ORIGINAL ARTICLE

Unique Autonomic Responses to Pain in Yoga Practitioners

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ABSTRACT

Objective: Autonomic nervous system activity is associated with neurobehavioral aspects of pain. Yogis use breathing, relaxation, and mindfulness to tolerate pain, which could influence autonomic responses. To evaluate how the link between autonomic responses and pain is altered by other factors, we compared perceptual and autonomic responses to pain between yogis and controls.

Methods: Nineteen yogis and 15 controls rated warm and painfully hot stimuli (1-cm^2 thermode on calf), with visual anticipatory cues indicating certainly painful, certainly nonpainful, or uncertainly either painful or nonpainful. Heart rate, skin conductance, respiration, and blood pressure were measured.

Results: At baseline, yogis breathed slower and deeper than did controls, with no differences in other autonomic measures. During the task, perceptual ratings did not differ between groups in either the certain or uncertain conditions. Nevertheless, yogis had higher phasic skin conductance responses in anticipation of and response to all stimuli, but particularly during painful heat in uncertain contexts (uncertain: 0.46 [0.34] μS; certain: 0.37 [0.28] μS; $t(18) = 3.962$, $p = .001$). Furthermore, controls showed a decrease in heart rate to warm (−2.51 [2.17] beats/min) versus painful stimuli (0.83 [1.63] beats/min; $t(13) = 5.212$, $p < .001$) and lower respiratory sinus arrhythmia during pain compared with warm trials, whereas yogis had similar reactions to painful and nonpainful stimuli.

Conclusions: Autonomic responses to pain differed in yogis and healthy volunteers, despite similar pain ratings. Thus, autonomic reactivity to pain may be altered by environmental and psychological factors throughout an individual's life.

Key words: pain, yoga, autonomic nervous system.

INTRODUCTION

Pain is inherently a subjective experience that is influenced by environmental factors throughout the life-span, including early childhood adverse experiences (1). Nevertheless, there has been a quest for an "objective" measure of pain, including methods such as brain imaging and recording autonomic responses. Such measures are particularly important in nonverbal populations, including infants, anesthetized patients, and patients with dementia or paralysis. Measurements of autonomic responses, most frequently heart rate (HR) and skin conductance, have frequently been used as a nonverbal methods of assessing pain (2). A number of studies have shown an increase in HR and/or skin conductance in response to the application of a noxious stimulus (3–5), yet these measures do not reliably correlate with pain perception (3,6,7). This may be because factors other than pain, such as attention and cognitive demands, also lead to the modulation of the autonomic response and associated changes in HR and skin conductance (8,9), and it is not clear how such factors interact with pain in influencing the autonomic nervous system (ANS).

Yoga is a practice that uses meditative techniques in combination with movement and breathing to create a state of highly focused inward attention (10) and influences both autonomic functioning and pain perception, with evidence that yoga has an impact on the ANS (11–15). In addition, clinical evidence in pain patients, as well as one experimental study in healthy subjects, shows that practicing yoga can influence pain perception and tolerance (16,17). Furthermore, it seems that yoga practitioners (yogis) use different cognitive strategies from those used by controls for tolerating pain (16). Studies have begun to elucidate neurological and physiological alterations that point to enhanced emotional self-regulation, stress coping, and cognitive control in yoga practitioners, which could mediate improved pain modulation (reviewed in Refs. (1,10)). Neuroimaging data

ANOVA = analysis of variance, **ANS** = autonomic nervous system, **DBP** = diastolic blood pressure, **ECG** = electrocardiogram, **HR** = heart rate, **HRV** = heart rate variability, **NN50** = the number of pairs of successive NN (R-R) intervals that differ by more than 50 ms, **QRS complex** = name for the graphical deflections seen on a typical ECG, made of Q, R, and S waves, **pNN50** = the proportion of NN50 divided by the total number of NN (R-R) intervals, **RMSSD** = root mean square of successive differences, **R-R interval** = cycle length between R waves, **RSA** = respiratory sinus arrhythmia, **SBP** = systolic blood pressure, **SCL** = skin conductance level, **SCR** = skin conductance response, **SDRR** = standard deviation of RR intervals

show that long-term yoga practitioners demonstrate protection from age-related gray matter decline (18), a signature that has been suggested to accelerate in patients with chronic pain and other stress-related disorders such as posttraumatic stress disorder (for review, see Refs. (1,19).

