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Conceptual Conditioning: Mechanisms Mediating Conditioning Effects on Pain

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Abstract

Classical conditioning can profoundly modify subsequent pain responses, but the mechanisms that drive this effect are unresolved. In pain-conditioning studies, cues are typically conditioned to primary aversive reinforcers; hence, subsequent pain modulation could reflect learned precognitive associations (i.e., those involving neural plasticity independent of expectations and other forms of conceptual thought) or conceptual expectancies. We isolated conceptual contributions using a thermal pain-conditioning procedure in which different conditioned stimulus (CS) cues were repeatedly paired with *symbolic representations* of high and low noxious heat. In a subsequent test phase, identical noxious stimuli evoked larger skin conductance responses (SCRs) and pain ratings when preceded by CS cues associated with high temperature than by those associated with low temperature. These effects were mediated by participants' self-reported expectancies. CS cues associated with high temperature also evoked larger anticipatory SCRs than did CS cues associated with low temperature, but larger anticipatory SCRs predicted smaller subsequent heat-evoked SCRs. These results provide novel evidence that conditioned modulation of pain physiology can be acquired through purely conceptual processes, and that self-reported expectancies and physiological threat responses have opposing effects on pain.

Keywords

classical conditioning, pain modulation, expectancy, skin conductance response, multilevel mediation analysis

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People's prior experiences strongly affect how they perceive and respond to stimuli and events (e.g., Greene, Botros, Beck, & Fei-Fei, 2015). Among the many varieties of percepts and judgments influenced by past experience, effects on pain have particularly important clinical and translational implications. Predicting and avoiding pain shapes our behavior in profound ways, and pain is the major reason people seek medical care. Ratings of pain experience are a primary outcome in clinical trials, and they form the basis for medical diagnosis and treatment decisions (Dworkin et al., 2003; Levy, 1996).

One of the most powerful ways to influence pain is classical conditioning. A vast literature on conditioning reports the induction of learning in animals by pairing a cue (the conditioned stimulus, or CS) with a primary aversive or appetitive outcome (e.g., shocks or food, respectively; this is the unconditioned stimulus, or US). Whereas in many studies the researchers are interested primarily in behavioral and physiological responses to the cues themselves, pain-conditioning studies have demonstrated robust effects of cues on responses to noxious stimuli in both animals and humans (for reviews, see Atlas & Wager, 2013; Fanselow, 1986). However, the nature of the learning that underlies pain modulation—and specifically the degree to which conceptual thoughts and expectations are involved—is not well understood.

Both responses to cues and the effects of cues on subsequent pain are often assumed to reflect learning that is *precognitive* (i.e., learning that involves plasticity in neural circuits independent of expectations and other forms of conceptual thought, and not open to introspection). Findings consistent with this idea have shown that some types of conditioned autonomic and behavioral responses,

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including analgesia, can be acquired in decerebrate animals and humans (Berntson & Micco, 1976; Berntson, Tuber, Ronca, & Bachman, 1983) or in the isolated spinal cord (Grau, Salinas, Illich, & Meagher, 1990). Such findings suggest that such responses rely on associative learning in subcortical systems. In addition, conditioned responses are often mediated by synaptic plasticity in specific pathways and are robustly produced in invertebrates (Antonov, Antonova, Kandel, & Hawkins, 2001; Glanzman, 1995; Hawkins, Abrams, Carew, & Kandel, 1983; Johansen, Cain, Ostroff, & LeDoux, 2011).

Human pain modulation also appears to be highly dependent on the pairing of cues and reinforcers, which suggests that precognitive learning may be critical. The paradigm that produces the most reliable effects on human pain involves two phases: In the *learning* phase, different cues are repeatedly reinforced with either high-pain or low-pain stimuli. In the subsequent *test* phase, all cues, whether associated with high pain or low pain, are followed by identical noxious stimuli. This procedure allows a test of the cues' effects on pain. Typically, pain responses in the test phase are larger after cues associated with high pain than after cues associated with low pain (Atlas, Bolger, Lindquist, & Wager, 2010; Atlas & Wager, 2012; Colloca, Petrovic, Wager, Ingvar, & Benedetti, 2010; Voudouris, Peck, & Coleman, 1990). In contrast, verbal suggestion alone does not typically produce robust pain-modulation effects (Colloca et al., 2008, 2010; de Jong, van Baast, Arntz, & Merckelbach, 1996; Montgomery & Kirsch, 1997; Voudouris et al., 1990), which suggests that pain modulation may require experience-based, precognitive learning.

However, another aspect of conditioned modulation of pain suggests the opposite: Precognitive associations are not enough, and cognitive expectations are required. Formal mediation analyses have found that the effects of conditioned cues on reported pain are mediated by participants' self-reported expectancies (Montgomery & Kirsch, 1997). In addition, conditioning does not typically produce pain modulation when people are unaware of the association between the cue and pain (Carlino et al., 2015; Montgomery & Kirsch, 1997; Watson, El-Deredy, Bentley, Vogt, & Jones, 2006). These findings suggest that conditioned modulation of pain is mediated by *conceptual* representations (i.e., by conscious, reportable pain expectancies).

One way these apparently discrepant findings may be reconciled is that both conditioning and conceptual processes are required, but neither alone is sufficient to modulate pain. Previous studies have not provided clear tests of this hypothesis: Virtually all pain-conditioning studies use primary aversive stimuli as USs, which inherently confounds precognitive and conceptual effects. Conditioning may induce both precognitive synaptic plasticity and conceptual expectancies in parallel (Kirsch, 2004; Montgomery & Kirsch, 1997). At first blush, a few paradigms do not appear to involve primary reinforcers. In social-conditioning studies, for example, representations of other individuals' pain are used as USs (Colloca & Benedetti, 2009; Vogtle, Barke, & Kroner-Herwig, 2013). However, other people's expressions of pain may serve as primary reinforcers, because they are affective, arousing stimuli in their own right; hence, the mechanisms by which they induce learning may be precognitive rather than conceptual. Thus, what is actually learned during aversive conditioning—and which types of learning processes (i.e., precognitive or conceptual) mediate conditioned modulation of pain—remains unknown.

In this study, we used two approaches to dissociate the roles of precognitive associations and conceptual representations in conditioned modulation of pain. First, we investigated whether conditioned cue effects on heat-pain responses could be obtained without any differential primary reinforcement of cues for high pain and low pain. Such pain modulation would necessarily be conceptual. To this end, we developed a *symbolic-conditioning* paradigm, in which the reinforcers that served as USs during learning were linked with pain only via conceptual processing. Visual cues (CS-high and CS-low cues), each consisting of a specific geometric shape, were repeatedly paired with pictures of thermometers displaying high and low temperatures, respectively. During a subsequent test phase, the CS-high and CS-low cues were followed by identical noxious heat stimuli (i.e., the cues were never differentially reinforced with actual pain), and we examined the cues' effects on physiological pain responses (heat-evoked skin conductance response, or SCR) and reported pain. If precognitive associations drive conditioned modulation of pain, symbolic conditioning would not be expected to affect pain responses during the test phase. If, however, conceptual representations drive conditioned modulation of pain, symbolic conditioning might affect subsequent pain responses.

