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Pain or nociception? Subjective experience mediates the effects of acute noxious heat on autonomic responses - corrected and republished

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Abstract

Nociception reliably elicits an autonomic nervous system (ANS) response. Because pain and ANS circuitry interact on multiple spinal, subcortical, and cortical levels, it remains unclear whether autonomic responses are simply a reflexive product of noxious stimulation regardless of how stimulation is consciously perceived or whether the experience of pain mediates ANS responses to noxious stimulation. To test these alternative predictions, we examined the relative contribution of noxious stimulation and individual pain experience to ANS responses in healthy volunteers who underwent 1 or 2 pain assessment tasks. Participants received 8 seconds of thermal stimulation of varied temperatures and judged pain intensity on every trial. Skin conductance responses and pupil dilation responses to stimulation served as measures of the heat-evoked autonomic response. We used multilevel modelling to examine trial-by-trial relationships between heat, pain, and ANS response. Although both pain and noxious heat stimulation predicted skin conductance response and pupil dilation response in separate analyses, the individual pain experience statistically mediated effects of noxious heat on both outcomes. Furthermore, moderated mediation revealed that evidence for this process was stronger when stimulation was perceived as painful compared with when stimulation was perceived as nonpainful, although this difference emerged late, in the 4-second period after thermal stimulation. These findings suggest that pain appraisal regulates the heat-evoked autonomic response to noxious stimulation, documenting the flexibility of the autonomic pain response to adjust to perceived or actual changes in environmental affordances above and beyond nociceptive input.

Keywords: Pain, Noxious stimulation, Arousal, Autonomic nervous system, Heat, Skin conductance, Pupil dilation, Nociception

1. Introduction

The autonomic nervous system (ANS) is remarkably responsive to painful stimulation. More than 4 decades of research show that nociceptive stimulation elicits autonomic reactions, with studies demonstrating widespread effects across stimulus modalities (eg, heat, electrical, and pressure stimulation) and physiological outcomes (eg, cardiovascular reactivity, respiration, skin conductance, and pupil dilation; see Ref. 53, for a review). Although nociception refers to neural encoding of impending or actual tissue damage (ie, noxious stimulation), pain refers to the subjective experience of actual or impending harm.[42,43] Although nociceptive stimulation usually leads to pain, pharmacological and brain lesion research shows that one can exist without the other.[30,48,70] Several human neuroimaging studies support this finding, showing that pain and noxious stimulation intensity are dissociable at the level of brain activity.[2,5,24,50,68,95]

However, it remains unknown whether psychological processes, such as pain appraisal, mediate autonomic responses to painful stimulation or whether autonomic responses preliminarily reflect ascending nociceptive signals. Although multiple studies show the sensitivity of the ANS to noxious stimulation,[53] few mechanistic studies have investigated the specificity of the ANS response to pain, and most studies in humans have failed to dissociate nociception from pain. Despite the lack of formal investigation, the International Association for the Study of Pain (IASP) refers to autonomic responses as a "consequence of encoding" and links autonomic responses with nociception.[42] Indeed, nociceptive pathways functionally intersect with the ANS on multiple subcortical and spinal levels (see Ref. 11, for a review), so nociceptive stimuli might evoke an autonomic response automatically as part of a coordinated defensive response, irrespective of awareness or the conscious pain experience. However, research on the nociceptive flexion response indicates that psychological factors such as expectation and affect can shape spinally mediated reflexive responses.[78,79,82,92] Thus,

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experienced pain might shape the autonomic response to noxious stimulation, as cortical regions that regulate pain are also involved in ANS modulation (see Ref. 10, for a review). Physiological arousal can be directly influenced by top-down psychological processes, such as emotional reappraisal,[38] implicit and explicit learning,[4,34,64,73,94] and stress.[47,55] Consistent with these findings, manipulating attention or emotion can also affect pain experience and the autonomic response to noxious stimulation.[18,21,44,54,77] Thus, taken together, the question remains open whether pain appraisal is necessary for noxious stimulation to activate the ANS.

To address this question, we experimentally manipulated heat stimulation intensity in 2 tasks and measured the effects on self-reported pain and heat-evoked autonomic responses using palmar skin conductance response (SCR). Skin conductance response constitutes a well-established marker of the sympathetic threat response.[14,32,39,88] To replicate findings, we collected increased pupil diameter as another marker of physiological arousal,[17] although pupil diameter can be influenced by both sympathetic and parasympathetic activity. Both markers are reliably influenced by painful stimulation.[53] We used multilevel modelling to compare the associations of noxious stimulation and pain intensity with evoked autonomic responses and to test a process model in which noxious stimulation predicted autonomic responses through increased pain. Furthermore, we compared this process model for both painful and nonpainful stimulation to test whether the subjective categorization of thermal stimuli as painful or innocuous regulated the influence of heat on autonomic responses.

2. Method

2.1. Participants

One hundred eighteen healthy adult volunteers from a community sample at the National Institutes of Health (NIH) provided informed consent. Procedures were approved by the NIH Institutional Review Board (protocol number: 15AT0132) and followed the ethical guidelines for human subject research in the Declaration of Helsinki. Eligible participants were required to be between the ages of 18 and 50 years and fluent in English. Participants were not invited if they had chronic pain or a history of chronic pain or regularly used medication affecting pain; any major neurological or psychiatric conditions; any conditions that affected pain sensitivity or somatosensation; or any dermatological conditions on the testing region. Participants were not enrolled if they used recreational drugs or were pregnant, and urine screens were used to confirm eligibility. Based on a clinical assessment on arrival in the laboratory, 2 participants were deemed ineligible. The remaining 116 participants underwent sensory testing to assess heat-evoked pain and autonomic responses.

Participants completed at least 1 of 2 different pain assessment tasks: the 2-step pain assessment (TSPA) and the adaptive staircase calibration (ASC). Fifty participants completed both tasks, whereas 41 completed only the TSPA and 25 completed only the ASC. As the samples were only partially overlapping, and all variables were measured and manipulated within subjects, we considered the 2 pain assessment tasks independently for the purpose of the current analyses. Seventy-five participants completed the ASC (52.86% women; M_{age} = 27.96 years, SD_{age} = 7.93; 40.00% Black/African American, 37.14% Caucasian/White, 14.29% Asian, 5.71% Hispanic/Latino, and 2.86% mixed race), and 91 participants completed the TSPA (58.24% women; M_{age} = 28.02 years, SD_{age} = 7.37; 39.77% Caucasian/White, 37.50% Black/African American, 12.50% Asian, 6.82% Hispanic/Latino, and 3.41% mixed/other race).

2.2. Stimuli and apparatus

Participants received different levels of thermal heat stimulation on 8 sites of the nondominant inner volar forearm through a 16 × 16-ATS thermode (Medoc Ltd, Ramat Yisha, Israel). During both pain assessment tasks, we recorded skin conductance using the Biopac MP150 EDA-100C module (Biopac Systems, Inc, Goleta, CA) through 2 shielded silver–silver chloride electrodes. We prepared electrodes with NaCl electrolyte gel (GEL101) as recommended by Biopac Systems for skin conductance measurement. We attached electrodes at the hypothenar muscles of participants' nondominant hands. During the ASC, we also recorded pupillometry data from the participant's right eye using the Eyelink 1000 Plus eye tracking system (SR Research, Ltd, Ottawa, Canada). We used a 9-point calibration and validation procedure to calibrate gaze position and pupil measurement. Visual stimuli (eg, fixations) and behavioral responses (eg, pain ratings) were presented and collected using Experiment Builder software (SR Research) for the ASC and E-Prime 2.0 software (Psychology Software Tools, Pittsburgh, PA) for the TSPA.

2.3. Procedures

We first describe the procedures that were common to the ASC and TSPA tasks, followed by the unique procedures associated with each separate task.

2.3.1. General procedures

We recruited participants through flyers posted in NIH buildings and distributed through email list servers, ClinicalTrials.gov, and the NIH patient recruitment office. All potential participants underwent a phone screen to assess eligibility, and eligible participants were invited to the NIH's outpatient clinic. On arrival, participants provided informed consent. As per NIH guidelines, participants went through a basic nursing assessment and received a physical examination if they had not received one at the NIH within the past year. Following clinical assessments, participants completed the pain assessment procedures described below (for procedures, see **Fig. 1**). In addition to characterizing autonomic and pain responses to noxious stimuli, we used this session to establish eligibility for subsequent pain experiments. Participants also completed personality and affect questionnaires before, during, and after the session that we did not analyze for the purpose of the present research questions. Participants received monetary compensation contingent on the individual length of the session.