In the current study, we took advantage of expected differences in cognitive techniques of yogis and nonyogis to evaluate the interaction between pain-evoked and cognitively modulated changes in autonomic activity. Hypothesizing that yogis may have different perceptual and/or autonomic responses to pain compared with nonyogis, we compared subjective pain reports and objective autonomic measures between these two groups. Previous studies have found that responses to pain differ when pain is presented in certain and uncertain contexts (20,21). Thus, to further vary cognitive and emotional demand that could influence autonomic functioning, we compared responses both during expectation periods and during stimulation in which pain was certain or uncertain.

METHODS

Participants

Nineteen yogis and 15 physically active people ("controls") completed the study between January 2014 and October 2015. All participants provided informed consent and were monetarily compensated in accordance with approval from the National Institutes of Health central nervous system institutional review board.

Exclusion criteria included major stressful life events in the last 3 months, irregular sleep schedule, use of nicotine or recreational drugs in the last 6 months, daily consumption of more than 400 mg of caffeine, weekly consumption of more than 7 (women) or 14 (men) alcoholic drinks, pregnancy or breastfeeding, colorblindness, chronic pain, major medical condition, chronic systemic disease, psychiatric disorder, condition affecting cardiac or vascular function, or taking within the last 3 months medication that could interfere with study measures.

Inclusion criteria for yogis included a minimum of 6 years of yoga experience, practice of at least 4 hours per week on average in the last 3 years, and training in a yoga style that encompassed postures, breathing exercises, and relaxation, with at least 50% of their practice devoted to postures. Practitioners were excluded if they practiced other mind-body techniques such as tai chi or karate, more than 1 hour per week combined.

Control subjects qualified if they were physically active, exercising at least 4 hours per week including walking, but had not practiced yoga, meditation, or martial arts at all in the last year or more than once per week for more than 4 months in their lifetime.

Groups were matched on age, sex, body mass index, physical activity level (excluding yoga), education, household income, ethnicity/race, and reproductive status. All participants were instructed to refrain from exercising or consuming caffeinated products for 2 hours before testing, and to refrain from drinking alcohol or taking any medications outside their usual regimen for 24 hours prior. Regularly cycling women (including those using hormonal contraceptives) were always tested in the first 12 days of their menstrual cycle, unless they were on monophasic hormonal contraception, in which case they were tested at any time.

Testing Procedure

In a single session, subjects were recorded for 5 minutes of baseline autonomic measures, trained on use of the rating scales, calibrated for a heat stimulus–evoking moderate pain, familiarized with the thermal stimuli, and finally tested on the thermal task (Figure 1A). Testing was performed by a female experimenter (C.V.) who was blinded to participants' yoga experience.

Baseline Autonomic Measures

To establish baseline measurements of HR, respiration, electrodermal activity, and blood pressure, participants were connected to physiological recording devices and 5 minutes of baseline measures was recorded (baseline block; Figure 1A) while participants were in a semireclined position in a chair. They were instructed to relax and gaze at a crosshair on a screen for the 5 minutes without moving or speaking.

Intensity and Hedonics Rating Scales

Participants were trained to rate the intensity and hedonics (pleasantness or unpleasantness) of stimuli using visual analog scales. The intensity scale ranged from 0 (no heat sensation) to 200 (intolerable pain), with 100 labeled as pain threshold. The unpleasantness scale ranged from −100 (extremely unpleasant) to +100 (extremely pleasant), with 0 as neutral. In previous studies, these scales were sensitive to subtle psychological manipulations (22,23).