Our second approach was analytic: We used multilevel mediation analyses to examine whether self-reported expectancies formally mediated the effects of conditioned cues on heat-evoked SCR. If so, this would show that expectancies mediate conditioning effects not only on reported pain (Montgomery & Kirsch, 1997) but also on pain physiology, which is less likely to be affected by demand characteristics (Orne, 1962) and decision biases.

Another aim of this study was to compare the effects of self-reported expectancy with the effects of anticipatory (cue-evoked) SCR, an autonomic preparatory response. Distinguishing the effects of these two types of anticipatory responses on subsequent pain may shed light on a seemingly inconsistent finding in human and animal pain-conditioning studies. As mentioned earlier, human participants typically show stronger pain responses after cues

associated with high pain than those associated with low pain. In contrast, in rodent studies, pain-predictive conditioned cues typically *reduce* the animal's pain response (a phenomenon called *conditioned analgesia*; Chance, White, Krynock, & Rosecrans, 1977). This phenomenon has been attributed to fear- or threat-induced analgesia in animals (Fanselow, 1986). One possible explanation for this apparent discrepancy is that physiological threat responses suppress pain through an automatic compensatory process, whereas expectancies have the opposite effect (i.e., increased pain with increasing expected pain). The former effect may dominate in animal studies and the latter may dominate in human studies, which could lead to the apparent discrepancy. To examine this hypothesis, we measured trial-to-trial variation in participants' self-reported pain expectancy and anticipatory SCR amplitude. We tested whether each of these anticipatory responses mediated the effects of conditioned cues on heat-evoked SCRs and pain ratings—and, if so, in which direction.

Method

Participants

We tested 30 healthy participants with no history of psychiatric, neurological, or pain disorders and no current pain. All participants gave written informed consent and received $12 per hour for their participation. The experiment was approved by the institutional review board of the University of Colorado Boulder. We chose a sample size of 30 on the basis of the large to very large effect sizes observed in our lab in previous heat-pain conditioning studies with primary reinforcers (e.g., Atlas et al., 2010; Schafer, Colloca, & Wager, 2015). Sample sizes of about 30 provide approximately 80% power to detect an effect size (Cohen's *d*) of 0.54 or larger, which is near the lower bound of what we expected to find in our symbolic-conditioning paradigm according to our hypothesis that the conditioning effects on pain in previous studies were mediated by conceptual representations.

One participant decided to stop before the end of the test phase because she found the heat stimuli too painful. In addition, 1 participant had to be excluded because of thermode failure. Finally, we excluded the data of 2 other participants who did not show SCRs to the heat stimuli (mean SCR amplitude < 0.05 μS). Thus, the final data set was based on 26 participants (mean age = 25 years, range = 18–55 years; 18 men, 8 women).

General procedure

Participants first completed a calibration procedure to ensure that they were not abnormally sensitive or insensitive to thermal stimuli. During the calibration, we delivered three thermal stimuli (peak temperature = 45–50 °C; ramp rate = 40 °C/s; 1.0 s at peak temperature) to each of five sites on the volar surface of the participants' left inner forearms, using a contact heat-evoked-potential stimulator (27-mm diameter Peltier thermode; Medoc Ltd., Ramat Yishai, Israel). After each stimulus, participants rated their experienced pain using a visual analog scale (VAS). The anchors were *no pain* and *worst-imaginable pain*. Participants then completed the conditioning phase followed by the test phase.

Conditioning phase

At the beginning of the conditioning phase, we instructed participants that they would learn associations between specific shapes and specific heat levels. We also told them that the heat levels would be displayed on a thermometer during the first part of the experiment (the conditioning phase) and applied to their forearms during the second part of the experiment (the test phase).

Each conditioning trial (Fig. 1a) started with a 0.5-s fixation screen, after which one of six geometric shapes (the CSs; a square, parallelogram, diamond, triangle, trapezoid, or pentagon) appeared at the center of the screen for 2 s. Following the CS, participants indicated which heat level they expected on a 100-unit vertical VAS with lower and upper anchors of *baseline skin temperature* and *extremely hot*, respectively. Participants rated the heat level they anticipated by using a computer mouse to drag a slider on the VAS. The VAS was displayed alongside a picture of a blank thermometer. After a 1- or 2-s anticipation period in which the fixation screen was displayed again, the thermometer reappeared for 3 s, this time indicating a specific heat level (the US). For a given participant, three of the shapes (CS-high cues) were always followed by a thermometer indicating a high heat level, and the other three shapes (CS-low cues) were always followed by a thermometer indicating a low heat level. We used five different heat levels, in random order, in both the high-heat and the low-heat categories; hence the specific heat levels displayed varied across trials (the average displayed heat levels following each CS-low and CS-high cue were 37.0% and 81.6% of the thermometer scale's range, respectively). The next trial started after an intertrial interval ranging from 1.5 to 3.5 s, during which a blank screen was displayed. Participants completed 120 conditioning trials (20 trials per CS, in random order) and were allowed to take a short break after every 30 trials.

To examine potential interactions between the effects of conceptual conditioning and the level of explicit information about cue meaning, we presented each cue in one of three conditions: (a) no instructions, (b) one-time instructions (before the start of the conditioning phase) about the heat level associated with the cue, or (c) per-trial instructions (before the CS cue) about the upcoming heat level. The per-trial instructions consisted of the presentation of