2.3.2. Thermal stimulation

Participants experienced thermal stimuli delivered via thermode during both tasks. Before the tasks, we informed participants that they could stop thermal stimulation at any point if stimulation was intolerable either by asking the experimenter to stop the stimulus or by removing the thermode from their forearm. Target temperatures ranged from 34 to 50°C in 0.5°C increments. Each stimulation trial started with a 1.5-second on-ramp phase, during which thermal stimulation rose from 32°C to the target temperature level, stayed at the target temperature for 5 or 7 seconds, and ended with a 1.5-second off-ramp phase to return to baseline. The first 12 participants in the ASC experienced 10-second thermal stimulation, before we switched to 8-second thermal stimulation, as it is common in previous research.[36,59] We tested whether trial length influenced the SCR and pupil dilation response (PDR) to heat stimulation in the ASC

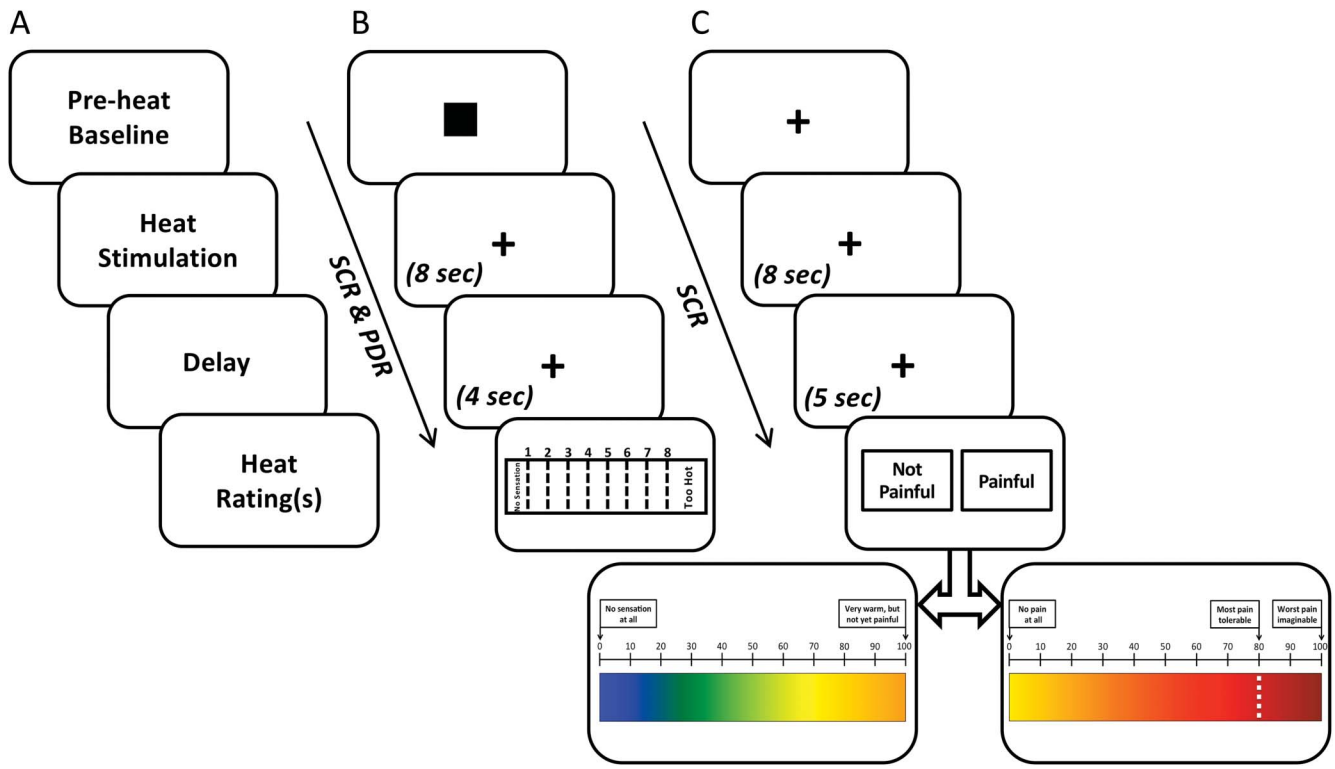

Figure 1. Trial structure: (A) general trial structure, (B) trial in the adaptive staircase pain assessment, and (C) trial in the 2-step pain assessment. We measured skin conductance responses (SCRs) and pupil dilation responses (PDRs) in the adaptive staircase pain assessment and SCR in the 2-step pain assessment.

(see supplementary material, available online at http://links.lww.com/PAIN/A778). Consequently, we combined SCR and PDR data across participants in the ASC. All participants in the TSPA experienced 8-second thermal stimulation. Triggers were sent to Biopac to mark the onset and offset of each thermal stimulus, which permitted analyses of time-locked physiological responses. To minimize fear-potentiated startle responses resulting from uncued aversive stimulation,[16,37] we signaled heat onset to participants by displaying a fixation cross on the computer screen (**Fig. 1**). Experimenters were extensively trained to manually deliver heat at fixation onset. Any variation in experimenters' reaction times is likely to be offset by the slow 1.5-second ramp from baseline temperature to peak stimulation, thus mitigating the possibility that startle could have contributed to our results.

2.3.3. Perceived intensity ratings

After the offset of the thermal stimulus, participants rated the perceived intensity using visual analogue scales (VASs) described below. Participants provided ratings using a mouse and stated each rating aloud to facilitate the experimenters' recording of participants' ratings. The verbal ratings allowed experimenters to avoid repeating temperatures participants deemed to be intolerable and—in the case of the ASC—continuously adjust temperature delivery (see specific procedures below). Experimenters instructed participants to rate pain based on the entire heat trial and checked in with the participants periodically to ensure that participants were using scales as intended. Each VAS had an anchor to denote maximum tolerable pain. If a participant found a specific stimulus to be intolerable, the participant rated the stimulus as higher than this level. Experimenters adjusted subsequent thermal stimuli (irrespective of task) on this skin site to be 0.5 to 1° lower than the temperature of the thermal stimulus rated as intolerable if subsequent temperatures were equal or higher than the stimulus rated as intolerable. To account for such changes, we used the adjusted—not original—temperature variable in all analyses. Furthermore, some participants revised verbal ratings after having provided computer ratings. Sometimes, participants clicked the mouse incorrectly or corrected a rating. However, our computer set-up did not allow participants to change their rating once imputed. Because of these occasional revisions, we analyzed adjusted temperatures and participants' verbalized pain ratings when testing the present research questions. Results and conclusions reported below did not differ substantially when we analyzed responses recorded by a computer mouse rather than verbal ratings.

2.3.4. Specific procedures

The ASC and TSPA were counterbalanced across subjects. Because of occasional time constraints and interim changes to our experimental protocol (eg, the TSPA task was added partway through our protocol as a secondary pain assessment), not all participants completed both pain assessment tasks. We included the ASC because this pain assessment task is an essential part in our clinical protocol to assess participants' eligibility for subsequent experiments. The ASC constitutes an adaptive, unidimensional measure of pain, designed to tailor heat stimulus delivery to the pain range of each participant; thus, the ASC is well suited to quickly and reliably check pain threshold and tolerance levels to determine participation eligibility.[13,22] In addition, we have used this task in our previous research.[3,5,6] However, because the ASC is adaptive, it does not distinguish well between painful and nonpainful temperatures. Furthermore, 1 concern with a continuous VAS is that it conflates pain and innocuous

intensity. Therefore, we also included the TSPA in our experimental protocol, which allowed us to distinguish between painful and nonpainful stimulation.[89] Because we did not have prior data on the direct relationship between the tasks, and because the literature suggests that nonadaptive tasks such as the TSPA may be less reliable than adaptive tasks such as the ASC,[58] we opted to include both pain assessment tasks in our analyses.

