a non-painful electrocutaneous stimulus (US$_{NP}$). The onset of the two stimuli

156   (electrocutaneous + vibrotactile) occurred simultaneously at all times. Allocation of tactor to CS$^+$

157   or CS$^-$ was counterbalanced across participants in a randomized order. The lateral tactor was only

158   used for calibration purposes and remained at that (neutral) location during the whole experiment.

159

160   **Measures and Outcomes**

161   **Manipulation Check.**

162   In order to check whether the conditioning procedure was successful, US expectancy

163   ratings were conducted at several time points during the study (see Procedure). Participants were

164   required to answer the questions (i) *"To what extent do you expect the stimulus package to be*

165   *painful (rather than non-painful) if the stimulus package includes the upper vibration?"* and *"To*

166   *what extent do you expect the stimulus package to be painful (rather than non-painful) if the*

167   *stimulus package includes the lower vibration?"* on a numerical rating scale (NRS) ranging from

168   0 = "I do not expect that it will feel painful" to 10 = "I fully expect that it will feel painful".

169

170   **Positive and Negative Affect.**

171   The Positive and Negative Affect Schedule (PANAS; Watson et al. 1988) is a self-report

172   measure assessing positive and negative affect. Participants indicate the extent to which 20

173   different emotional states (e.g. guilty, inspired, restless) are currently applicable to them on a 5-

174   point Likert scale (1 = not at all, 5 = very much). The two subscales have good psychometric

175   properties, including good convergent and discriminant validity (Crawford & Henry 2004). In the

176   present sample, there was a high internal consistency for the positive affect subscale (α = .88).

177   However, that for the negative affect subscale was rather low (α = .60).

178

179   **Participant perceptions.**

180   At the end of the experiment, participants answered a number of questions in order to assess

181   (1) whether they were naïve to the purpose of the experiment (i.e. blinding check), (2) whether

182   they perceived differences between the vibrations of the two CS tactors, (3) how they perceived

183   the timing of the vibrations relative to the electrocutaneous stimuli, (4) whether they noticed a

184   relationship between the vibrotactile CSs and the painfulness of the electrocutaneous USs (i.e.

185   contingency awareness/awareness of the conditioning paradigm), and (5) whether they

186   experienced a direct association between their adaptation of the stimulus intensity and the actual

187   intensity during the calibration phases.

188

189   **Outcome.**

190   The primary outcome of this study was the percentage of trials during test phase in which

191   at-pain-threshold electrocutaneous stimuli (US_{PT} at test phase) were experienced as painful, when

192   the CS^+ was active, as compared to when the CS^- was active. For that purpose, participants

193   indicated whether or not each 'stimulus package', i.e. the combination of either vibration with the

194   electrocutaneous stimulation, was painful or non-painful and rated the intensity of the stimuli on

195   the Sensation and Pain Rating Scale (SPARS, initially called the FESTNRS; Madden et al. 2016b)

196   within a maximum of 15 seconds after stimulus onset. The SPARS captures both painful and non-

197   painful experiences as it runs from -50 = "no sensation", through 0 = pain threshold ("the exact

198   point at which you feel transition to pain") to +50 = "most intense pain you can imagine".

199   Participant ratings on the SPARS show a curvilinear relationship to stimulus intensity, with a

200   slightly steeper slope at the extremes of the scale and stable stimulus-response characteristics

201   across the range of the scale (Madden et al. April, 2018). A visual scale showing the range and the

202   anchors was presented on a computer screen on which participants could select any number within

203   this range. Furthermore, participants reported which tactor they felt (upper vs. lower) during each

204   trial by clicking one of two buttons.

205

206   **Procedure**

207   The procedure of this study was adapted from Madden et al. (2016a). The study has a

208   within-subject design and consists of four main phases (see also Fig. 2). The protocol has been

209   locked on the online database Augias from KU Leuven from which we did not deviate.

210   **Pilot study.**

211   A pilot study with 17 participants was conducted in order to test and optimize the methods

212   and procedures to be used in the full-scale experiment. The general procedure did not have to be

213   changed and can be found below. However, the ideal number of pulse trains for delivering non-

214   painful, at-pain-threshold and painful stimuli had to be tested resulting in a 1-4-7 grading, which

215   provided easily differentiable intensities.

216   Importantly, pain thresholds proved very unstable, with both habituation and sensitization

217   occurring throughout the experiment. In order to counteract this, recalibration phases were

218   included between acquisition and test blocks. Regarding sensitization, participants were asked to

219   (re-)calibrate to -5 instead of 0 so as to ensure that the stimulus would be closer to just non-painful

220   rather than to just painful. Additionally, it was decided to use a 3-pulse train as the at-pain-

221   threshold stimulus (US_{PT}) in the test phase because the 5-pulse train which had been calibrated to

222   -5 was consistently rated as painful during testing. We also tried to minimize context changes

223   between (re-)calibration phases and test phases. However, given the frequent (re-) calibrations, a

224   fast and precise calibration procedure was needed. The pilot study showed that self-adjustment

225   through the participant by means of clicking "more" and "less" buttons displayed on a computer

226   screen was more efficient than the commonly used adaptive staircase procedure (e.g. Madden et

227   al. 2016a).

228   A final aspect that was addressed in the pilot study was the intensity and distance between

229   the tactors. The distance between the tactors was increased from 70 mm (Madden et al. 2016a) to

230   80 mm and the vibration intensity was calibrated to a level that would permit good

231   distinguishability.

232   **Preparation.**

233   Prior to testing, participants received written information about the study without details

234   about the study goals and exact procedures so as to ensure blinding. Participants were screened for

235   exclusion criteria via e-mail. Upon arrival at the lab, participants once more received general study

236   information. They had the opportunity to ask questions and were instructed to carefully read and

237   sign the informed consent form and an exclusion criteria form afterwards. Participants were seated

238   in front of a computer, straddling a chair. Before the electrodes were attached on the left side of

239   the lower back, participants crossed their arms over their chest, looked upwards, and bent

240   backwards. The line of greatest curvature or 'hinge' was identified. Moving along this line, the

241   two electrodes were taped to the skin 40 mm left of the spine once the participant had resumed a

242   comfortable resting posture. The tactors were placed 40 mm above, 40 mm below and 15 mm to

243   the left of the electrodes.