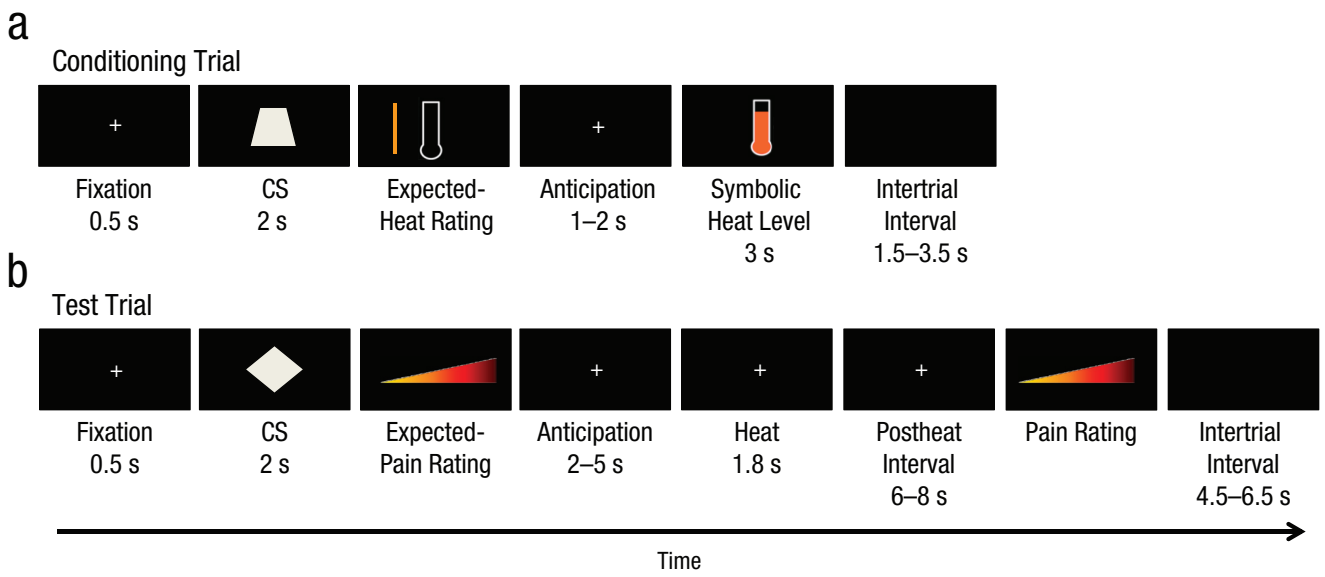

Fig. 1. Examples of a conditioning trial and a test trial. All conditioning trials were completed before the beginning of the test phase. In a conditioning trial (a), participants saw a fixation screen, after which one of six shapes (the conditioned stimulus, or CS) appeared. A vertical visual analog scale (VAS), represented in the figure as a vertical line (lower anchor: *baseline skin temperature*; upper anchor: *extremely hot*), then appeared next to a blank thermometer. To indicate the heat level they expected, participants used a computer mouse to drag a slider on the VAS. The fixation screen was displayed again, and then the thermometer reappeared, this time symbolically indicating a specific heat level (the unconditioned stimulus). Finally, a blank screen was displayed until the next trial began. In one third of conditioning trials, the conditioned stimulus was preceded by a 1-s instruction screen displaying "HIGH" or "LOW" (see Method and the Supplemental Material available online). In a test trial (b), participants saw the fixation screen, followed by one of the shapes (shown here) or the word "HIGH" or "LOW." They then indicated, on a horizontal VAS, how much pain they expected in the trial. The fixation screen reappeared. At this point, heat was applied to participants' forearms. After a pause, participants used the horizontal VAS to rate how much pain they experienced. Finally, a blank screen was displayed until the next trial began.

the word "LOW" or "HIGH" for 1 s immediately before the onset of the CS. Thus, for a given participant, each shape was always assigned the same combination of instruction condition and heat-level outcome; for example, the square might always be paired with the no-instruction condition and be followed by a low heat level (i.e., a CS-low/no-instruction cue). Each participant completed 40 trials (20 CS-high and 20 CS-low trials) of each of the three instruction conditions, intermixed in random order. The allocation of the geometric shapes (CSs) to the different conditions was counterbalanced across participants. Because the instruction manipulation did not substantially influence the effects of CS type on any of the dependent variables, we report test-phase results pooled across the three instruction conditions (the slight variations that were found as a function of instruction condition are reported in the Supplemental Material available online).

Test phase

Each test trial (Fig. 1b) started with a 500-ms fixation screen. Then, one of the CSs from the preceding conditioning phase or the word "LOW" or "HIGH" was presented for 2 s. After this, participants indicated how much pain they expected on that trial using a horizontal 100-unit VAS with anchors of *no pain* and *worst-imaginable pain*. After 2 to 5 s, a contact heat-evoked-potential stimulus (peak temperature = 47 or 48 °C; ramp rate = 40 °C/s; 1.0 s at peak temperature) was delivered to the volar surface of the left inner forearm. Between stimuli, the thermode maintained a baseline temperature of 32 °C. The VAS reappeared on the screen 6 to 8 s after offset of the heat stimulus, and participants rated how much pain they had experienced on that trial. Then, after an intertrial interval ranging from 4.5 to 6.5 s, during which an empty screen was displayed, the next trial started.

Unbeknownst to the participants, the temperature of all heat stimuli was either 47 °C or 48 °C, counterbalanced across trials, and had no relation to the preceding cue. Participants completed 10 trials for each of the CSs or words, in random order (i.e., 80 test trials in total). After every 16 trials, we moved the thermode to a new site on the participants' forearms. To reduce the impact of site-specific habituation effects (Jepma, Jones, & Wager, 2014), we administered one initial 48 °C stimulus to each skin site before starting the first trial on that site.

SCRs

We recorded participants' electrodermal activity throughout the experiment using 11-mm Ag/AgCl electrodes (Biopac Systems, Goleta, CA) attached to the medial phalanges of

the left index and middle fingers. The hand with the SCR electrodes attached rested on a table, and participants were instructed to sit still and to not move their left hands during the task. Data were sampled at 500 Hz.

In offline analyses, we applied a 5-Hz low-pass filter to the skin conductance signal and down-sampled the signal to 50 Hz. We calculated the CS-evoked, thermometer-evoked, and heat-evoked SCRs by subtracting the mean signal during the 2 s before stimulus onset from the time course of the signal during the 8 s after stimulus onset (for the visual stimuli) or 10 s after stimulus onset (for the contact heat stimuli). We defined trial-specific peak SCR amplitudes as the maximum value of the SCR signal during the 0- to 8-s interval after CS or thermometer onset and as the maximum value during the 4- to 10-s interval after contact-heat onset.

We included the data from all trials in the main analyses to prevent introducing a sampling bias by selectively removing the most extreme values. However, to check for extreme values, we examined the distribution of each participant's single-trial SCR amplitudes, and we defined potential outliers as values that fell more than 3 standard deviations from the mean (on average, 1.7 trials for CS-evoked SCR and 1.1 trials for heat-evoked SCR). To examine whether any observed effects might have been driven by these extreme SCR values, we excluded these trials and repeated our mediation analyses (see Mediation Results section).

Statistical analyses

We used the Multilevel Mediation toolbox (http://wagerlab.colorado.edu/tools; Atlas et al., 2010; Wager et al., 2009) to conduct multilevel regression and mediation analyses on the single-trial behavioral data and SCR amplitudes.

Regression analyses for the conditioning phase. To examine explicit learning of the CS-heat associations during the conditioning phase, we modeled the effects of CS type (CS-high vs. CS-low) and the CS Type × Time interactions on expected heat ratings. Time was modeled using the mean-centered linear and quadratic effects of trial number (counted separately for each CS). We also contrasted CS Type × Time interaction effects for (a) the no-instruction condition and the combination of the one-time and per-trial instruction conditions and for (b) the one-time instruction condition and the per-trial instruction condition. To test for the acquisition of conditioned SCRs to the CSs, we repeated these analyses on CS-evoked SCR amplitudes. In this analysis, we also accounted for potential adaptation of SCRs over time by modeling the linear and quadratic effects of time (trial number), and we tested whether there was a main effect of instruction condition. Finally, we tested the effects of symbolic heat level (high or low), the linear and quadratic effects of time, and the Heat Level × Time interactions (linear and quadratic) on participants' thermometer-evoked SCR amplitudes.

Regression analyses for the test phase. To test for conditioning effects on heat-evoked SCRs and pain ratings during the test phase, we modeled the effects of stimulus temperature, CS type (CS-high vs. CS-low), the Temperature × CS Type interaction, and the CS Type × Instruction interactions. To test whether the effects of CS type changed over time (e.g., because of extinction learning), we also modeled the CS Type × Time (trial number) interactions. To account for potential site-specific or site-nonspecific pain-adaptation effects (Jepma et al., 2014), we also modeled the linear and quadratic effects of both site-specific and site-nonspecific repetition. After running regression models that included all the regressors mentioned, we excluded covariates of no interest that did not predict the dependent variable (ps > .10). We report the results from these reduced models, which are very similar to those from the initial full models.