2.3.5. Adaptive staircase calibration

Using an established pain calibration task,[3,5,6,89] participants experienced 24 heat stimulation trials on 8 skin sites and provided pain ratings on a VAS ranging from 0 (*no sensation at all*) to 10 (*most pain imaginable*). The scale also included anchors for nonpainful warmth (1), pain threshold (2), moderate pain (5), and maximum tolerable pain (8). All participants initially received heat stimulation at 41, 44, and 47°C (in this order), and then for the remaining 21 trials, we used iterative linear regression to identify and apply temperatures predicted to elicit low, medium, or maximum tolerable pain (ie, VAS ratings of 2, 5, and 8, respectively). All participants received the same sequence of low, medium, and maximum tolerable pain stimulation (with temperatures based on the iterative fit to each individual). The sequence ensured that each skin site received a low, medium, and maximum painful temperature, and that each stimulus level was followed by every possible stimulus level. Thus, stimulus levels were not randomized across participants, but temperatures varied for each individual. Each skin site received 3 stimulations over the course of this task.

To facilitate pupillary measurements, we programmed a computer-based task using Experiment Builder (see **Fig. 1B** for trial structure). To begin each trial, the participant fixated on a black square in the center of the computer screen. Following steady fixation of 500 ms, a cross appeared, and the experimenter triggered the thermal stimulus through Pathways software at the temperature determined by the iterative regression. Four seconds after heat offset, the pain rating scale appeared, and the participant provided their rating using a mouse and then stated the rating aloud. An experimenter then moved the thermode to the next skin site. We recorded pupil dilation, gaze position, skin conductance, respiration, heart rate, and electrocardiogram activity during the ASC task. We analyzed heat-evoked SCRs and pupil dilation for the purpose of the current investigation.

2.3.6. Two-step pain assessment

We adapted this task from Wager et al.[89] Unlike in the ASC, most participants in the TSPA (exceptions see below) received the same fixed sequence of 32 predetermined temperatures. For each heat stimulus, participants provided both categorical and continuous ratings (see **Fig. 1C** for trial structure). Five seconds after heat offset, participants rated whether the stimulus was "*Not Painful*" or "*Painful*," and then rated the intensity of each stimulus using a 0 to 100 VAS. If the stimulus had not been painful, participants rated intensity from 0 (*no sensation at all*) to 100 (*very warm, but not yet painful*). If the stimulus had been painful, participants rated intensity from 0 (*no pain at all*) through 80 (*most pain tolerable*) to 100 (*worst pain imaginable*).

We applied predetermined temperatures between 34 and 50°C, with approximately 50% of the thermal stimuli predicted to be nonpainful and 50% predicted to be painful, using a pain cutoff of 44°C. Stimulus lists were pseudorandom and generated using random samples with restrictions, such that each skin site received 4 stimulations that were at least 1°C apart, with 2 stimuli from the nonpainful range and 2 stimuli from the painful range. We changed lists over the course of the study to refine thermal stimulus sampling from above and below the pain threshold and to adequately capture the lower bound of the nonpainful temperature interval. As a consequence, 81 participants had the same fixed temperature sequence, whereas 10 participants had 1 of 2 other temperature sequences. Instead of randomizing temperatures within participants, participants received the same sequence of temperatures to ensure that individual participants received stimulation across the targeted temperature range and did not receive a disproportional amount of high or low temperatures. Temperatures only varied across subjects within a sequence if a participant found a stimulation to be intolerable. In this case, all subsequent stimulations on that site were required to be below the intolerable temperature (see general procedures above). We recorded skin conductance, respiration, heart rate, and electrocardiogram activity during the TSPA task. For the current analyses, we focused on heat-evoked SCRs.

2.4. Peripheral autonomic responses

To measure the autonomic response to heat stimulation, we focused on SCRs for both tasks and examined PDRs during the ASC task. Data were preprocessed and extracted using custom code in MATLAB 2015b (MathWorks, Inc, Natick, MA). Technical difficulties prohibited us from collecting autonomic arousal data for a subset of participants (skin conductance for 2 ASC participants; pupil dilation for 4 ASC participants; skin conductance for 3 TSPA participants), reducing sample sizes to: $n = 73$ for analyses of skin conductance during the ASC; $n = 71$ for pupil dilation during the ASC; and $n = 88$ for skin conductance during the TSPA. Research participants were blind to temperature levels or reported pain levels when coding for artifacts in individual trials.

2.4.1. Skin conductance

We first preprocessed skin conductance data in AcqKnowledge (Biopac Systems, Inc, Goleta, CA). Data were smoothed with a 1000 samples moving average function and filtered with a 25-Hz FIR low-pass filter. Data were then imported to MATLAB, where we downsampled data to 250 Hz and used event markers to extract and visualize skin conductance levels from 1 second before heat onset until 4 seconds after offset (12 seconds total) to account for a delayed SCR, similar to previous work.[59] Skin conductance responses were baseline-corrected by subtracting the mean skin conductance level over the second before heat onset. Finally, trained research assistants visually inspected each trial for artifacts. Based on these inspections, we excluded on average 26.76% of trials per ASC participant and 21.77% of trials per TSPA participant.

2.4.2. Pupil dilation

Because we did not collect data on absolute pupil sizes for all participants, we report changes in pupil dilation in arbitrary units (a.u.). To import ASC pupillary data, we used MATLAB-based algorithms.[45] Initial inspections revealed that extreme values surrounded each period identified as a blink, so we interpolated from 100 ms before to 100 ms after each blink. Data were then aligned to event markers, and we used custom MatLab code to visually inspect pupillary data on each trial to both verify interpolation and identify any artifacts that were not automatically detected. We deleted artifacts and excluded any trials with at least 50% missing data ($M = 1.6\%$ of trials excluded per

participant). We then downsampled data to 250 Hz and applied a 25-sample Savitzky–Golay smoothing filter.[12,83] Similar to SCR analyses, we baseline-corrected PDR data for each trial by subtracting the mean pupil dilation level over the 0.5 seconds preceding heat onset.

2.4.3. Heat-evoked autonomic responses

We used custom MATLAB code to align continuous time series data with event markers and extract heat-evoked SCR and PDR data. We calculated different measures of SCR and PDR to heat stimulation. For each trial, we extracted baseline-corrected responses from 0.5 seconds after heat onset to 4 seconds after heat offset (to account for delayed autonomic responses). We used the trapezoidal rule–based MATLAB function *trapz.m* to calculate trial-by-trial area under the curve (AUC) across this window as a summary measure of the heat-evoked autonomic response for both outcome measures (SCR and PDR). When calculating AUC in the pupil dilation data, we interpolated across missing data. To gain further insight on the dynamics of heat-evoked autonomic responses, we also computed the following: (1) trial amplitude (difference between overall maximum and preceding minimum); (2) response latency (duration between heat onset and maximum amplitude); and (3) AUC for 3 phases of heat stimulation (*early* = 0.5-4 seconds after onset; *peak* = 4 seconds after onset to heat offset; and *after* = the 4 seconds after offset). Trials with SCR amplitudes that did not exceed 0.02 μs were set to 0 and considered nonresponses, similar to previous work.[36] Unlike the other autonomic response measures, the SCR and PDR amplitude distributions were strongly right-skewed. To reduce non-normalcy, we thus square-root transformed SCR and PDR amplitudes. To better account for multiple fluctuations in pupil dilation and skin conductance across a trial, we focused on full-trial AUC analyses for the main manuscript; however, we present results using additional autonomic response measures in the Supplementary Materials (available online as supplemental digital content at http://links.lww.com/PAIN/A778). Conclusions were similar across autonomic response measures, and we discuss any discrepancies in the main manuscript.

2.5. Analytic strategy

As temperature, pain, and autonomic responses varied within subjects, we tested our hypotheses using multilevel models, which incorporate an intercept and slope for each participant and allow for different numbers of observations across participants. To test main effects and interactions without controlling for competing variables, we used custom-built code in Matlab (the *glmfit_multilevel.m* function, available at https://github.com/canlab/CanlabCore) to implement linear mixed models. We used multilevel logistic regression to examine pain categorization in the TSPA (ie, to predict whether stimuli were labeled painful or innocuous), as implemented with the *glmer* function in R Studio 0.99.473 (R Studio, Inc, Boston, MA). In all analyses, we person-centered predictors and, when testing interaction effects, also included the main effects of variables comprising interaction terms in our statistical predictor models.

2.5.1. Multilevel mediation and mediated moderation analyses

To examine process models that linked temperature, subjective intensity, and autonomic responses, we used multilevel mediation.[9,46,52] Multilevel mediation and mediated moderation was assessed in Matlab using the Mediation Toolbox (https://github.com/canlab/MediationToolbox).[3,90] See **Figure 2** for a graphical depiction of hypothetical mediation and mediated moderation models. We implemented a mediation model with data from the ASC task to test whether stimulus temperature affected the autonomic heat response through pain intensity (**Fig. 2A**). In this mediation model, path a represents the effect of stimulus temperature on pain intensity and path b represents the effect of pain intensity on the heat-evoked autonomic response while controlling for temperature. Path c represents the overall effect of stimulus temperature on the heat-evoked autonomic response without controlling for pain intensity, whereas path c' represents the direct effect of temperature on the autonomic response, while controlling for the indirect path from temperature through pain intensity to autonomic responses (multiplicative path ab).