244   **Calibration phase.**

245   In this phase, participants' pain thresholds for the electrocutaneous stimulus (5-pulse train)

246   were calibrated and participants could practice reporting on the SPARS. First, they received a 0.1

247   mA stimulus paired with the lateral tactor as a neutral vibrotactile stimulus and were asked to rate

248   their experience. Next, they were instructed to adjust the stimulus intensity to match their personal

249   ratings of -30, -10, +10, +30 and finally 0 (threshold) by clicking on "more" or "less" buttons

250   displayed on the computer screen. All electrocutaneous stimuli in the calibration phase were

251   delivered simultaneously with the lateral tactor. Next, participants received and rated six electrode-

252   tactor combinations with intensities between those previously determined as -30 and +30, and

253   again adjusted the intensity to their threshold and finally to -5. This last intensity served as the pain

254   threshold so as to ensure that it was closer to just non-painful rather than to just painful (to account

255   for anticipated perceptual sensitization) and was subsequently used for all single pulses and pulse

256   trains.

257   **Baseline phase.**

258   Before the conditioning procedure, baseline ratings of the CSs were obtained. Participants

259   received ten vibration-only trials, i.e. the upper and lower tactor were activated five times each,

260   rated each trial on the SPARS and indicated which tactor was activated. Participants who were

261   unable to discriminate the tactor locations (<50% correct) were excluded at this point in the

262   procedure. Subsequently, participants provided expectancy ratings.

263   **Acquisition phase**.

264   During the acquisition phase, participants received twelve painful electrocutaneous stimuli

265   (7-pulse trains – US_P) paired with the tactor that was assigned to become the CS^+. Similarly, the

266   other tactor (CS^-) was paired with the non-painful electrocutaneous stimulus (single pulses –

267   US_{NP}). The trials were delivered in three blocks of eight trials each and were pseudo-randomly

268   ordered with no more than two identical trials delivered successively. The reinforcement

269   contingency was set to 100%. Participants were instructed to rate each stimulus package on the

270   SPARS. After each block, participants were asked to give expectancy ratings and to readjust the

271   intensity of the 5-pulse trains (in combination with the lateral tactor) to -5 in order to counteract

272   habituation and sensitization. These newly determined at-pain-threshold intensities were then used

273   as stimulus intensity in the subsequent block.

274   **Test phase.**

275   In total, the test phase consisted of three blocks of 36 trials each (108 trials in total) with

276   the following stimulus packages being delivered: 15 CS^+/US_{PT} trials and 15 CS^-/US_{PT} trials as

277   main outcomes, 30 CS^+/US_P trials and 30 CS^-/US_{NP} trials as reinforcement in order to prevent

278   extinction, and 9 CS^+-only trials and 9 CS^--only trials. These stimulus packages were delivered in

279   random order with no more than two identical trials being delivered successively. After each trial

280   participants provided intensity ratings on the SPARS and indicated the tactor location. Again, they

281   gave expectancy ratings and readjusted the intensity of the 5-pulse trains to -5 between blocks.

282   Furthermore, after the final test block, participants were instructed to adjust the intensity of the 5-

283   pulse train to their pain threshold (0 on SPARS) two times, one time in combination with the upper

284   tactor and the other time with the lower tactor. Whether recalibration was done with the upper or

285   the lower tactor first was counterbalanced across participants.

286   Finally, participants filled in the PANAS and the five questions about their perceptions of

287   the study. After this, the electrodes and vibrotactors were removed. Participants were debriefed,

288   assigned their course credit and thanked for their participation.

289

290   **Statistical Analysis**

291   Data were analysed using SPSS 25.0 (IBM Corporation, 2017). Plots were generated in R

292   v3.4.3 (The R Foundation for Statistical Computing), in RStudio v1.1.414 (RStudio Inc). The

293   following packages were used: readr (Wickham et al. 2017), magrittr (Bache & Wickham 2014),

294   tidyverse (Wickham 2017), and ggplot2 (Wickham 2009).

295   Prior to analysis, data were checked for normality, sphericity, outliers and missing values.

296   The only missing values were found for the indication of tactor localisation. These were omitted

297   for the analyses on tactor localisation mistakes. When appropriate, non-parametric tests were

298   applied. Follow-up pairwise comparisons with Bonferroni adjustment were applied to ANOVAs

299   with significant results.

300   To rule out any baseline differences in vibration intensities of the two tactors, a Wilcoxon

301   signed rank test was conducted on baseline intensity ratings, with Condition (CS^+ vs. CS^-) as

302   within-subject factor. To test whether manipulations were successful, expectancy ratings as well

303   as intensity ratings were analysed by means of two separate 2 (Condition: CS^+ vs. CS^-) x 3 (Phase:

304   baseline, acquisition, test) repeated measures (RM) ANOVAs. Additionally, blinding for the study

305   goal, contingency awareness for the CS-US pairings, perceived control over the stimulus

306   calibrations as well as quality of the vibrotactile stimuli and simultaneity of the vibrotactile and

307   electrocutaneous stimuli were checked.