### Choosing a Moderately Painful Temperature and Task Familiarization

To familiarize each participant with rating the heat stimuli and to determine an individually calibrated "moderately painful" temperature ("thermal calibration"; Figure 1A), we applied an ascending series of 6-second heat stimuli between 38°C and 50°C, alternating across four locations on the inner surface of the right calf (organized proximal to distal), using a 3×3-cm CHEPS thermode (Medoc Ltd; Advanced Medical System, Ramat-Yishai, Israel). The calf was chosen for stimulation to keep subjects' hands free for electrodermal recordings and manual pain ratings. Subjects rated the intensity of each stimulus, and a moderately painful stimulus (intensity rating between 150 and 180) was selected for testing in the thermal task. Participants were not told that only one painfully hot and one warm stimulus would be used in the thermal task. The moderately painful stimulus chosen for subjects ranged from 44°C to 50°C, and with no group difference ($t(32) = 1.000$, $p = .325$; yogis: 47.87°C [1.23°C], controls: 47.33°C [1.88°C]). A 40°C stimulus was chosen as the nonpainful warm stimulus for all subjects because this temperature was clearly perceptible but always below pain threshold.

Thermal Task

Sitting in a semireclined position, participants viewed a screen while thermal stimuli were applied across four proximal to distal delineated locations of the inner left calf (Figure 1B). Each trial started with a 6-second initial blank screen wait period ("Wait1"), followed by a 6-second green, red, or yellow cross that indicated with certainty or not whether the following thermal stimulus was painfully hot ("pain") or nonpainful warm ("warm"). The red cross signified upcoming pain (certain-pain condition), green indicated upcoming nonpainful warmth (certain-warm condition), and yellow meant either pain or warm would occur (uncertain-pain or uncertain-warm condition, 50% probability of each undisclosed to participants). Next, participants viewed a picture of the thermode while being stimulated with either the warm 40°C temperature or the "moderately painful" temperature calibrated to that individual. This was followed by a 4-second wait period ("Wait2"), indicated by a black cross, which was included to capture delayed physiological responses (3). The participants then had 16 seconds to rate intensity and hedonics while the VAS scales were displayed on the screen. E-Prime 2.0 software (Psychology Software Tools, Pittsburgh, PA) was used to electronically control visual stimulus presentation and thermal stimulus parameters.

Physiological Data Acquisition, Preprocessing, and Analysis

Physiological data were acquired at a sampling rate of 1000 Hz (MP 150 amplifier system, AcqKnowledge 4.3 software; Biopac Systems, Inc, Goleta, CA) and preprocessed by an experimenter blinded to the subject's group. For all preprocessing filters, a Blackman −61-dB window was used.
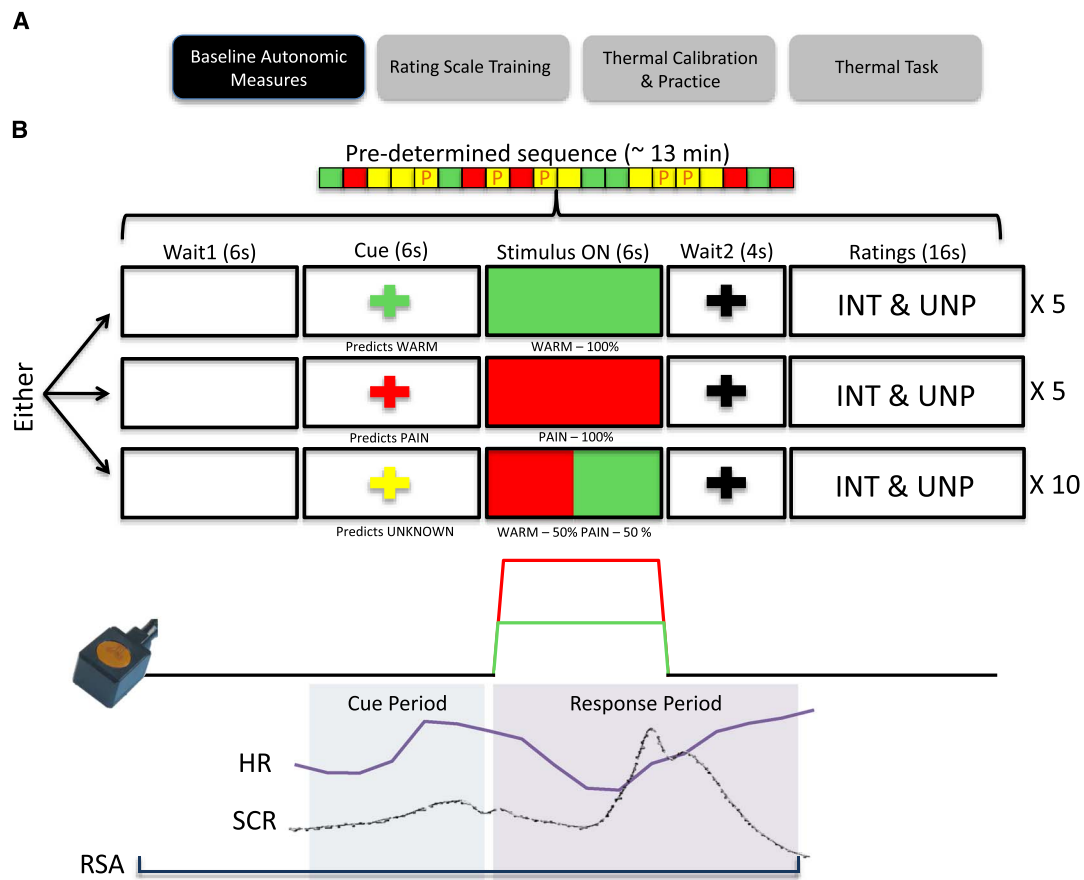
FIGURE 1. Experimental design. A, Testing session timeline. Black blocks refer to 5 minutes of physiological recording, without stimulation. B, Thermal task. Each trial contained the following periods: Wait1, cue, stimulus, Wait2, and rating. The thermode was moved among four locations at the start of each rating period. Physiological measures were acquired for the entire 13-minute duration of the task, but were analyzed within the cue period and the response period (stimulus + Wait2). All subjects received the same sequence of trial conditions (green, certain-warm; red, certain-pain; empty yellow, uncertain-warm; yellow with "P," uncertain-pain). Participants were asked to not move or speak for the duration of the task except during rating periods. A representative trace from HR and SCR are presented for the cue and response periods (bottom). HR = heart rate; SCR = skin conductance response; RSA = respiratory sinus arrhythmia; INT = intensity; UNP = pleasantness/unpleasantness.