Somewhat outdated, the traditional approach to mediation has focused on whether controlling for a variable affects the significance of the overall effect, suggesting mediation.[8] Within this framework, a reduced association between temperature and autonomic responses to nonsignificance when controlling for pain means that pain fully explains this association (ie, suggesting "full" mediation). By contrast, a partial reduction points to process variable(s) not accounted for in addition to pain (ie, suggests "partial" mediation). However, this approach is problematic because it does not account for sample size, which strongly influences the significance of overall and direct effects.[80] It has thus become customary to test indirect effects to infer mediation instead of interpreting differences in significance levels between overall and direct effects.[41,46,63] Consequently, we refrain from interpreting nonsignificant direct effects between temperature and autonomic responses when controlling for pain but point out theoretical implications in the case of partial mediation through pain.

In the TSPA task, we used mediated moderation[9] to test whether categorizing stimulation as painful relative to nonpainful influenced this process. As shown in **Figure 2B**, the mediated moderation model tested whether stimulus categorization (nonpainful vs painful) interacted with temperature and intensity (experienced warmth or pain) to form path coefficients. The Mediation Toolbox uses nonparametric bootstrapping, which is more robust in handling violations to normality than traditional, parametric approaches,[46] and downweights participants based on subject-level variance.[90] We used 5000 bootstrapping samples in each mediation analysis. Results between multilevel mediation and linear mixed effects models (which did not incorporate weighting or bootstraps) may diverge slightly because of this difference. Nevertheless, conclusions were nearly identical across different analytic strategies; we note any substantial differences throughout the article.

2.5.2. Model constraints

To estimate regression model parameters, we had to restrict analyses to participants who provided sufficient data to estimate first-level regression estimates. When testing associations with the SCR and PDR during the ASC task, we limited analyses to participants with at least 3 valid measurements in the predictors and predictands. These constraints reduced sample size to 61 participants when testing associations with SCR, to 71 participants when testing associations with PDR, and to 58 participants when testing associations between SCR and PDR indicators.

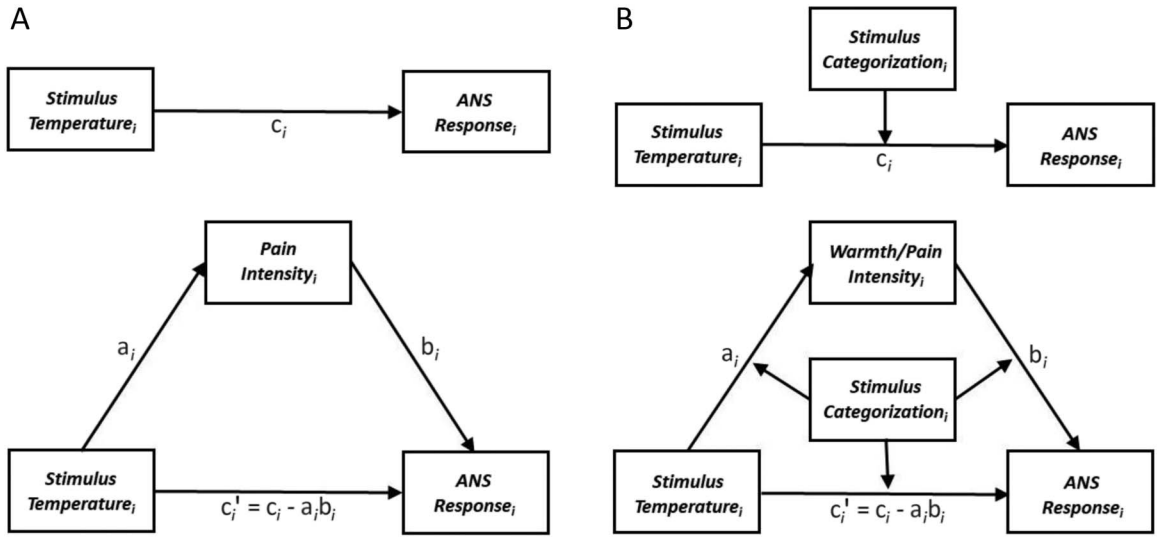

Figure 2. Theoretical process models: (A) *Mediation model*, in which the effect of stimulus temperature on the autonomic nervous system (ANS) response is statistically mediated by pain intensity. (B) *Mediated Moderation model*, in which the interaction effect between stimulus temperature and categorization in predicting the ANS response is statistically mediated by the interaction between stimulus categorization and intensity (experienced warmth or pain). In the mediation model, path c represents the overall effect of temperature on the ANS response, path a represents the effect of temperature on intensity, path b represents the effect of intensity on the ANS response, and path c' represents the direct effect of temperature on the ANS response, while subtracting the multiplicative indirect effect of temperature through intensity on the ANS response (path ab). In the mediated moderation model, paths represent the interaction effects of these model variables with the moderator stimulus categorization (nonpainful vs painful). Individual-level models (*i*) are aggregated to the group-level using nonparametric bootstrapping.

When testing associations with the SCR in the TSPA, we limited analyses to participants who had at least 7 valid measurements in predictors and predictands. We also made sure that for participants to be included in these analyses, participants had categorized at least 3 thermal stimuli as painful and at least 3 thermal stimuli as nonpainful. These constraints reduced sample size to 64 participants when testing interaction effects on SCR, to 66 participants in the post hoc simple slope analyses limited to painful stimulation, and to 68 participants in the post hoc simple slope analyses limited to nonpainful stimulation.

3. Results

We present results for both pain assessment tasks. First, we report basic relationships between temperature, pain, and autonomic responses separately for each task (**Table 1**). Next, we report results of our central analyses testing whether pain intensity mediates the effect of temperature on heat-evoked autonomic responses both in the ASC and TSPA. Finally, we compared this mediation model for temperatures categorized as painful and nonpainful. For bivariate correlations (averaged across participants) between temperature, warmth and/or pain intensity, and autonomic responses, see **Table 2** (ASC) and **Table 3** (TSPA).

Table 1

Associations between SCR and PDR measures in the adaptive staircase calibration.

Indicator	SCR predicting PDR			
	B	SE	t	*P*
AUC	98.45	22.16	4.44	<0.001
AUC (0.5-4 s)	95.39	44.27	2.15	0.035
AUC (4-8 s)	117.57	23.54	4.99	<0.001
AUC (8-12 s)	88.04	16.76	5.25	<0.001
Amplitude	5.47	0.70	7.83	<0.001
Latency	0.25	0.05	5.44	<0.001

N = 58 healthy adult volunteers. Analyses are restricted to participants who provided both at least 3 valid PDR and SCR measurements. Response amplitudes were square-root transformed to account for non-normalcy in distributions. Regression coefficients are unstandardized. SEs are standard errors of unstandardized regression coefficients.
AUC, area under the curve; PDR, pupil dilation response; SCR, skin conductance response.

3.1. Adaptive staircase calibration: basic relationships

3.1.1. Association between the skin conductance response and pupil dilation response

In the ASC task, SCR and PDR measures were strongly associated. Skin conductance response AUC was associated with PDR AUC (B = 98.45, SE = 22.16, t = 4.44, $P < 0.001$). We also found significant relations across all additional autonomic response measures, ie, amplitude, latency, and during all phases of heat stimulation (**Table 1**).

3.1.2. Effects of stimulus temperature on pain and autonomic responses

As expected, increases in stimulus temperature were associated with increases in perceived pain intensity (B = 0.82, SE = 0.04, t = 23.23, $P < 0.001$). Temperature also predicted increased autonomic arousal, with effects on both SCR AUC (B = 196.26, SE = 21.83, t = 8.99, $P < 0.001$) and PDR AUC (B = 50,564.69, SE = 4788.44, t = 10.56, $P < 0.001$) (for other SCR and PDR measures, see Supplemental Table 1, available online as supplemental digital content at http://links.lww.com/PAIN/A778). The upper panel of **Figure 3** depicts SCR and PDR across time as a function of temperature. Phase analyses revealed that effects on SCR and PDR were present during peak heat stimulation (AUC from 4 to 8 seconds) and after heat stimulation (AUC from 8 to 12 seconds), and for effects on PDR (but not SCR) during early heat stimulation (AUC 0.5-4 seconds) (see Supplemental Table 1, available online as supplemental digital content at http://links.lww.com/PAIN/A778).