308   Subsequently, in order to test the main hypothesis, a binary variable reflecting non-painful

309   (≤ 0) and painful (> 0) at-pain-threshold trials was computed, based on which a second variable

310   reflecting the percentage of US_{PT} trials rated as painful was created. Next, the percentage of US_{PT}

311   trials rated as painful was compared across the two conditions, i.e. between the 15 CS^+/US_{PT} and

312   15 CS^-/US_{PT} stimulus packages, by means of a Wilcoxon signed rank test - this was the primary

313   analysis. As secondary analyses, a 2 (Condition: CS^+/US_{PT} vs. CS^-/US_{PT}) x 3 (Test Block: 1, 2, 3)

314   RM ANOVA was conducted to examine time effects, and a Wilcoxon signed rank test was

315   conducted to compare the intensity ratings for the CS^+/US_{PT} and CS^-/US_{PT} trials. As additional

316   measures of shifts in perception, two Wilcoxon signed rank tests were used to (1) compare the

317   intensity ratings for the CS^+-only and CS^--only trials and (2) compare the recalibrated at-pain-

318   threshold intensities (from the final re-calibration phase) when paired with the CS^+ compared to

319   CS^-. For the latter analysis, the recalibrated intensities for the CS^+ and CS^- were divided by the

320   respective group mean recalibrated intensities. In order to control for mistakes on the tactor

321   localisation task, these analyses were repeated with perceived rather than actual tactor location,

322   i.e. trials on which the CS^+ location was wrongly perceived as the CS^- location were considered

323   CS^- trials in the analyses, and vice versa. Lastly, the relationship between the primary outcome and

324   negative affect (PANAS), contingency awareness, and expectancy, were explored by means of

325   analyses of covariance (ANCOVAs), with total scores of the positive and negative subscales of

326   the PANAS, the binary variable contingency awareness (yes/no) and the expectancy ratings given

327   for the upper and lower tactor during test phase.

328

329   **Results**

330   Fifty-one participants (7 males) aged 18 to 31 years (M = 19.39, SD = 2.55) were included.

331   Two participants were excluded mid-procedure for having no clear non-painful range and

332   perceiving almost all of the stimuli as painful. Four other participants were excluded due to

333   technical problems that may have rendered their data unreliable. None were excluded dor inability

334   to discriminate between the vibrotactile stimuli. Consequently, the analyses were conducted on the

335   final sample of 45 participants (6 males). Participant characteristics are described in Table 1. The

336   mean intensity of at-pain-threshold stimuli at the initial calibration was 3.18 mA (SD = 1.945).

337

338   **Table 1.**

339

340   **Blinding and Contingency Awareness**

341   At the completion of the experiment, 40/45 participants were unaware of the goal of the

342   study and 17 were unaware of the contingency, i.e. either they reported to have noticed no

343   correlation at all between the location of the vibration and whether the electrocutaneous stimulus

344   was painful or not, or they identified the CS⁻ to be associated with the painful stimuli. Fourteen

345   participants experienced differences between the vibrations from the upper and the lower tactor,

346   ten of whom perceived the CS⁺ as "stronger", "sharper", and "longer" and four of whom did not

347   specify the perceived difference. Four participants indicated that the US and CS did not have a

348   simultaneous onset but that they perceived either the vibrotactors (n = 3) or the electrocutaneous

349   stimulus (n = 1) first. Lastly, 29 participants reported that they experienced control over the

350   calibration whereas 16 reported that they did not.

351

352   **Manipulation Checks**

353   There was initially no significant difference between the intensity ratings for the two

354   vibrotactors at baseline (Wilcoxon signed-rank test: Z = -1.084; p = .278; CS⁺ = -32.22; CS⁻ = -

355   33.52). Similarly, expectancy ratings at the end of baseline did not differ significantly between the

356   two vibrotactors (Wilcoxon signed-rank test: Z = -1.302; p = .193; CS⁺ = 1.93; CS⁻ = -1.51).

357   Expectancy ratings for CS⁺ and CS⁻ across the three phases are depicted in Fig. 3.

358   Across acquisition and test phase, CS^+/US_P trials were rated as more intense than CS^-/US_{NP}

359   trials (main effect of Condition, $F(1,44)$ = 225.148; $p < .001$; η_p^2 = .837; 95% CI = [23.062;

360   30.218]; CS^+/US_P: $M = 9.630$, $SE = 1.024$; CS^-/US_{NP}: $M = -17.010$, $SE = 1.434$) and this increase

361   in intensity ratings was greater for CS^+/US_P trials than for CS^-/US_{NP} trials (Phase x Condition

362   interaction, $F(1,44) = 11.901$, $p = .001$; $\eta_p^2 = .213$). Follow-up pairwise comparisons indicated that

363   intensity ratings for CS^+/US_P trials and CS^-/US_{NP} trials increased from acquisition phase to test

364   phase ($p < .001$).

365   In order to check whether the classical conditioning procedure was successful, a 2

366   (Condition: CS^+ vs CS^-) x 3 (Phase: baseline, acquisition, test) RM ANOVA was performed on

367   expectancy ratings. This analysis revealed a significant Condition x Phase interaction ($F(2,43) =$

368   14.927, $p < .001$; $\eta_p^2 = .410$): follow-up pairwise comparisons revealed that expectancy ratings for

369   both CS^+ and CS^- increased significantly from post-baseline to post-acquisition as well as from

370   post-acquisition to post-test (both $p < .001$). Finally, participants who were contingency aware

371   showed a greater increase over time in the difference between expectancy ratings for CS^+ and CS^-

372   (see Fig. 3; Condition x Phase x Contingency Awareness interaction, $F(2,42) = 9.372$, $p < .001$;

373      $\eta_p^2 = .308$).

374

375   **Main Hypothesis: Effects on Intensity Ratings of Pain Threshold Stimuli**

376   Pain intensity ratings for CS^+/US_P and CS^-/US_{NP} trials, for CS^+/US_{PT} and CS^-/US_{PT} trials

377   as well as for CS^+ only and CS^- only trials are presented in Table 2. The test of the current study's

378   main hypothesis showed that participants rated more US_{PT} trials as painful when they were paired

379   with the CS^+ (81.5%, $SD = 21.03$) than when they were paired with the CS^- (72.7%, $SD = 24.69$;

380   Wilcoxon signed rank test: $Z = -3.033$ $p = .002$). In absolute numbers, 12.22 ($SD = 3.154$) out of

381   15 CS^+/US_{PT} trials were rated as painful compared to 10.91 ($SD = 3.704$) of the 15 CS^-/US_{PT} trials.