Electrodermal Activity and Phasic Skin Conductance Response

Skin conductance was recorded using the EDA100C-MRI amplifier (Biopac Systems). Two disposable Ag-AgCl electrodes were placed at the distal phalanxes of the index finger (ground lead) and the middle finger (positive lead) of the nondominant hand.

A 1-Hz low-pass filter was applied to all skin conductance recordings to eliminate noise. Mean tonic skin conductance level (SCL) was analyzed in 5-second blocks. A phasic skin conductance response (SCR) channel was created for thermal task recordings by applying a 0.05-Hz high-pass filter, allowing for analysis of specific responses normalized to an individual's baseline SCL drift (24). We extracted the maximal magnitude of SCRs occurring within the cue and response periods, and calculated the mean magnitude of all trials within each condition. Because SCRs occur at least 1 to 4 seconds after stimulus onset (3,24), we derived the magnitude of stimulus-related SCRs from the maximum phasic response that occurred within the 10-second period encompassing stimulus delivery and the post-stimulus wait period (stimulus + Wait2 = "response period"; see Figure 1B).

Respiration Waveform and Respiration Rate

Two respiration transducers were snugly fit at thoracic (~5 cm below the armpits) and abdominal levels (slightly above the navel) and connected to

RSP100C amplifiers (Biopac Systems). Low-pass 10-Hz filters were applied, and signals were centered so that peaks and valleys were not restricted. Before analysis, the waveforms were resampled to 62.5 Hz, and a band-pass filter of 0.05 to 1 Hz was applied to center the entire waveforms around zero and remove artifacts. There were two participants (both yogis) whose breathing rates were so slow at baseline that this filter distorted the signal; for these cases, a filter of 0.04 to 1 Hz was sufficient to zero and refine the signal without losing data.

To calculate respiration rate and amplitude, we used procedures recommended in the Biopac Users Guide (https://www.biopac.com/wp-content/uploads/BioHarness_Guide.pdf). We created a "rate channel" from either the thoracic or abdominal waveform, depending on which was more accurately centered on zero. The rate channel captured instantaneous breathing rate in cycles per minute, used to extract the mean breathing rate for different periods. For both waveforms, the amplitude of each respiration cycle was determined by subtracting the minimum value of the exhalation from the peak of the inhalation (in volts).