Table 2

Mean bivariate correlations (SDs) across participants between temperature, pain, and autonomic response measures in the adaptive staircase calibration.

Measure	1.	2.	3.
1. Temperature	—		
2. Pain	0.83 (0.15)	—	
3. SCR (AUC)	0.44 (0.22)	0.45 (0.23)	—
4. PDR (AUC)	0.38 (0.18)	0.37 (0.21)	0.29 (0.28)

Correlations are calculated within-person and averaged across participants. SDs of correlations are reported in parentheses. Because of averaging, significance levels of correlations are not calculated. Correlation means and SDs are calculated using Fisher's z-transformation, to account for non-normalcy in the distribution of correlations.
AUC, area under the curve; PDR, pupil dilation response; SCR, skin conductance response.

3.1.3. Effects of pain on autonomic responses

The lower panel of **Figure 3** shows the effects of perceived pain (irrespective of temperature) on the baseline-corrected SCR and PDR in the ASC task. Analyses revealed that pain intensity predicted both SCR AUC (B = 227.98, SE = 26.21, t = 8.70, $P < 0.001$) and PDR AUC (B = 48,382.89, SE = 5381.81, t = 8.99, $P < 0.001$). Consistent with these findings, analyses revealed associations between pain and additional SCR and PDR measures (ie, amplitude, latency, and during all phases of heat stimulation), although the effect of pain on SCR during early heat stimulation was not significant (see Supplemental Table 1, available online as supplemental digital content at http://links.lww.com/PAIN/A778).

3.2. Two-step pain assessment: basic relationships

3.2.1. Effects of stimulus temperature and categorization on intensity ratings

Similar to the ASC task, we found that stimulus temperature predicted perceived intensity during the TSPA (B = 3.87, SE = 0.19, t = 20.55, $P < 0.001$), when testing the simple association between temperature and perceived intensity without considering stimulus categorization as a moderating variable. Furthermore, a multilevel logistic regression analysis showed that higher temperatures were more likely to be categorized as painful (B = 1.12, SE = 0.08, z = 13.77, $P < 0.001$). An additional multilevel logistic regression analysis without person-centering temperature allowed us to determine the pain threshold across all participants at 44.8°C. Accordingly, temperatures below that threshold were more likely to be categorized as nonpainful, whereas temperatures above were more likely to be categorized as painful.

Table 3

Mean bivariate correlations (SDs) across participants between temperature, intensity (warmth or pain), and SCR for nonpainful (below diagonal) and painful (above diagonal) stimulation in the 2-step pain assessment.

Measure	1.	2.	3.
1. Temperature	—	0.77 (19)	0.42 (0.28)
2. Warmth/pain	0.53 (0.29)	—	0.41 (0.31)
3. SCR (AUC)	0.10 (0.29)	0.17 (0.30)	—

Correlations are calculated within-person and averaged across participants. SDs of correlations are reported in parentheses. Because of averaging, significance levels of correlations are not calculated. Correlation means and SDs are calculated using Fisher's z-transformation, to account for non-normalcy in the distribution of correlations.
AUC, area under the curve; SCR, skin conductance response.

However, when testing the moderating role of stimulus categorization, temperature effects on subjective intensity were qualified by a stimulus temperature × stimulus categorization interaction (B = 4.73, SE = 0.69, t = 6.81, $P < 0.001$). Post hoc simple slope analyses revealed that stimulus temperature predicted perceived intensity more when the stimulation was categorized as painful (B = 8.48, SE = 0.52, t = 16.29, $P < 0.001$) than when the stimulation was categorized as nonpainful (B = 4.06, SE = 0.38, t = 10.73, $P < 0.001$). These analyses suggest that stimulus temperature and subjective intensity are related, with painful temperatures relating to perceived intensity to a greater extent than nonpainful temperatures.

3.2.2. Effects of stimulus temperature, perceived intensity, and categorization on autonomic responses

Figure 4 depicts the SCR for painful and nonpainful stimulation across time as a function of temperature (upper panel) and perceived intensity (lower panel). Both stimulus temperature and perceived intensity predicted SCR AUC to a greater extent when temperatures were categorized as painful relative to nonpainful, as painful vs nonpainful stimulus categorization interacted both with temperature (B = 404.25, SE = 94.82, t = 4.26, $P < 0.001$) and perceived intensity (B = 21.84, SE = 7.13, t = 3.06, $P = 0.003$). Interactions on additional SCR measures are reported in Supplemental Table 1 (available online as supplemental digital content at http://links.lww.com/PAIN/A778). To further probe these interactions, we conducted post hoc analyses, testing the associations of temperature and perceived intensity with autonomic responses *separately* for stimulation categorized as painful and stimulation categorized as nonpainful.

3.2.3. Autonomic responses to stimuli judged as painful

Replicating findings from the ASC task, both stimulus temperature (B = 412.77, SE = 95.07, t = 4.34, $P < 0.001$) and pain (B = 29.63, SE = 6.15, t = 4.82, $P < 0.001$) predicted SCR AUC in separate analyses. These effects were present on all additional SCR measures, with the exception of the SCR during early heat stimulation (see Supplemental Table 1, available online as supplemental digital content at http://links.lww.com/PAIN/A778).

3.2.4. Autonomic responses to stimuli judged as nonpainful

Associations were weaker when temperatures were categorized as nonpainful. Temperature did not predict SCR AUC, although the effect approached significance (B = 31.95, SE = 17.76, t = 1.80, $P = 0.077$); by contrast, perceived intensity predicted SCR AUC when stimuli were judged as nonpainful (B = 7.25, SE = 2.57, t = 2.82, $P = 0.006$). It is noteworthy, though, that temperature predicted SCR AUC for nonpainful stimulus when using nonparametric bootstrapping (see analyses below). Associations of temperature with other SCR measures were more consistent. Additional analyses revealed that stimulus temperature predicted SCR amplitude and latency, perhaps because objective temperature predicted increased SCR only during peak heat stimulation and after heat offset (see Supplemental Table 1, available online as supplemental digital content at http://links.lww.com/PAIN/A778).

3.3. Process models linking autonomic responses with temperature and perceived pain

The above analyses demonstrate that objective stimulus intensity (ie, temperature) and perceived intensity (ie, pain) both predict

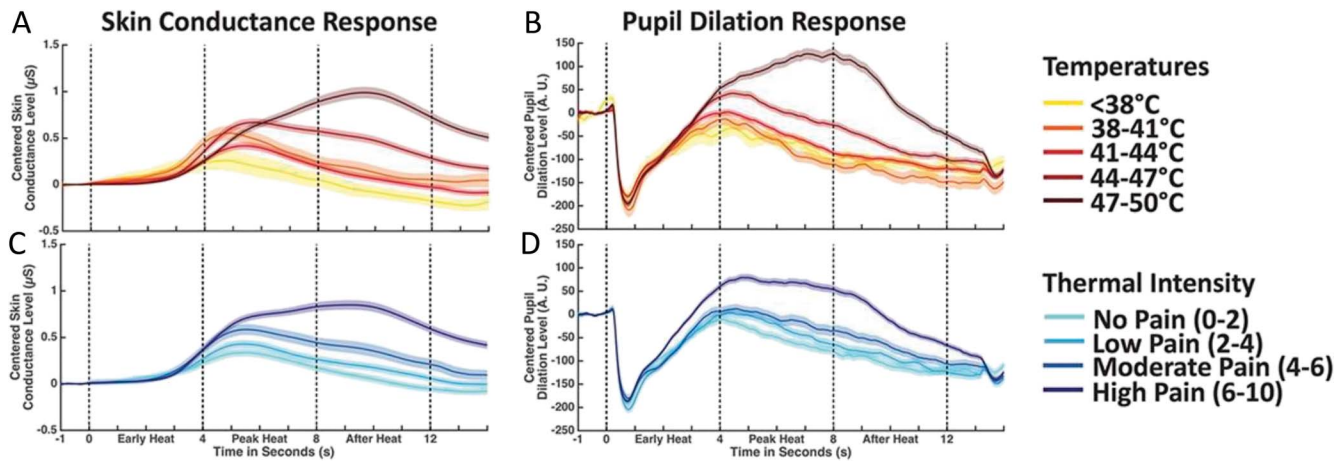

Figure 3. Averaged baseline-centered autonomic arousal responses (SCR and PDR) across heat trials in the adaptive staircase pain assessment. (A) Skin conductance response and (B) PDR, both by actual stimulus temperature. (C) Skin conductance response and (D) PDR, both by perceived thermal intensity. Pupil dilation is measured in arbitrary units (a.u.). For the purpose of visualization, responses on the thermal intensity VAS are divided up into 4 categories, to reflect a "No Pain" (0-2 VAS) category, and 3 pain categories: Low Pain (2-4 VAS), Moderate Pain (4-6 VAS), and High Pain (6-10 VAS). Analyses used continuous, rather than categorical, pain intensity ratings. PDR, pupil dilation response; SCR, skin conductance response; VAS, visual analogue scale.