382   This difference was still apparent when tactor localisation mistakes were controlled for (83.05%

383 [SD = 19.22] of CS$^+$/US$_{PT}$ trials vs 70.24% [SD = 26.29] of CS$^-$/US$_{PT}$ trials rated as painful, Z = -

384 3.768; p < .001). The examination of time effects showed that participants rated more US$_{PT}$ trials

385 as painful when they were paired with the CS+ than when they were paired with the CS- across all

386 three blocks of the test phase (main effect of Condition, $F(1,44)$ = 10.201; p = .003; η_p^2 = .188),

387 and that this difference decreased over the three blocks of the test phase (main effect of Block

388 ($F(2,43)$ = 3.360; p = .044; η_p^2 = .135; no Block x Condition interaction, $F(2,43)$ = .332; p = .719).

389

390 **Table 2.**

391

392 **Secondary Analyses: Effects on Intensity Ratings**

393   Participants rated US$_{PT}$ trials as significantly more painful when the US$_{PT}$ was paired with

394 the CS$^+$ (M = 6.585, SD = 5.128) than when the US$_{PT}$ was paired with the CS$^-$ (M = 4.113, SD =

395 4.989; Wilcoxon signed rank test: Z = -3.302; p = .001). This difference was still apparent when

396 tactor localisation mistakes were controlled for (Z = -3.515; p < .001; $M_{CS+/UST}$ = 6.881, SD =

397 4.556; $M_{CS-/UST}$ = 3.779, SD = 5.703).

398   Exact mean intensity ratings for CS$^+$/US$_P$ and CS$^-$/US$_{NP}$ trials during acquisition and test

399 phase are shown in Fig. 4. Overall, participants rated stimulus intensities higher in the test phase

400 than in the acquisition phase. When examining the exact mean intensity ratings, a 2 (Condition:

401 CS$^+$/US$_P$ vs CS$^-$/US$_{NP}$) x 2 (Phase: acquisition, test) repeated measures ANOVA showed that there

402 was a significant Condition x Phase interaction effect ($F(1,44)$ = 11.901; p = .001; η_p^2 = .213).

403 Pairwise comparisons revealed that intensity ratings differed significantly between acquisition and

404 test phase ($M_{acquisition}$ = -6.120, SE = 1.159; M_{test} = -1.259, SE = .874; p < .001) as well as between

405   the vibrotactors (M_{CS+} = 9.630, SE = 1.024; M_{CS-} = -17.01, SE = 1.434; p < .001). None of these

406   results changed notably when contingency awareness was added to the analysis as a covariate.

407

408   **Exploratory Analyses**

409   Intensity ratings for the CS⁺-only and CS⁻-only trials did not differ between the two

410   conditions (Wilcoxon signed rank test: Z = -1.155; p = .248). This did not change when accounting

411   for tactor localisation mistakes on the CS-only trials (Z = -1.307; p = .191). Recalibrated stimulus

412   intensities, i.e. for the 5-pulse train electrocutaneous stimulus paired with the lateral vibrotactor,

413   increased significantly from first (calibration phase) to last (before final test block) calibration

414   moment (Z = -5.842; p < .001), with a mean intensity of 3.18 mA (SD = 1.945) at first calibration

415   and 6.39 mA (SD = 4.716) at the last recalibration. However,, final recalibrations for CS⁺ and CS⁻

416   did not differ (Z = -.035; p = .972), with a mean intensity of 7.82 mA (SD = 5.045) for the CS⁺ and

417   7.69 mA (SD = 5.014) for the CS⁻.

418   The ANCOVAs revealed no effects of negative affect, contingency awareness, or

419   expectancy ratings on the percentage of CS⁺ and CS⁻ trials rated as painful, on the intensity ratings

420   of the CS⁺-only and CS⁻-only trials, or on the final recalibrations with CS⁺ and CS⁻ (all p-values >

421   .05).

422

423   **Discussion**

424   The aim of the present study was to investigate whether simultaneous classical conditioning

425   can modulate pain reports of at-pain-threshold stimuli. This knowledge may inform whether

426   classical conditioning could be one of the mechanisms underlying the development or maintenance

427   of chronic pain. It was expected that, after an acquisition phase in which non-painful vibrotactile

428   stimuli (CSs) were repeatedly paired with either painful or non-painful electrocutaneous stimuli,

429   US_{PT}'s would be experienced as painful on more of the CS^+ trials than the CS^- trials, confirming

430   that pain threshold ratings can be modulated through classical conditioning.

431   The results confirmed our hypothesis: at-pain-threshold electrocutaneous stimuli evoked a

432   conditioned response, i.e. they were 6% more likely to be reported as painful and were rated on

433   average as more painful, if they were presented with CS^+ vibrotactile stimuli than if they were

434   presented with the CS^- vibrotactile stimuli. Besides, the exact pain ratings for CS^+/US_{PT} trials were

435   significantly higher than those for CS^-/US_{PT} packages. Notably, the majority of CS^-/US_{PT} trials

436   were also perceived as painful. The calibration and classical conditioning procedures were

437   successful, as indicated by significant increases in intensity ratings across all three phases as well

438   as in pain expectancies from post-baseline to post-acquisition for the CS^+ but not the CS^-, even

439   though many participants were not explicitly contingency aware. Neither the CS-only trials nor

440   the final recalibrations were significantly affected by classical conditioning. Recalibrated pain

441   threshold intensities increased throughout the experiment, indicating habituation.

442   The present findings are largely in line with the study by Madden et al. (2016a) and the

443   study by Williams & Rhudy (2007), in that exposure to non-nociceptive vibrotactile stimuli was

444   found to influence pain thresholds through classical conditioning. The results specifically parallel

445   those by Madden et al. (2016a), as even the difference between intensity ratings for CS^+/US_{PT} and

446   CS^-/US_{PT} trials was roughly the same in both studies, while CS-only trials were unaffected by the

447   conditioning procedure. Interestingly, despite the inclusion of re-calibration procedures, a higher

448   percentage of at-pain-threshold trials, including 72% of CS^-/US_{PT} trials, was perceived as painful

449   in this study than in the study by Madden et al. (2016a). Nevertheless, by finding comparable

450   results while using different nociceptive stimuli (electrocutaneous vs. laser), this replication clearly

451   strengthens the notion that classical conditioning can modulate judgements about whether an event

452   is painful or not. Furthermore, both studies used a simultaneous conditioning paradigm in which

453   CS and US were presented at the same time. This approach excludes expectation or changes in

454   arousal – to which shifts in pain thresholds have been attributed in other classical conditioning

455   studies (Harvie et al. 2016a; Miguez et al. 2014) – as the mechanism of effect. Indeed, the

456   simultaneous approach seems well suited to model chronic pain as the real-world CS (e.g. tactile

457   cue) and US (nociceptive signalling) are likely to occur at the same time (Madden et al. 2016b),

458   thus enhancing the validity of our findings. Finally, as half of our participants were not contingency

459   aware, the present study also supports the claims by Harvie et al. (Harvie et al. 2016a) and Jensen

460   et al. (Jensen et al. 2015) that expectations or conscious awareness of the learned association are

461   not required for classically conditioned shifts in pain thresholds to occur.