Multilevel mediation analyses for the test phase. We next used multilevel mediation analyses to test whether the effects of CS type on pain responses in the test phase were formally mediated by participants' CS-evoked SCRs or pain-expectancy ratings. Our mediation analyses tested whether there was an effect of CS type (CS-high vs. CS-low) on CS-evoked SCR amplitude and pain-expectancy ratings (paths $a1$ and $a2$, respectively), whether CS-evoked SCRs and pain-expectancy ratings were predictive of heat-evoked SCRs and pain ratings when we controlled for CS type (paths $b1$ and $b2$, respectively), and whether the relationship between CS type and heat-evoked SCRs or pain ratings (path c) decreased when we controlled for CS-evoked SCR or pain-expectancy ratings: $c - c'$, equivalent to $a \times b + \text{cov}(a,b)$. This last test was a test of mediation. We controlled for stimulus temperature and for the linear and quadratic effects of site-specific and site-nonspecific repetition by including these effects as covariates in the mediation models. We tested the significance of all effects using a bootstrap procedure (100,000 bootstrap samples). Bootstrapping does not require the assumptions of normality or equality of variance for valid inference, which is a primary motivation for its widespread use in mediation tests.

Results

Conditioning phase

Participants gradually learned the heat levels predicted by each CS-high and CS-low cue, as expected (see Fig. S1A in the Supplemental Material). Most of the

learning, as indexed by expected heat ratings, occurred over approximately the first seven trials for each cue. When explicit instructions about cue meaning were provided in addition to the information provided by the conditioning process, participants immediately predicted the heat levels associated with the CS-high and CS-low cues, also as expected (see Fig. S1A in the Supplemental Material). These effects were reflected in significant effects of CS type, the CS Type × Time interactions, and the CS Type × Time × Instruction (no instruction vs. instruction) interaction (all *p*s < .001; see Table S1 in the Supplemental Material for effect sizes of all significant predictors).

The CSs evoked small SCRs (~5–10 times smaller than those evoked by noxious heat stimuli during the subsequent test phase) that did not differ across cues (p = .228). The CSs that had been paired with explicit instructions about their associated heat levels evoked smaller SCRs than the CSs for which no prior information had been provided (p = .015; Fig. S1B and Table S1 in the Supplemental Material), which possibly reflects enhanced cognitive processing of the CSs for which associated heat levels could be learned only from experience. There were no CS Type × Time or CS Type × Instruction interactions (all *p*s > .250; see Fig. S2 in the Supplemental Material), which indicates that SCRs to the CS-high and CS-low cues did not differ between early learning or late learning in any of the instruction conditions.

The thermometer pictures also evoked small SCRs that did not differ as a function of displayed heat level (p > .250; Fig. S3 in the Supplemental Material). In addition, there were no Heat Level × Time interactions (linear or quadratic) on thermometer-evoked SCR amplitude (all *p*s > .250).

In summary, participants did not show a differential SCR to the symbolic USs (i.e., the high and low heat levels displayed on thermometers). Although they successfully learned the heat-predictive values of the CSs, they also did not acquire increased SCRs to CS-high compared with CS-low cues during the conditioning phase. These findings confirm that the symbolic representations of high and low heat were neither primary aversive USs nor spontaneously acquired CSs to heat pain, which would be expected to elicit differential SCRs. Thus, any CS effects on subsequent pain responses were unlikely to reflect second-order conditioning of the CSs to previously acquired associations between the thermometers and heat pain (Gewirtz & Davis, 2000); instead, they must be driven by the CSs' conceptual representation of high and low heat acquired during the symbolic-conditioning procedure.

Test phase

Our main results concern the effects of symbolic conditioning on participants' responses to painful heat in the test phase. The effects of CS type (CS-high vs. CS-low) on both heat-evoked SCRs and reported pain were highly significant in all three instruction conditions. Therefore, we report results pooled across instruction conditions (the slight variations that were found as a function of instruction condition are reported in Additional Results in the Supplemental Material).

Conditioning effects on heat-evoked SCRs. Heat-evoked SCRs were larger on CS-high than on CS-low trials (p = .007; Fig. 2a, Table 1). Thus, the symbolically conditioned cues affected participants' autonomic response to the noxious heat stimuli. As expected, heat-evoked SCRs were greater following heat stimuli of 48 °C than those of 47 °C (p < .001; Table 1), but there was no Temperature × CS Type interaction (p > .250). The effect of CS type on heat-evoked SCR amplitude decreased over the course of the test phase (CS Type × Time interaction; p = .005) and was absent during the last part of the test phase (Fig. 2b). This decrease may have resulted from habituation to heat-evoked SCRs overall (strong negative repetition effects; Table 1), which produced a floor effect.

Conditioning effects on heat-evoked pain ratings. As expected, participants reported higher pain after 48 °C heat stimuli than after 47 °C heat stimuli (p < .001; see Table 1 for regression results for all significant predictors). In addition, pain ratings were higher on CS-high trials than on CS-low trials (p < .001; Fig. 2c, Table 1). There was also a CS Type × Temperature effect (p = .009); the effect of CS type was larger for the 48 °C stimuli than for the 47 °C stimuli. In addition, there was a trend for a negative CS Type × Time interaction (p = .080), which indicates a slight decrease of the effect of CS type on pain ratings over the course of the test phase. However, the effect of CS type on pain rating was still highly significant at the end of the test phase (Fig. 2d shows pain ratings for each quartile of the test phase and both heat stimuli).

Mediation results. We next used multilevel mediation analyses to test whether the effects of CS type on autonomic and self-reported pain responses were formally mediated by trial-to-trial variation in anticipatory (CS-evoked) SCR amplitude or pain-expectancy ratings. We first examined whether anticipatory SCR amplitude or pain-expectancy ratings mediated the effect of CS type on heat-evoked SCR amplitude (Fig. 3a). Both anticipatory SCRs and pain-expectancy ratings were higher after CS-high cues than after CS-low cues (Fig. 3c and 3d; path $a1$: p = .038; path $a2$: p < .001). Anticipatory SCR amplitude negatively predicted heat-evoked SCR amplitude when we controlled for CS type (path $b1$, p < .001) but did not significantly mediate the effects of CS type on heat-evoked SCR ($a \times b1$, p > .250). In contrast, pain-expectancy ratings positively predicted heat-evoked SCR amplitude when we controlled for CS type (path $b2$,