autonomic responses when modeled separately, consistent with previous work.[36] Robust effects of temperature and subjective pain/intensity were present in both tasks, although we found differences based on pain categorization in the TSPA. Next, we tested whether subjective pain/intensity mediated the effect of temperature on the heat-evoked autonomic response, or whether temperature predicted the autonomic response independently from subjective pain/intensity. Unlike the linear mixed models reported above, we tested these hypotheses using multilevel nonparametric bootstrapping.[46]

3.3.1. Pain partially mediates temperature effects on autonomic responses in the adaptive staircase calibration task

In the ASC, we tested mediation models in which actual stimulus intensity (ie, temperature) indirectly predicted autonomic responses through perceived pain. We found significant partial mediation of both SCR and PDR AUC (**Fig. 5**). When controlling for stimulus temperature, pain continued to predict SCR AUC (path b = 115.85, SE = 24.28, t = 4.77, $P < 0.001$). By contrast, the effect of temperature on SCR AUC dropped from path c = 159.58, SE = 13.21, t = 12.08, $P < 0.001$ to c' = 65.28, SE = 20.82, t = 3.13, $P < 0.001$. As expected, the indirect effect of stimulus temperature through pain on SCR AUC was significant (path a × b = 85.57, SE = 18.54, t = 4.62, $P < 0.001$). Similarly, when controlling for stimulus temperature, pain continued to predict PDR AUC (b = 23,518.24, SE = 5185.64, t = 4.54, $P < 0.001$). The effect of temperature on PDR AUC dropped significantly when controlling for pain, from c = 40,169.60, SE = 2901.98, t = 13.84, $P < 0.001$ to c' = 19,126.27, SE = 4981.80, t = 3.84, $P < 0.001$, as a significant indirect effect from stimulus temperature through pain on PDR AUC indicated (a × b = 17,519.85, SE = 3943.52, t = 4.44, $P < 0.001$). We found similar partial mediation patterns for all other autonomic response measures, with the exception of SCR AUC during peak

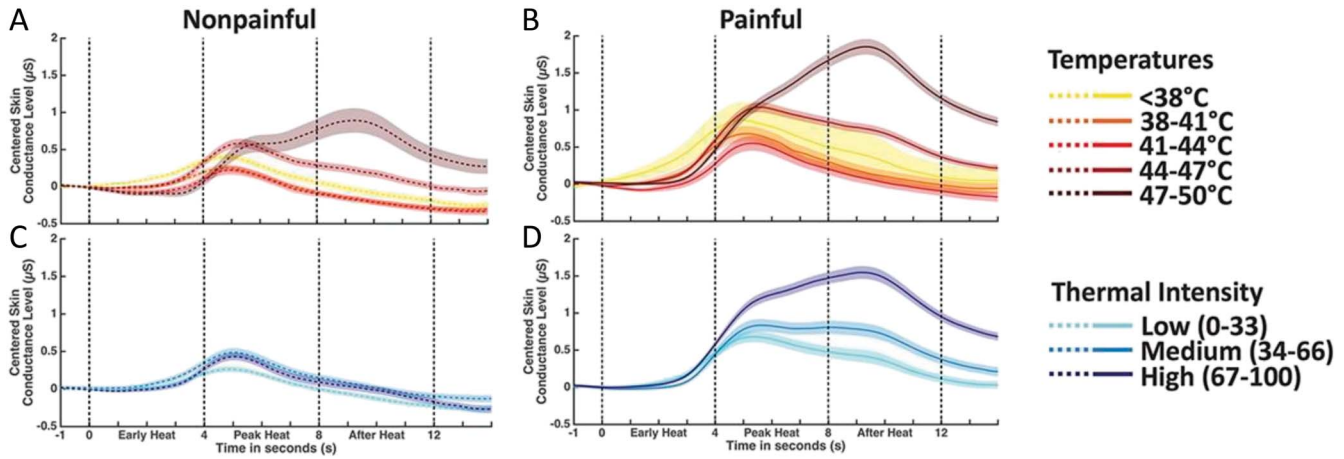

Figure 4. Averaged baseline-centered skin conductance response across heat trials in the 2-step pain assessment. Top: Skin conductance response by actual stimulus temperature, when thermal stimuli were rated as (A) nonpainful or (B) painful. Bottom: Skin conductance response by perceived thermal intensity, when thermal stimuli were rated as (C) nonpainful or (D) painful. For the purpose of visualization, responses on the thermal intensity VAS are divided up to reflect perceived nonpainful and painful intensity using 3 warmth categories and 3 pain categories: Low (0-33), Medium (34-66), and High (67-100). Analyses used continuous, rather than categorical, warmth and pain intensity ratings. VAS, visual analogue scale.

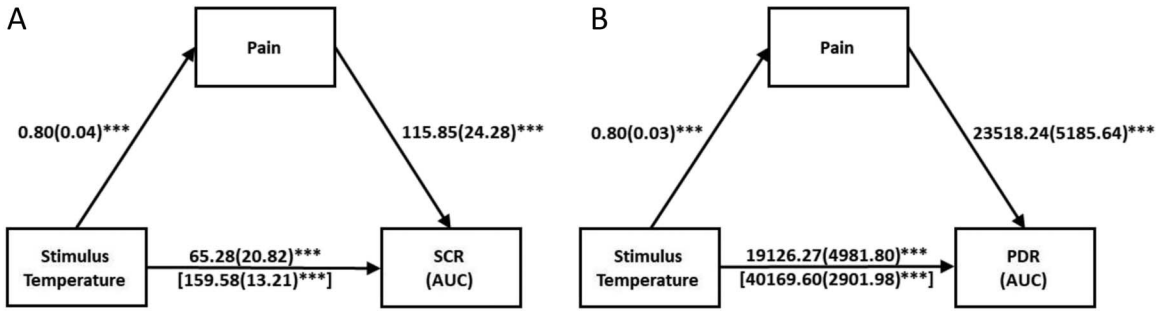

Figure 5. Modelling the association of actual stimulus intensity with (A) skin conductance response (SCR) and (B) pupil dilation response (PDR) through perceived pain (adaptive staircase pain assessment). Path coefficients are unstandardized regression coefficients and their SEs, derived using nonparametric bootstrapping. Statistics in square brackets indicate the associations of stimulus temperature with autonomic responses without controlling for pain. We calculated measures of SCR and PDR, calculating area under the curve (AUC) from 0.5 to 12 seconds after heat onset. See Supplemental Table 2 for more comprehensive statistical information (available online as supplemental digital content at http://links.lww.com/PAIN/A778). ***$P < 0.001$.

heat and for PDR AUC during early heat, in which pain fully mediated the effect of temperature on autonomic responses (see Supplemental Table 2, available online as supplemental digital content at http://links.lww.com/PAIN/A778).

3.3.2. Intensity mediates temperature effects on autonomic responses in the TSPA, depending on pain categorization: moderated mediation

Next, we extended the mediation model tested in the ASC. We found evidence for a mediated moderation model in which stimulus temperature predicted autonomic responses through perceived stimulation intensity, when testing effects on SCR amplitude and latency (see Supplemental Table 3, available online as supplemental digital content at http://links.lww.com/PAIN/A778), with the model showing stronger evidence of mediation during painful than nonpainful stimulation. By contrast, we did not find support for mediated moderation when testing SCR AUC (**Fig. 6**). When controlling for the temperature × stimulus categorization interaction, the perceived intensity × stimulus categorization interaction did not predict SCR AUC (b = 4.69, SE = 4.65, t = 1.01, $P = 0.314$). By contrast, the temperature × stimulus categorization interaction continued to predict SCR AUC when controlling for the perceived intensity × stimulus categorization interaction, only slightly dropping from c = 280.46, SE = 40.54, t = 6.92, $P < 0.001$ to c' = 214.68, SE = 56.02, t = 3.83, $P < 0.001$. The discrepancies in significant mediated moderation between the SCR measured as AUC compared with the SCR measured as amplitude and latency may be explained by the fact that we found evidence for mediated moderation measuring SCR AUC after heat stimulation, but not during early or peak heat (see Supplemental Table 3, available online as supplemental digital content at http://links.lww.com/PAIN/A517), suggesting that differences in the strength by which perceived stimulation intensity accounts for the effect of temperature on autonomic responses between painful and nonpainful stimulation emerged after the heat stimulus had subsided. Next, we decomposed the model and tested separate simple mediation models for stimuli categorized as painful and nonpainful.