462   Importantly, our findings contradict those by Madden et al. (2016b), who did not find an

463   effect of classical conditioning on pain thresholds. One difference between the two studies is the

464   type of US, namely electrocutaneous versus thermal stimuli. We specifically chose to use a

465   different nociceptive stimulus than both studies by Madden and colleagues (Madden et al. 2016a;

466   Madden et al. 2016b), not only in order to investigate whether the previous findings extend to other

467   stimulus modalities, but also to help interpret their contradictory findings. The electrocutaneous

468   stimulus in the present study is considered to be less discrete than laser stimuli but more discrete

469   than contact heat stimuli (Plaghki & Mouraux 2003). Previous investigations of conditioned fear

470   of touch confirm that the spatial precision of somatosensory cues influences the likelihood of

471   associative learning (Harvie et al. 2016b) and it seems possible that a similar mechanism is relevant

472   here. The three stimulus types have different characteristics: laser stimuli isolate nociceptors

473   (Mouraux & Iannetti 2009) and therefore lack ecological validity, whereas the other two modalities

474 stimulate both nociceptive and non-nociceptive mechanisms; thermal stimuli are more

475 physiological than electrocutaneous stimuli, which activate mechanoreceptors as well as

476 nociceptors but via their axons as well as receptors and nerve endings (Handwerker & Kobal 1993),

477 but chronic pain seldom develops after repeated exposure to noxious heat stimuli. This difference

478 in discreteness between different types of nociceptive stimulation may also be a possible

479 explanation for the diverging results given by Madden et al. (2016b). One path forward that might

480 overcome these limitations is to use proprioceptive stimuli (related to movement) as the

481 conditioning stimuli (Meulders et al. 2011).

482   The present study has several limitations that require attention. First of all, participants

483 were frequently unsure about their indication of tactor location and many reported difficulties at

484 the end of the experiment. In these cases, some tended to base their choice of tactor location on

485 whether the accompanying electrocutaneous stimulus was painful or not, which resulted in

486 location-mistakes on 12.19% of all trials, on 15.85% of all CS^+/US_{PT} trials and even 27.11% of all

487 CS^-/US_{PT} trials and which might indicate a reversed form of conditioning. That is, the simultaneous

488 presentation of vibrotactile and electrocutaneous stimuli meant that each contained information

489 about the occurrence of the other. Consequently, the electrocutaneous stimuli may have served as

490 CS (providing information about the tactor) on some trials. A possible explanation for this error

491 rate might be the close proximity of the tactors. We tried to improve discriminability between

492 tactors by increasing the distance between them from 70 mm (as in the study by Madden et al.

493 2016a) to 80 mm, which led to fewer mistakes than in the pilot study, yet the problem still occurred

494 occasionally. A second point of criticism is that the vibrotactors made an audible sound that could

495 have guided participants' attention to the tactor location. Nevertheless, we decided not to use white

496 noise to mask it because of practical reasons: the calibration procedure was not compatible with

497 continuous white noise delivered via headphones, and switching off the white noise during

498 calibration would have formed an obvious change of context that could have interfered with

499 conditioning. Moreover, white noise would have caused additional exhaustion of the participants.

500 However, as mentioned previously, participants did not seem to be able to use the emitted sounds

501 to locate the vibrotactors, as mistakes were made nonetheless. Another limitation is that not only

502 most of the CS^+/US_{PT} trials but also the majority of CS^-/US_{PT} trials were perceived as painful. As

503 we consider the pain threshold in itself to be arbitrary (i.e., either just painful or just not painful),

504 we would expect not more than 50% of CS^-/US_{PT} trials to be rated as painful. The elevated

505 percentage of painful CS^-/US_{PT} trials may indicate a suboptimal calibration of the electrocutaneous

506 stimulus or sensitization throughout the experiment. Yet, this should not have affected the overall

507 results of our study.

508 The present findings bear important clinical implications: it is well established that classical

509 conditioning can drive pain expectancies and pain-related fear, and while such an effect has long

510 been suggested for pain itself the scientific basis for this claim has been lacking. By showing that

511 it is possible to modulate pain threshold ratings through classical conditioning in the laboratory

512 independent of the other pain-related constructs mentioned previously, it becomes more plausible

513 that similar mechanisms occur in chronic pain. Specifically, in the acute pain phase patients may

514 learn to associate the nociceptive input with other concurrent somatosensory stimuli implicitly or

515 explicitly, such that borderline painful stimulation becomes more likely to be perceived as painful

516 thus promoting maladaptive behaviours, cognitions and, eventually, chronicity.

517 Future studies to replicate and extend our findings about classical conditioning of pain

518 would be useful, as studies on this matter are still rare. Our sample was highly homogenous as it

519 consisted of healthy students, primarily females around 20 years of age. In order to draw any firm

520   conclusions about classical conditioning of chronic pain, replication in other populations that

521   resemble demographics of individuals suffering from chronic pain more closely is necessary.

522   Additionally, the present study focused on discrete locations of stimulation, but it might be

523   valuable to investigate whether the conditioned shift in report of pain thresholds does also

524   generalize to proximal or distal areas, in order to better understand the spreading of pain.

525   Furthermore, as mentioned previously, it would be interesting to investigate whether the

526   discreteness of the US affects pain conditioning by directly comparing the different types of

527   nociceptive stimulation. Lastly, future studies may include psychophysiological measures in

528   addition to self-reports of pain to get a more complete picture of the effects of pairing noxious and

529   non-noxious stimuli on pain perception and other pain-related outcomes.