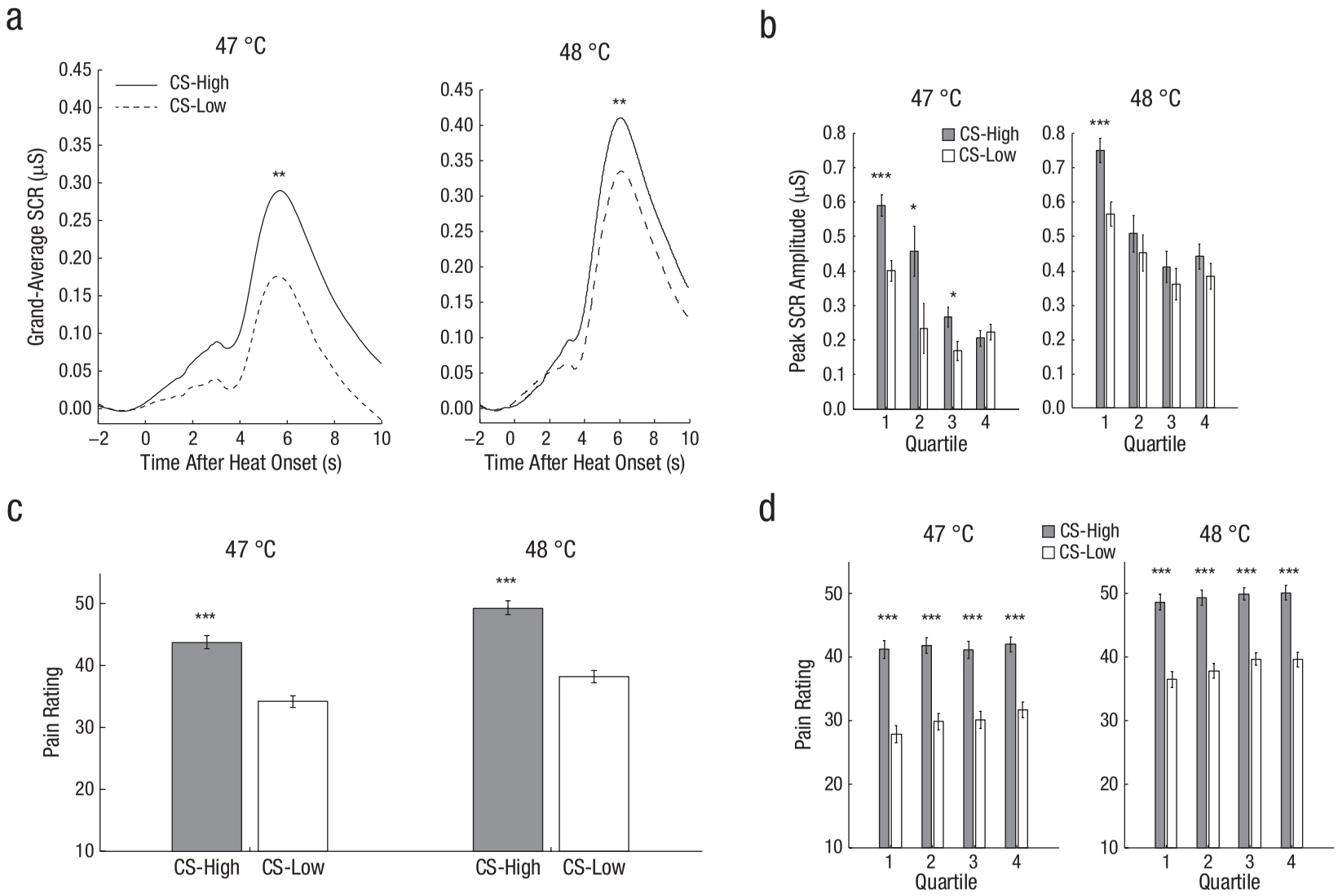

Fig. 2. Conditioned modulation of pain responses in the test phase. Grand-average heat-evoked skin conductance responses (SCRs) are plotted (a) as a function of time after heat onset for both conditioned stimulus (CS) types (CS-high and CS-low), separately for 47 °C and 48 °C heat stimuli. Peak heat-evoked SCR amplitude is graphed (b) as a function of quartile of the test phase (i.e., time during the test phase) for both CS types, separately for 47 °C and 48 °C heat stimuli. The graph in (c) shows the mean pain ratings for the two cue types, separately for 47 °C and 48 °C heat stimuli, and the graph in (d) shows mean pain ratings for each cue type in each quartile of the test phase, separately for 47 °C and 48 °C heat stimuli. Error bars represent ±1 within-subjects *SE*. Asterisks represent significant differences between CS-high and CS-low cues (*$p < .05$, **$p < .01$, ***$p < .001$).

Table 1. Results of Regression Analyses Predicting Heat-Evoked Skin Conductance Response Amplitude and Pain Rating in the Test Phase

Outcome and predictor	*b*	*SEM*	Cohen's *d*	*t*(25)	*p*
Heat-evoked skin conductance response					
Intercept	0.39	0.09	0.85	4.62	< .001
Temperature	0.06	0.01	1.18	4.12	< .001
CS type	0.03	0.01	0.59	2.94	.007
CS Type × Time	–0.0008	0.0003	0.52	3.21	.005
Site-nonspecific repetition	–0.07	0.02	0.69	4.27	< .001
(Site-nonspecific repetition)^2	0.02	0.01	0.39	2.72	.01
Site-specific repetition	–0.01	0.002	0.98	4.15	< .001
(Site-specific repetition)^2	0.001	0.0004	0.49	2.87	.01
Pain rating					
Intercept	38.6	3.14	2.41	12.3	< .001
Temperature	2.77	0.28	1.94	9.85	< .001
CS type	7.05	0.95	1.46	7.39	< .001
Temperature × CS Type	0.29	0.1	0.57	2.87	.009
CS Type × Time	–0.02	0.01	0.39	1.83	.08
CS Type × Instruction Condition (no-instruction condition > per-trial and one-time instruction conditions)	–1.18	0.46	0.50	2.56	.02
CS Type × Instruction Condition (per-trial instruction condition > no-instruction and one-time instruction conditions)	–1.49	0.86	0.34	1.74	.09
Site-nonspecific repetition	0.64	0.38	0.33	1.7	.10
(Site-specific repetition)^2	0.02	0.01	0.39	1.99	.06

Note: Temperature was coded as –1 = 47 °C and 1 = 48 °C. Conditioned-stimulus (CS) type was coded as –1 = CS-low and 1 = CS-high.

$p < .001$), and fully mediated the effects of CS type on heat-evoked SCRs ($a \times b2$: $p = .006$; path c': $p > .250$).

We used two other mediation models to investigate whether pain-expectancy ratings and anticipatory SCR mediated the effects of the CS on pain ratings (Fig. 3b). The effects of CS type on anticipatory SCR (path $a1$) and expected pain (path $a2$) were as described in the previous paragraph. Anticipatory SCR amplitude did not predict pain rating when we controlled for CS type (path $b1$, $p > .250$) and did not mediate the effects of CS type on pain ratings ($p = .192$). Pain-expectancy ratings positively predicted pain ratings when we controlled for CS type (path $b2$, $p < .001$) and formally mediated the effects of CS type on pain ratings ($a \times b2$, $p < .001$). When we controlled for pain-expectancy ratings, the effects of CS type on pain ratings remained marginally significant (path c', $p = .045$), which implies a partial mediation rather than a full mediation.