When we only modeled trials categorized as painful, we replicated the partial mediation model from the ASC task: Perceived intensity partially mediated the association between stimulus temperature and SCR AUC (**Fig. 6**). When controlling for stimulus temperature, pain predicted SCR AUC (b = 12.21, SE = 4.38, t = 2.81, $P = 0.006$), while the effect of temperature on SCR AUC while controlling for pain dropped from c = 279.01, SE = 32.48, t = 8.60, $P < 0.001$ to c' = 174.79, SE = 48.17, t = 3.63, $P < 0.001$. As expected, the indirect effect of stimulus temperature through pain on SCR AUC was significant (a × b = 79.84, SE = 31.63, t = 2.52, $P = 0.021$). We found the same pattern of partial mediation for painful trials for all other SCR indicators, with the exception of SCR AUC during peak heat (see Supplemental Table 3, available online as supplemental digital content at http://links.lww.com/PAIN/A517), which showed full mediation, replicating findings with regard to SCR AUC during peak heat in the ASC task (see above).

Similarly, we found evidence for mediation when we tested the mediation model for trials categorized as nonpainful (**Fig. 6**). When controlling for temperature, perceived warmth predicted SCR AUC (b = 4.49, SE = 1.63, t = 2.75, $P = 0.005$). Unlike when testing simple associations above, temperature did predict the SCR when using nonparametric bootstrapping: When controlling for perceived warmth, the effect of temperature on SCR AUC dropped from c = 35.72, SE = 11.40, t = 3.13, $P < 0.001$ to c' = 7.63, SE = 12.42, t = 0.62, $P = 0.527$; the indirect effect from temperature through perceived warmth to SCR AUC was significant (a × b = 13.04, SE = 5.36, t = 2.43, $P = 0.017$). We found support for mediation on all other SCR measures, including SCR latency and amplitude (see Supplemental Table 3, available online as supplemental digital content at http://links.lww.com/PAIN/A778).

3.4. Supplementary analyses

For detailed methods and results, see the online supplement (available at http://links.lww.com/PAIN/A778). First, we ruled out the possibility that statistical mediation observed with linear modelling (ie, intensity as a mediator of temperature effects on ANS responses) was due to nonlinearities in the effects of temperature on intensity and/or ANS responses. However, assuming a power function underlying the sensory response to heat stimulation[76,86] and thus log-transforming model variables to move nonlinear associations between these variables into the linear space[1,23,76] did not substantially change associations with SCR and PDR AUC both in the ASC and TSPA (see Supplemental Table 4, available at http://links.lww.com/PAIN/A778).

Second, we ruled out the possibility that associations of stimulus categorization were confounded with temperature by testing the associations of stimulus categorization with SCR measures within restricted temperature ranges (see Supplemental Table 5, available at http://links.lww.com/PAIN/A778). Particularly in the medium

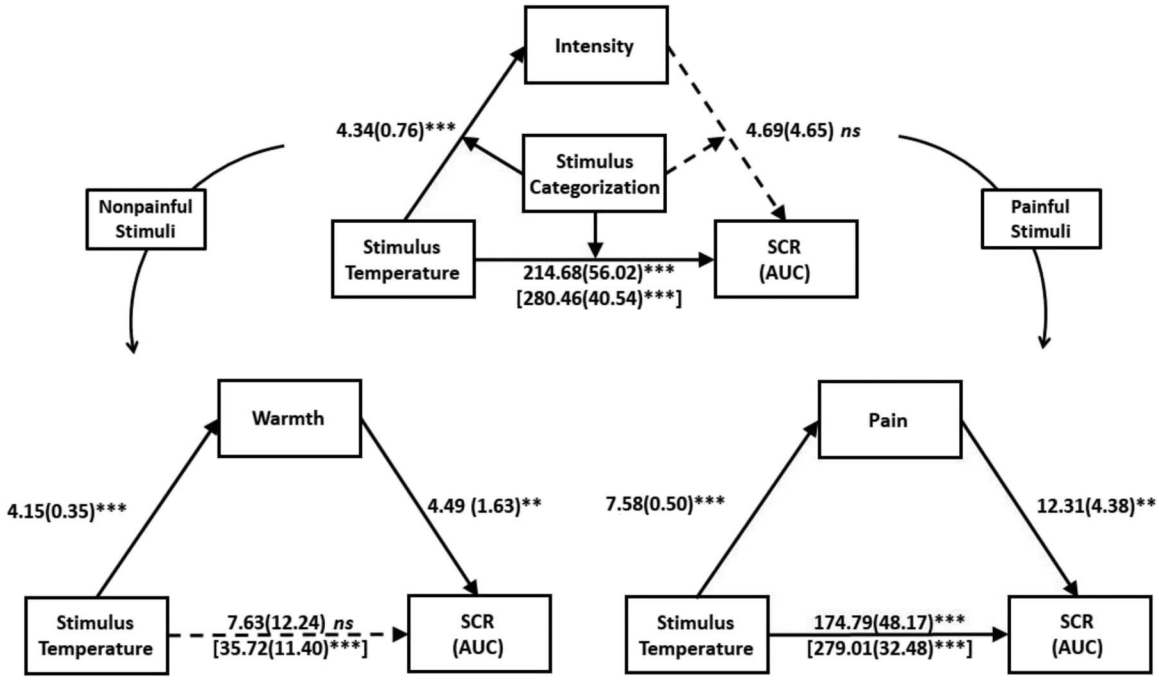

Figure 6. Modeling the association of actual stimulus intensity with skin conductance response (SCR) through perceived pain (2-step pain assessment). Stimulus categorization (nonpainful vs painful) moderates this model. The model is decomposed into separate mediation models analyzing stimulation perceived as painful and nonpainful. Path coefficients are unstandardized regression coefficients with their SEs, derived using nonparametric bootstrapping. Statistics in square brackets indicate associations without controlling for intermediate variables. Solid paths indicate statistically significant associations, whereas dashed paths indicate statistically nonsignificant associations. We calculated measures of SCR, calculating area under the curve (AUC) from 0.5 to 12 seconds after heat onset. See Supplemental Table 3 for more comprehensive statistical information (available online as supplemental digital content at http://links.lww.com/PAIN/A778). $^{***}P < 0.001$ and $^{**}P < 0.01$.

and high temperature range (44-50°C), SCRs were consistently elevated across different SCR measures when thermal stimuli were characterized as painful relative to nonpainful. In addition, we found some evidence for elevated SCRs even in the low range (41-44°C) when testing differences in SCR AUC and amplitude in response to painful relative to nonpainful stimulation. By holding temperature intervals constant, we ruled out the possibility that effects of stimulus categorization on the autonomic response are due to a positive association between stimulus categorization and temperature.

The latter finding somewhat explains why visual inspections of the effects of temperature and pain in the ASC (**Figs. 3A and C**) suggested weaker overall effects on SCRs than in the painful trials of the TSPA (**Figs. 4B and D**). The TSPA probably manages better than the ASC to identify painful temperatures, which may have led to stronger overall SCRs. When using continuous variables, however, temperature and intensity predicted SCRs in separate analyses to a similar extent regardless of task, suggesting that these tasks despite methodological differences capture similar aspects of the autonomic response to nociception.