530

531   **Conclusion**

532   This study provides evidence that ratings of at-pain-threshold electrocutaneous stimuli can

533   be modified through classical conditioning. Our results, therefore, advance the debate about

534   whether or not pain itself can be classically conditioned and, although the study was conducted in

535   healthy participants, it contributes to our understanding of the development and maintenance of

536   chronic pain. From a scientific perspective, classical conditioning may pose a useful method for

537   modelling chronic pain in healthy participants. Furthermore, against the backdrop of broad

538   scientific knowledge about classical conditioning in other psychopathologies (Breivik 2016), the

539   present findings suggest that this line of research bears great potential to improve interventions

540   and preventive measures against chronic pain in the future.

541

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544   18-19 September 2017, for which JT received the "best presentation" prize. The authors would

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646

Figure 1

Set-up of vibrotactors and the electrodes on the back (adapted with permission from Madden et al. 2016a).

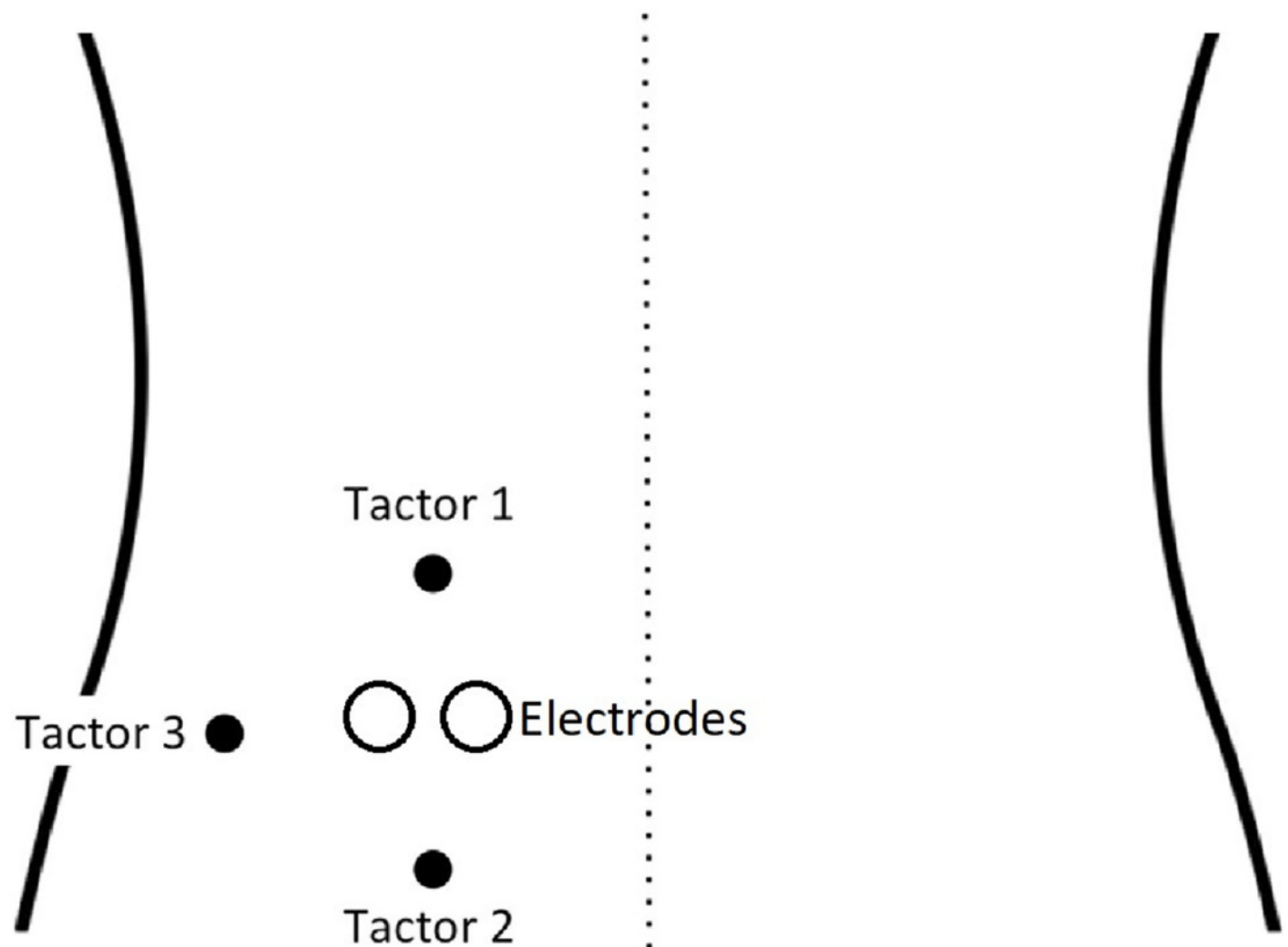



Figure 2

The four phases of the experimental procedure.

Dots indicate vibrotactile stimuli, dark dots show which vibrotactor was activated for each trial type. Circles indicate the field on which the two electrodes were placed (adapted with permission from Madden et al. 2016a).