The negative relationship between anticipatory and heat-evoked SCR amplitude could potentially be explained by an amplitude-dependent refractory period, such that a larger SCR to the CS leads to a larger suppression of the SCR to the subsequent heat stimulus. If this is the case, the negative relationship between anticipatory and heat-evoked SCRs should decrease when the interval between them increases. To test this hypothesis, we added the duration of the interval between CS onset and heat onset—which varied across trials because of the jittered heat-anticipation delay and variation in pain-expectancy rating times—as a covariate to the first mediation analysis. This did not change the results: If anything, path b (the negative effect of anticipatory SCR on heat-evoked SCR) became even stronger when we controlled for the interval between CS onset and heat onset ($p < .00001$).

In these main analyses, we included all trials to prevent the introduction of a sampling bias by selectively removing extreme values. However, to examine whether any of the observed effects might have been driven by extreme SCR values on specific trials, we repeated the mediation analyses after excluding trials on which SCR amplitudes were more than 3 standard deviations from the within-person mean. This yielded the same pattern of significant results for all tests, except that the effects of CS type on anticipatory SCR amplitude (path $a1$ in Figs. 3a and 3b) was just short of significance after the extreme values were excluded ($p = .13$). Exclusion of extreme values changed neither the negative relationship between anticipatory SCR and heat-evoked SCR nor the effects of

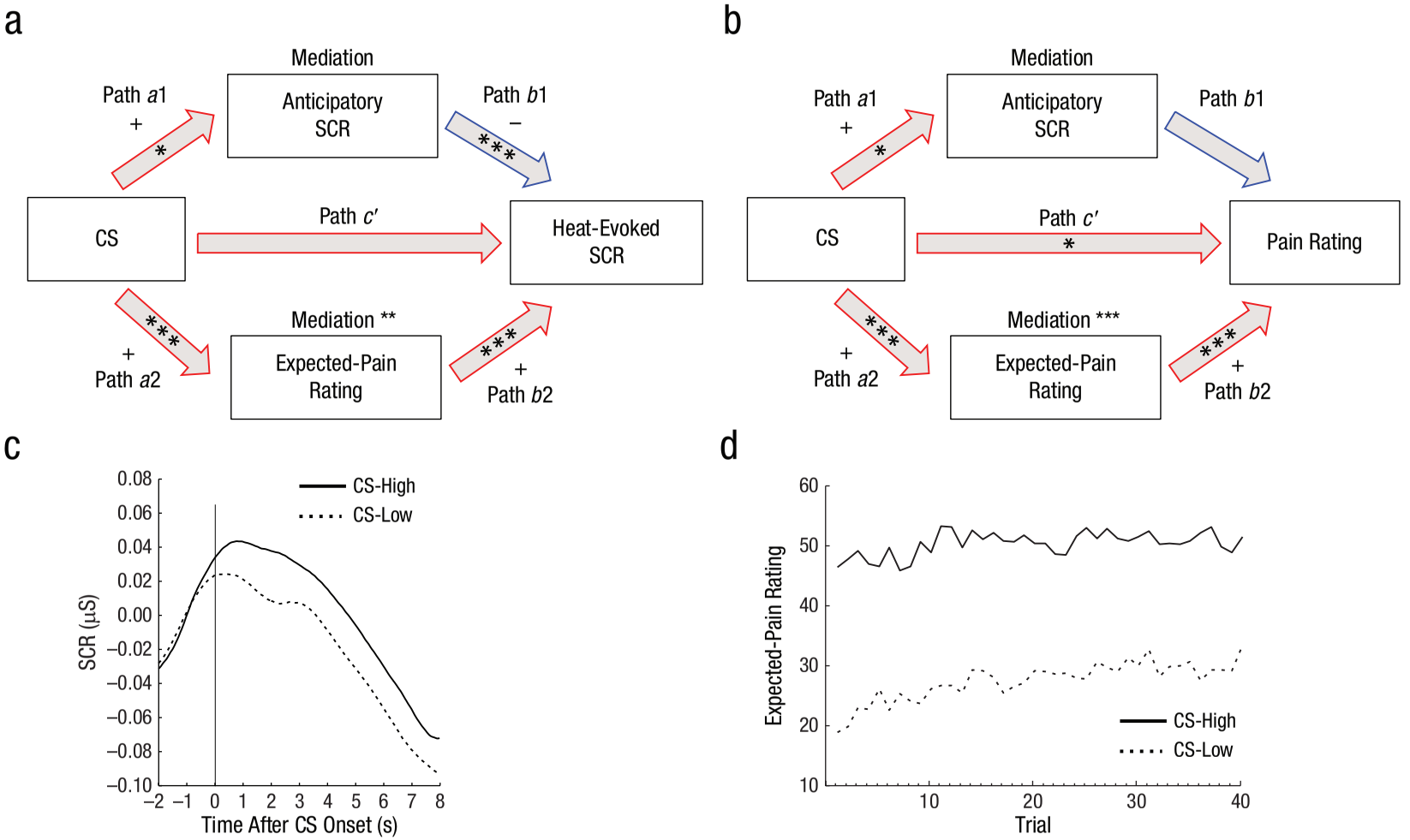

Fig. 3. Mediation results. The mediation model in (a) shows the effect of the type of conditioned stimulus (CS) on heat-evoked skin conductance response (SCR), as mediated by anticipatory SCR (paths *a*1 and *b*1) and expected-pain rating (paths *a*2 and *b*2). Path *c*′ indicates the effect of CS type on heat-evoked SCR when anticipatory SCR and expected-pain rating were included in the model. The mediation model in (b) shows the effect of CS type on pain rating, as mediated by anticipatory SCR (paths *a*1 and *b*1) and expected-pain rating (paths *a*2 and *b*2). Path *c*′ indicates the effect of the CS on pain rating when anticipatory SCR and expected-pain rating were included in the model. In both models (a and b), red and blue arrows indicate positive and negative paths, respectively. Asterisks indicate significant path coefficients (*$p < .05$, **$p < .01$, ***$p < .001$). Note that the two mediators (anticipatory SCR and expected-pain rating) were tested in separate mediation models. The displayed significance levels are based on these separate models, but including both mediators in a single model produced highly similar results. In (c), grand-average SCR in the test phase is graphed as a function of time before and after CS onset for both CS types. In (d), mean expected-pain rating in the test phase is plotted as a function of trial for both CS types.

CS type and expected-pain rating on heat-evoked SCR. The same pattern of results was found when we excluded trials on which SCR amplitudes were more than 2 standard deviations from the mean (on average, 4.9 trials for anticipatory SCRs and 4.3 trials for heat-evoked SCRs).