4. Discussion

Across 2 different heat pain assessment tasks, we found that perceived pain consistently predicted autonomic responses over and above objective noxious heat stimulation (ie, temperature). Furthermore, we found statistical evidence for a mediation model, in which perceived pain partially mediated the effects of noxious heat on autonomic responses—both SCRs and PDRs. Our TSPA task revealed that this process was in turn moderated by subjective categorization of the stimulus as painful or nonpainful. Evidence for this mediated moderation model emerged when testing effects on SCR amplitude and latency and SCR AUC after heat stimulation. Follow-up analyses revealed that when heat stimulation was perceived as painful, we observed the same relationships as in the ASC task: subjective pain partially mediated temperature effects on autonomic responses. When heat stimulation was perceived as nonpainful, we similarly found that subjective warmth mediated the effects of temperature on autonomic responses. However, the latter process was weaker, in particular when focusing on autonomic responses in the short period right after heat stimulation.

4.1. Theoretical and practical implications

These findings have important theoretical implications, highlighting the importance of pain appraisal in regulating the autonomic response to noxious stimulation. Although there is functional overlap between bottom-up and top-down nociceptive processes and the ANS both on subcortical and cortical levels of the central nervous system,[10,11] it remained unclear whether conscious appraisals of pain are necessary for noxious stimulation to elicit a defensive autonomic response. Although past research has tested the unique contribution of pain to autonomic responding[66] or tested autonomic responses to both painful and innocuous heat stimulation,[59,85] our findings are the first to show that (1) pain statistically mediates the effect of varying noxious stimulation on autonomic responses, (2) this process is reduced for innocuous heat stimulation, and (3) explicit category judgments (ie, labeling pain vs no pain) directly modulate autonomic responses, irrespective of objective stimulus intensity. This pattern of findings suggests that consciously experiencing pain is important—if not crucial—for initiating a defensive autonomic response to tissue threat. Conversely, our findings rule out

a purely reflexive account of autonomic responding, in which increased autonomic activity reflects objective properties of noxious stimulation irrespective of the pain experience. By contrast, our findings suggest that experienced pain plays an important role in modulating spinal or supraspinal ANS responses to noxious stimulation. Such findings pose a challenge to accounts linking nociception directly to ANS responses while minimizing the mediating role of pain appraisal[42,43] as well as to important translational work of pain in nonhuman primates and rodents, as they emphasize the role of language and appraisal in driving autonomic responses in humans. By contrast, our findings are consistent with classic work on the role of emotional appraisal and affective experience in shaping the physiological response to threat.[33,35,38,56,57,81,84] It is noteworthy that pain did not fully account for the association between pain and autonomic responses, which suggest that other spinal or supraspinal processes in addition to pain are also involved in the autonomic response to noxious stimulation. However, such potential additional processes do not diminish the central role that pain seems to play in the autonomic, nociceptive response.

As a consequence, our findings document the flexibility of the autonomic pain response to quickly adapt to perceived or actual changes in environmental demands. Receiving information about upcoming pain can alter the autonomic pain response.[27,28,62,74] Furthermore, pain sensitivity is malleable and can quickly increase or decrease over time, leading to both sensitization and habituation depending on the level of repeated noxious stimulation.[25,51,91] To respond appropriately, ANS responsiveness needs to be able to adapt to various perceived or actual environmental circumstances. Pain appraisal that adjusts to situational affordances while regulating the autonomic response allows for such autonomic flexibility.

Our results suggest that autonomic responses are highly sensitive to changes in subjective pain, which can be interpreted as support for the use of ANS markers as nonverbal proxies of pain in pharmaceutical, medical, and legal settings. However, biomarkers require not only sensitivity but also specificity.[72] Autonomic responses are known to reflect arousal, which is inherently nonspecific: Increased autonomic arousal is associated with other emotionally salient events, such as during anticipation of pain,[4,26,71] when experiencing rewarding or surprising outcomes,[19,20,61] and during other types of aversive or appetitive stimulation, such as pleasant and unpleasant imagery.[15] Thus, the broad relevance of physiological arousal limits the suitability of these measures for nonverbal biomarkers of pain. An exciting direction is to isolate more fine-grained aspects of autonomic responses that predict pain with model-based methods[7,36] or a combination of autonomic measures.[60,87] However, any measures that are highly sensitive to pain must also be compared with other arousing processes to demonstrate specificity before serving as a potential nonverbal biomarker of pain.

4.2. Limitations and future directions

Several avenues for future research remain. Our findings need to be replicated and extended using different pain induction methods, such as cold stimulation, pressure stimulation, and electric shock. When conducting these studies, it is important to include innocuous control stimulation conditions, to compare unique associations between subjective stimulation and autonomic responding both for painful and nonpainful stimulation. Only a fraction of research on the association between acute pain and autonomic responses includes adequate innocuous stimulation, which limits conclusions about how unique the contribution of pain to ANS responsivity really is.[53] Furthermore, future research needs to include direct measures of peripheral nociception, to account for intraindividual and interindividual differences in afferent nociceptive input beyond the effect of temperature. For example, a microneurographic approach would allow to directly measure peripheral nociceptive input stemming from noxious heat stimulation.[40,69] Similarly, the nociceptive flexion response following electric stimulation of the sural nerve captures the pain threshold on the spinal level and correlates well with subjective pain intensity.[93]

Similarly, results need to be generalized to other measures of autonomic responding, such as measures of respiration or cardiovascular reactivity. We had to exclude a relatively large amount of trials in the SCR data because of artifacts (>21% of trials). Fortunately, we mostly replicate findings across SCR and PDR data in the ASC, which gives us confidence that exclusion rates did not unduly influence our findings. However, replication of these findings using other ANS measures is desirable. In general, including multiple autonomic measures allows for extracting a latent autonomic arousal response[36] and thus increases confidence that autonomic measures indeed tap into the same aspect of autonomic responding. Including measures of cardiovascular reactivity in particular would allow for disentangling sympathetic from parasympathetic aspects of the autonomic pain response. Skin conductance and pupil dilation measures in our research likely reflected the sympathetic nervous system's threat response to pain.[14,17,88] Other autonomic measures, such as heart rate variability, are more likely to capture parasympathetic downregulation of the sympathetic pain response.[49] Combining these measures would help to determine whether the association between pain and autonomic responses is driven by increased sympathetic activation, decreased parasympathetic activation, or both.

In addition, more research on the causal link between pain and autonomic responses is needed. Our findings provide statistical evidence for the role of pain in regulating the autonomic response to noxious stimulation. However, our findings are correlational and need to be supplemented with experimental manipulations of pain. There is substantial evidence that psychological interventions aimed to alter the pain experience, such as mood inductions, manipulating pain expectations, or hypnosis, affect autonomic responses.[53] It remains unclear, however, whether these interventions reduce ANS responses by directly reducing pain or by reducing other psychological and physiological processes associated with a defensive autonomic response. As a consequence, experimental research on pain and autonomic activation has convincingly documented the power of the mind in changing the autonomic pain response. Nevertheless, direct manipulations of pain and autonomic responses, such as through pharmacological interventions, are needed to complement existing psychological research to conclusively pinpoint the role of pain in the autonomic response to noxious stimulation.

Finally, it remains unclear how well autonomic responses track experimentally induced pain for people suffering from chronic pain. A number of studies show that chronic pain conditions, such as fibromyalgia, irritable bowel syndrome, or painful bladder syndrome, are associated with altered tonic ANS activity (see Ref. 29,65, for reviews). Furthermore, people with chronic pain syndromes may have impaired interoceptive access into body and mind processes (see Ref. 31, for a review). Altered autonomic responses and a lack of conscious insight into one's pain experience could reduce the functional association between pain and autonomic responses. Such a dissociation would in turn affect the diagnostic value of autonomic responding as a pain measure for people suffering from chronic pain conditions. According to recent research, during longer-lasting tonic pain

episodes in the range of minutes, experienced pain stops predicting autonomic responses above and beyond noxious stimulation,[67] perhaps because sensory aspects of noxious stimulation become less important and affective-motivational factors start to dominate the pain experience.[75] Consistent with our findings, however, past research on acute (6 seconds) heat pain has shown that variations in pain continue to predict changes in autonomic responses (skin conductance and heart rate) when holding stimulus temperature constant.[59] Thus, more research is needed to probe the relative contribution of pain and noxious stimulation in people suffering from chronic pain conditions and healthy volunteers.

5. Conclusion

In summary, our findings show that pain appraisal plays an important role in the defensive autonomic response to noxious heat stimulation. In 2 pain assessment tasks, we find that autonomic responding is not simply a reflexive reaction to potential or actual tissue damage, but that the individual pain experience plays an important role in the autonomic responses to objective noxious stimulation.

Conflict of interest statement

The authors have no conflict of interest to declare.

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Appendix A. Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PAIN/A778 and http://links.lww.com/PAIN/A517.

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