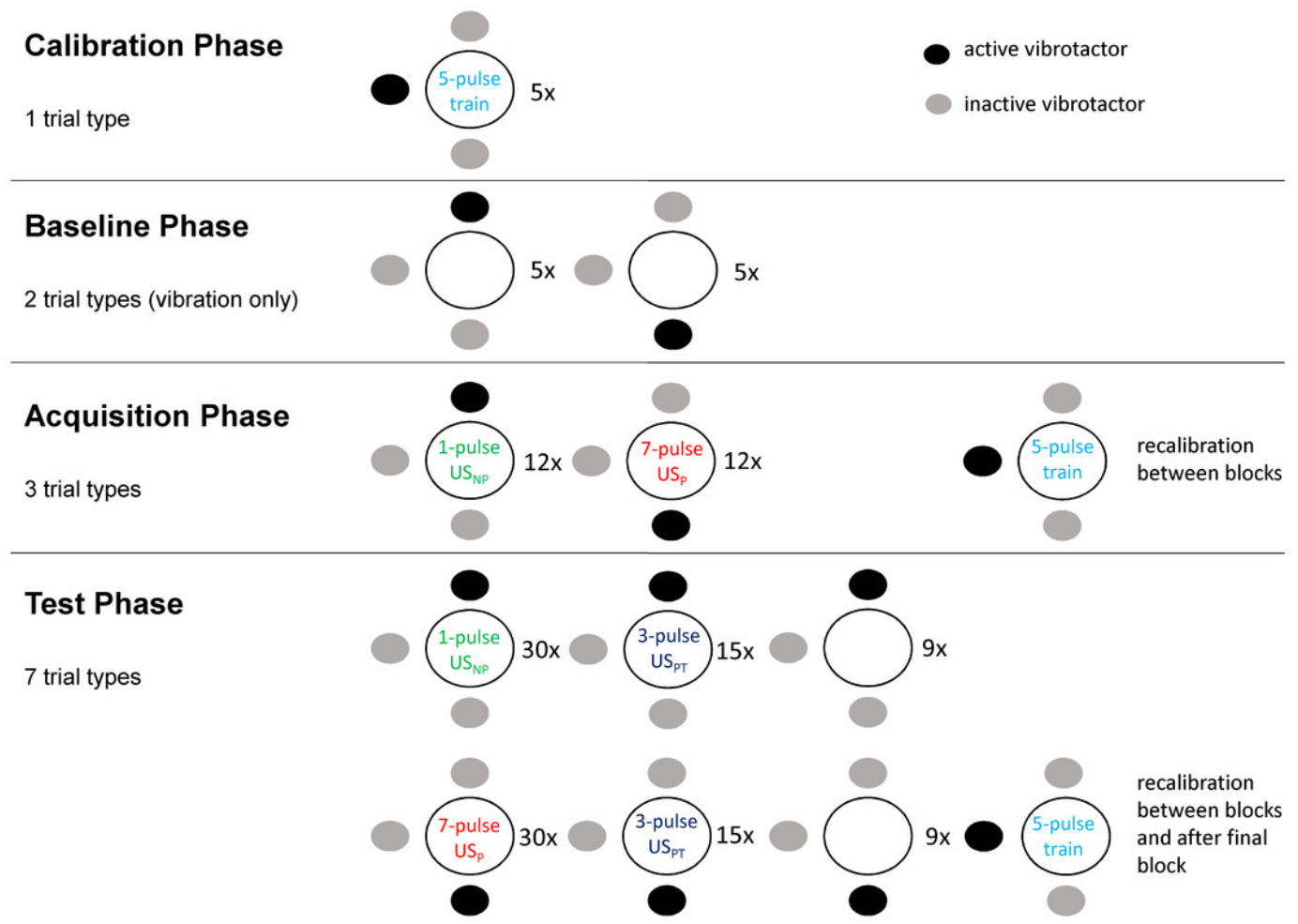



Figure 3

Scatter boxplot of expectancy ratings by CS type across baseline, acquisition and test phases (mean, standard error), panelled by contingency awareness.