Finally, we also examined the correlations among expected-pain ratings, experienced-pain ratings, heat-evoked SCR amplitude, and anticipatory SCR amplitude while controlling only for temperature and site-specific and site-nonspecific repetition. We did this separately for each participant and tested whether the correlation coefficients differed from 0 using one-sample *t* tests. Expected-pain ratings correlated positively with both pain ratings (mean $r = .71$, 95% confidence interval, or CI = [.64, .79]), $t(25) = 20.3$, $p < .001$, and heat-evoked SCR amplitude (mean $r = .16$, 95% CI = [.09, .23]), $t(25) = 4.7$, $p < .001$. Anticipatory SCR amplitude correlated negatively with heat-evoked SCR amplitude (mean $r = -.11$, 95% CI = [−.17, −.04]), $t(25) = 3.5$, $p = .002$, but did not correlate with pain rating (mean $r = .01$, 95% CI = [−.04, .06]), $t(25) = 0.55$, $p > .25$. These correlations corroborate the results for path *b* in our mediation analyses. We note that the SCR measures yielded relatively low correlations because of the low signal-to-noise ratio inherent in single-trial physiological data but are consistent across participants. Low *R* values mean simply that we could not predict outcomes very accurately for individual trials, but here we were concerned with the reliability of the effects tested in the population. Finally, there was no correlation between expected-pain rating and anticipatory SCR amplitude (mean $r = .004$, CI = [−.05, .05]), $t(25) = 0.18$, $p > .25$, which suggests that these measures reflect two independent anticipatory processes.

Discussion

Together, expectations and learning shape pain processing on the basis of environmental cues (Rescorla, 1988; Stewart-Williams & Podd, 2004), but the mechanisms driving these effects are largely unresolved. Our results demonstrate robust modulation of self-reported and physiological pain responses following a purely conceptual conditioning procedure. Furthermore, conditioned-cue effects on heat-evoked SCRs were fully mediated by reported expectations. These findings indicate that conceptual representations, rather than precognitive associations, are critical in conditioned pain modulation in humans.

Conditioning to conceptual reinforcers provides a unique way to disentangle precognitive and conceptual influences. One interesting question is how our findings relate to recent demonstrations of pain modulation following social-conditioning procedures in which USs were observations of other people's pain responses (Colloca & Benedetti, 2009; Vogtle et al., 2013). Whereas pain expressions in other people may serve as primary reinforcers because of their affective nature, the thermometer images that served as USs in our paradigm were not affectively arousing and were linked with pain only via conceptual processing. In addition, pain assessments in social-conditioning studies have focused on self-reported pain, which is sensitive to decision biases. For example, in what we believe is the only social-conditioning study to examine autonomic physiology, Colloca and Benedetti (2009) found heart-rate deceleration in response to cues associated with high pain compared with cues associated with low pain. This finding is likely to be related to anticipatory orienting rather than pain-evoked autonomic responses. Our study significantly extends this work by showing modulation of pain physiology following purely conceptual conditioning.

If conceptual representations are a crucial ingredient in pain modulation, why are verbal suggestions alone typically much less effective than suggestion combined with conditioning? The weak effect on pain of verbal suggestion alone is a recurring theme (Colloca et al., 2008, 2010; de Jong et al., 1996; Voudouris et al., 1990). One possibility is that expectancies created by one-time information are too weak. Repeatedly experiencing cue-outcome associations may strengthen people's trust in the cues' predictive value and thereby their capacity to modulate pain. Another interesting possibility is that conditioning results in more precise, and therefore more powerful, expectancies about cue-outcome associations. More precise expectancies would be expected to yield greater pain modulation in a Bayesian framework, because precise prior information is more influential than diffuse information when integrating expectations with incoming sensory data (Buchel, Geuter, Sprenger, & Eippert, 2014). Verbal suggestions of low or high pain are rather nonspecific and therefore may lead to diffuse, uncertain expectancies. According to this account, conditioning to certain conceptual representations—such as visually displayed heat levels—may counterintuitively produce stronger pain-modulation effects than conditioning to primary noxious stimuli, for which perceived intensity is more ambiguous. Future studies that directly compare effects of primary and conceptual conditioning could test this hypothesis.

A unique feature of our study is that we measured trial-to-trial variation in physiological responses to pain anticipation (anticipatory SCRs) as well as pain (heat-evoked SCRs) and the relationship between them. Anticipatory SCRs in the test phase were larger following CS-high cues than CS-low cues, which is consistent with anticipatory threat. Anticipatory SCRs and self-reported expectancies had opposite effects on pain responses, which suggests that anticipatory SCRs were not open to introspection. The suppressing effect of anticipatory SCRs on heat-evoked SCRs was unlikely to have been due to an amplitude-dependent refractory period, because (a) anticipatory

SCRs were much smaller than heat-evoked SCRs, (b) controlling for the delay between CS onset and heat onset did not weaken the effect, and (c) a previous study found a positive rather than negative relationship between SCR amplitudes evoked by two successive stimuli (Bach, Flandin, Friston, & Dolan, 2010). Instead, the opposing effects of self-reported expectancy and anticipatory SCR may reflect two distinct pain-modulatory effects: an expectancy-driven effect that causes assimilation of the pain response toward the expected pain and a precognitive compensatory mechanism through which an autonomic threat response has analgesic effects. The second effect may reflect the fear-conditioned analgesia that is prominent in the animal literature (Chance et al., 1977; Fanselow, 1986; Helmstetter & Fanselow, 1987) but not in human pain-conditioning studies (for an exception, see Flor & Grusser, 1999). Which of the two processes predominates may depend on the pain-related outcome measure (e.g., self-reports or autonomic responses), threat intensity (Rhudy & Meagher, 2000), and species (Kirsch, 2004). The expectancy effect dominated in our study, resulting in robust net assimilation of pain responses toward the predictive values of the cues.

In conclusion, the present study provides novel evidence that conceptual representations can influence pain physiology in a surprisingly robust way. Investigation of the neural mechanisms underlying the acquisition and expression of conceptually conditioned pain modulation is an important objective for future research. For example, is the expression of conceptually conditioned pain modulation mediated by brain systems that also mediate nociceptive pain or is it mediated by cognitive or valuation systems that do not interact with the nociceptive system (Woo, Roy, Buhle, & Wager, 2015)? This remains an open question. Finally, it will be interesting to directly compare the effects of conceptual and primary pain conditioning. We did not include the latter condition, which has been studied extensively in previous work, because our aim was to examine whether conceptual conditioning could modify pain responses at all. Having demonstrated this, further examination of the similarities and differences between conceptual and primary conditioning is an important next step.

Author Contributions

M. Jepma and T. D. Wager developed the study concept and designed the study. M. Jepma collected the data and performed the data analysis. M. Jepma drafted the manuscript, and T. D. Wager provided critical revisions. Both authors approved the final version of the manuscript for submission.

Declaration of Conflicting Interests

The authors declared that they had no conflicts of interest with respect to their authorship or the publication of this article.

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Supplemental Material

Additional supporting information can be found at http://pss.sagepub.com/content/by/supplemental-data

References

Antonov, I., Antonova, I., Kandel, E. R., & Hawkins, R. D. (2001). The contribution of activity-dependent synaptic plasticity to classical conditioning in *Aplysia*. *The Journal of Neuroscience*, *21*, 6413–6422.

Atlas, L. Y., Bolger, N., Lindquist, M. A., & Wager, T. D. (2010). Brain mediators of predictive cue effects on perceived pain. *The Journal of Neuroscience*, *30*, 12964–12977.

Atlas, L. Y., & Wager, T. D. (2012). How expectations shape pain. *Neuroscience Letters*, *520*, 140–148.