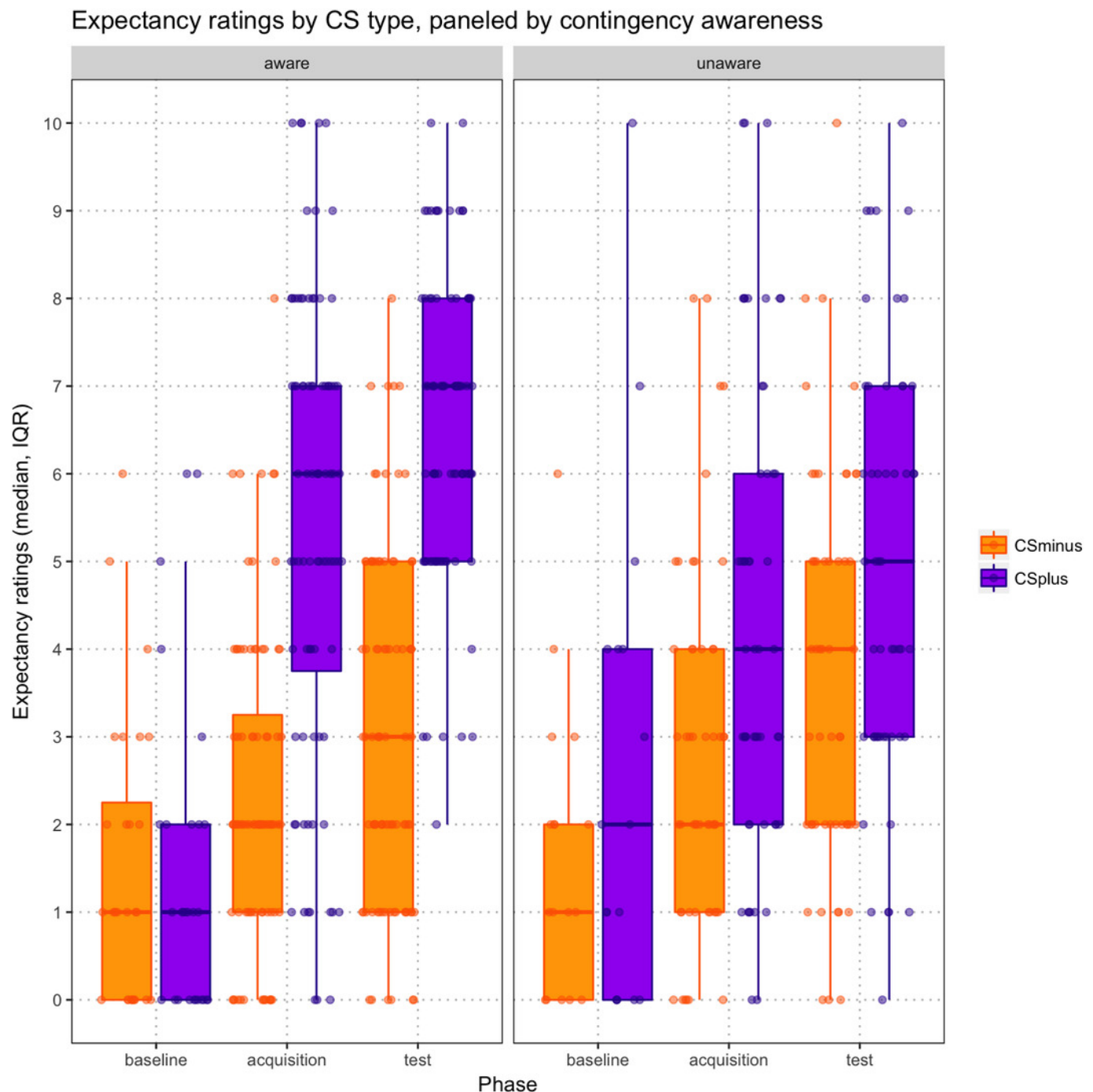
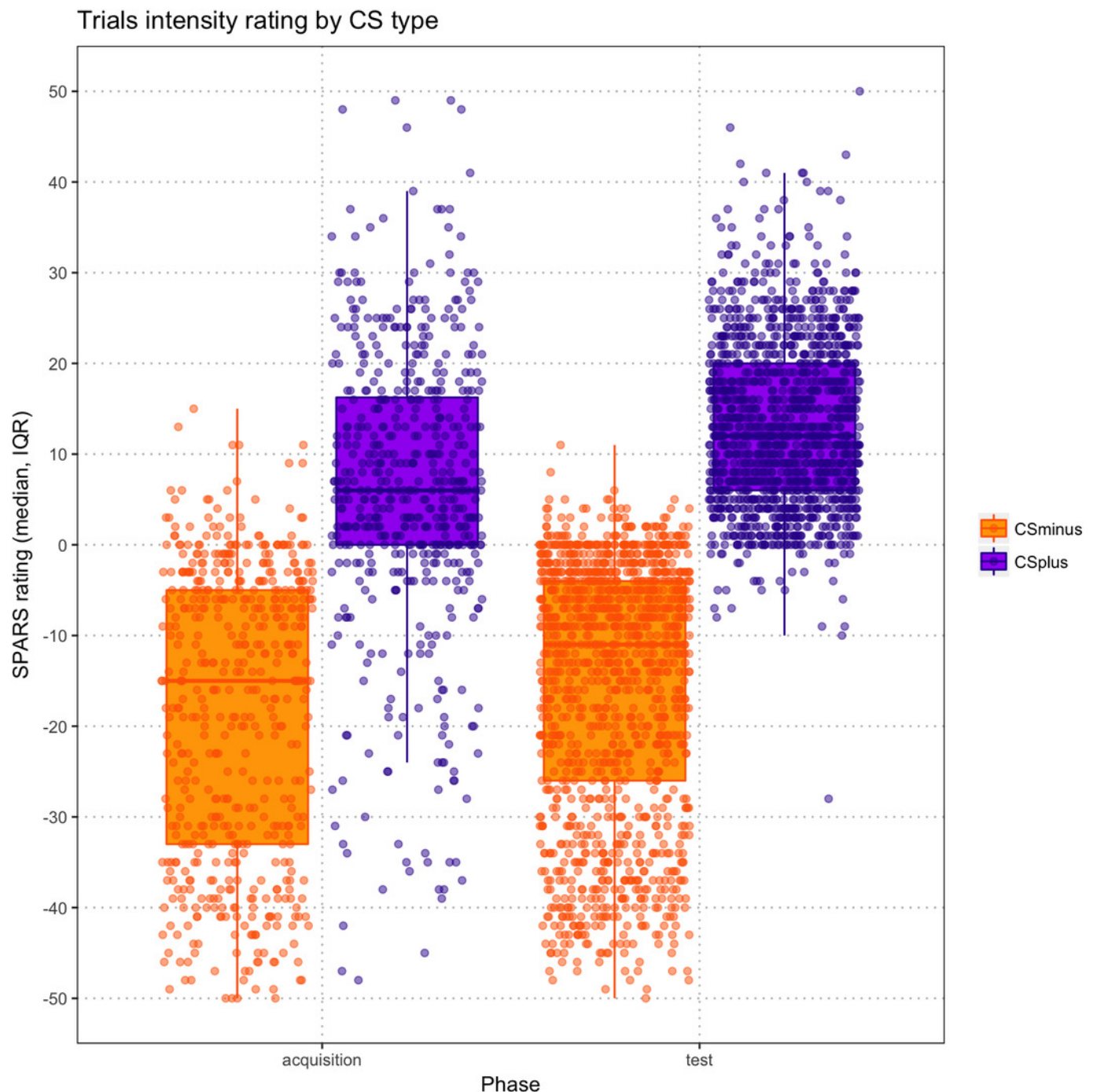



Figure 4

Scatter boxplots of exact mean intensity ratings (error bars = standard error) by CS type during acquisition and test phase.

Participant characteristics (n = 45).

[1]Clinical meaning of PANAS scores based on Crawford and Henry (2004).

Outcome	Mean (SD)	Range
Age	19.44 (2.668)	18-31
Positive state affect (PANAS)	26.69 (6.657)	14-39 (normal range)[1]
Negative state affect (PANAS)	13.36 (2.647)	10-19 (normal range)[1]

Mean stimulus intensity ratings (SD) for the three phases.

CS = conditioned stimulus; US_P = painful unconditioned stimulus; US_{NP} = non-painful unconditioned stimulus; US_{PT} = at-threshold unconditioned stimulus.

	Baseline phase	Acquisition phase	Test phase
CS+/US$_P$		3.722 (1.862)	12.213 (1.562)
CS-/US$_{NP}$		-19.181 (2.859)	-13.528 (2.376)
CS+/US$_{PT}$			6.174 (5.109)
CS-/US$_{PT}$			3.596 (3.714)
CS+ only	-32.811 (3.150)		-24.821 (15.464)
CS- only	-35.489 (2.822)		-27.222 (12.992)