Atlas, L. Y., & Wager, T. D. (2013). Expectations and beliefs: Insights from cognitive neuroscience. In K. Ochsner & S. M. Kossler (Eds.), *The Oxford handbook of cognitive neuroscience: Vol. 2. The cutting edges* (pp. 359–381). New York, NY: Oxford University Press.

Bach, D. R., Flandin, G., Friston, K. J., & Dolan, R. J. (2010). Modelling event-related skin conductance responses. *International Journal of Psychophysiology*, *75*, 349–356.

Berntson, G. G., & Micco, D. J. (1976). Organization of brainstem behavioral systems. *Brain Research Bulletin*, *1*, 471–483.

Berntson, G. G., Tuber, D. S., Ronca, A. E., & Bachman, D. S. (1983). The decerebrate human: Associative learning. *Experimental Neurology*, *81*, 77–88.

Buchel, C., Geuter, S., Sprenger, C., & Eippert, F. (2014). Placebo analgesia: A predictive coding perspective. *Neuron*, *81*, 1223–1239.

Carlino, E., Torta, D. M., Piedimonte, A., Frisaldi, E., Vighetti, S., & Benedetti, F. (2015). Role of explicit verbal information in conditioned analgesia. *European Journal of Pain*, *19*, 546–553.

Chance, W. T., White, A. C., Krynock, G. M., & Rosecrans, J. A. (1977). Autoanalgesia: Behaviorally activated antinociception. *European Journal of Pharmacology*, *44*, 283–284.

Colloca, L., & Benedetti, F. (2009). Placebo analgesia induced by social observational learning. *Pain*, *144*, 28–34.

Colloca, L., Petrovic, P., Wager, T. D., Ingvar, M., & Benedetti, F. (2010). How the number of learning trials affects placebo and nocebo responses. *Pain*, *151*, 430–439.

Colloca, L., Tinazzi, M., Recchia, S., Le Pera, D., Fiaschi, A., Benedetti, F., & Valeriani, M. (2008). Learning potentiates neurophysiological and behavioral placebo analgesic responses. *Pain*, *139*, 306–314.

de Jong, P. J., van Baast, R., Arntz, A., & Merckelbach, H. (1996). The placebo effect in pain reduction: The influence of conditioning experiences and response expectancies. *International Journal of Behavioral Medicine*, *3*, 14–29.

Dworkin, R. H., Backonja, M., Rowbotham, M. C., Allen, R. R., Argoff, C. R., Bennett, G. J., . . . Weinstein, S. M. (2003). Advances in neuropathic pain: Diagnosis, mechanisms, and treatment recommendations. *Archives of Neurology*, *60*, 1524–1534.

Fanselow, M. S. (1986). Conditioned fear-induced opiate analgesia: A competing motivational state theory of stress analgesia. *Annals of the New York Academy of Sciences*, *467*, 40–54.

Flor, H., & Grusser, S. M. (1999). Conditioned stress-induced analgesia in humans. *European Journal of Pain*, *3*, 317–324.

Gewirtz, J. C., & Davis, M. (2000). Using Pavlovian higher-order conditioning paradigms to investigate the neural substrates of emotional learning and memory. *Learning & Memory*, *7*, 257–266.

Glanzman, D. L. (1995). The cellular basis of classical conditioning in *Aplysia californica*—it's less simple than you think. *Trends in Neurosciences*, *18*, 30–36.

Grau, J. W., Salinas, J. A., Illich, P. A., & Meagher, M. W. (1990). Associative learning and memory for an antinociceptive response in the spinalized rat. *Behavioral Neuroscience*, *104*, 489–494.

Greene, M. R., Botros, A. P., Beck, D. M., & Fei-Fei, L. (2015). What you see is what you expect: Rapid scene understanding benefits from prior experience. *Attention, Perception, & Psychophysics*, *77*, 1239–1251.

Hawkins, R. D., Abrams, T. W., Carew, T. J., & Kandel, E. R. (1983). A cellular mechanism of classical conditioning in *Aplysia*: Activity-dependent amplification of presynaptic facilitation. *Science*, *219*, 400–405.

Helmstetter, F. J., & Fanselow, M. S. (1987). Effects of naltrexone on learning and performance of conditional fear-induced freezing and opioid analgesia. *Physiology & Behavior*, *39*, 501–505.

Jepma, M., Jones, M., & Wager, T. D. (2014). The dynamics of pain: Evidence for simultaneous site-specific habituation and site-nonspecific sensitization in thermal pain. *The Journal of Pain*, *15*, 734–746.

Johansen, J. P., Cain, C. K., Ostroff, L. E., & LeDoux, J. E. (2011). Molecular mechanisms of fear learning and memory. *Cell*, *147*, 509–524.

Kirsch, I. (2004). Conditioning, expectancy, and the placebo effect: Comment on Stewart-Williams and Podd (2004). *Psychological Bulletin*, *130*, 341–345.

Levy, M. H. (1996). Pharmacologic treatment of cancer pain. *The New England Journal of Medicine*, *335*, 1124–1132.

Montgomery, G. H., & Kirsch, I. (1997). Classical conditioning and the placebo effect. *Pain*, *72*, 107–113.

Orne, M. T. (1962). On the social-psychology of the psychological experiment: With particular reference to demand characteristics and their implications. *American Psychologist*, *17*, 776–783.

Rescorla, R. A. (1988). Pavlovian conditioning: It's not what you think it is. *American Psychologist*, *43*, 151–160.

Rhudy, J. L., & Meagher, M. W. (2000). Fear and anxiety: Divergent effects on human pain thresholds. *Pain*, *84*, 65–75.

Schafer, S. M., Colloca, L., & Wager, T. D. (2015). Conditioned placebo analgesia persists when subjects know they are receiving a placebo. *The Journal of Pain*, *16*, 412–420.

Stewart-Williams, S., & Podd, J. (2004). The placebo effect: Dissolving the expectancy versus conditioning debate. *Psychological Bulletin*, *130*, 324–340.

Vogtle, E., Barke, A., & Kroner-Herwig, B. (2013). Nocebo hyperalgesia induced by social observational learning. *Pain*, *154*, 1427–1433.

Voudouris, N. J., Peck, C. L., & Coleman, G. (1990). The role of conditioning and verbal expectancy in the placebo response. *Pain*, *43*, 121–128.

Wager, T. D., Waugh, C. E., Lindquist, M., Noll, D. C., Fredrickson, B. L., & Taylor, S. F. (2009). Brain mediators of cardiovascular responses to social threat: part I. Reciprocal dorsal and ventral sub-regions of the medial prefrontal cortex and heart-rate reactivity. *NeuroImage*, *47*, 821–835.

Watson, A., El-Deredy, W., Bentley, D. E., Vogt, B. A., & Jones, A. K. (2006). Categories of placebo response in the absence of site-specific expectation of analgesia. *Pain*, *126*, 115–122.

Woo, C. W., Roy, M., Buhle, J. T., & Wager, T. D. (2015). Distinct brain systems mediate the effects of nociceptive input and self-regulation on pain. *PLoS Biology*, *13*(1), Article e1002036. Retrieved from http://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1002036