Electrocardiogram, HR, and HR Variability

The electrocardiogram (ECG) was recorded using three electrodes on the chest, connected to the ECG100C-MRI (Biopac Systems) with a band-pass filter of 0.5 to 35 Hz. QRS complex labels were automatically applied to

R-peaks and manually inspected and corrected if needed. In the case of rare ectopic beats, the QRS label was removed and a peak was interpolated by splitting the time between the previous normal peak and the next normal peak in half.

For HR analysis, we created a "rate channel" derived from the QRS labels on the filtered ECG, which displayed beat-to-beat HR in beats per minute (bpm) for the entire recording and used to determine instantaneous HR throughout various periods of the study.

For HR variability (HRV), we evaluated respiratory sinus arrhythmia (RSA), frequency domain/total HRV power, and several time domain measures including the standard deviation of R-R intervals (SDRR), root mean square of successive differences (RMSSD), and percentage of adjacent R-R intervals that differ by more than 50 milliseconds (pNN50). These are all common indices for autonomic circulatory control (reviewed in Ref. (25)).

RSA was calculated in AcqKnowledge using the peak-valley method described by Grossman et al. (26), in which the shortest R-R interval is subtracted from the longest R-R interval in each respiratory cycle. For 5-minute blocks, we computed mean RSA for all complete respiration cycles that fell within the block. For the thermal task, we measured the mean RSA for all complete respiration cycles within the trial (excluding rating period; including Wait1 to Wait2).

Frequency domain analysis was performed using automated parameters in AcqKnowledge with a spline resampling frequency of 2 Hz, outputting power for low-frequency and high-frequency bands typical of HRV analyses (27). However, because several subjects (eight yogis and two controls) had a breathing rate that sometimes fell below 9 cycles per minute (0.15 Hz; the cutoff between these frequency bands), RSA partially shifted from the high-frequency band to low-frequency band. Because it is difficult to disentangle RSA's contribution to each frequency band, we report total frequency power, a measure that indicates predominantly parasympathetic tone (28).

Time domain analyses were performed by extracting all R-R intervals of each 5-minute block, and SDRR, RMSSD, and pNN50 calculated these measures for each block.

Real-Time Noninvasive Blood Pressure

Systolic and diastolic blood pressure (SBP, DBP) were continuously recorded using a noninvasive air pressure bladder attached to the proximal phalanx of the thumb and were monitored in real-time using CareTaker 4.1.0 (Empirical Technologies Corporation, Charlottesville, VA).

Calculating Change in HR and SBP for Thermal Task Conditions

We subtracted the HR or SBP at each second of the trial from the value at the last second of the Wait1 period, yielding relative HR and SBP responses to cues and stimuli. We then calculated for each trial condition the mean HR and SBP change within the 6-second cue period and the 10-second response period (stimulus + Wait2; see Figure 1B).

Statistical Analysis

All behavioral and physiological data were analyzed using SPSS (IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY), using a significance level of $p < .05$. Post hoc within-subject/pairwise t tests and independent-sample t tests were used to evaluate effects detected by mixed-model analyses of variance (ANOVAs). Values presented in text and tables are displayed as mean [standard deviation].

Five-Minute Baseline Physiological Measures

Independent-sample t tests were used to compare group means (yogis versus controls) for each physiological measure during baseline. Physiological measures for this analysis included mean HR, SBP, DBP, respiration rate, thoracic respiration amplitude, abdominal respiration amplitude, tonic SCL, and five measures of HRV (mean RSA, SDRR, RMSSD, pNN50, and frequency total power).

Physiological Responses to Cues During the Thermal Task

A 2 × (3) mixed-model ANOVA with one between-group factor with two levels (yogis, controls) and one repeated measure with three levels (cue: green/certain warm, red/certain pain, and yellow/uncertain) was used to assess phasic mean HR, SBP, and phasic SCR magnitude differences during the cue period.

Ratings and Physiological Responses to Thermal Stimuli During the Thermal Task

A 2 × (2 × 2) mixed-model ANOVA with one between-group factor with two levels (yogis, controls) and two repeated measures with two levels each (context: certain, uncertain; stimulus: warm, painful) was performed to evaluate intensity and hedonic ratings as well as physiological responses during each response period, including phasic mean HR, mean SBP, and phasic SCR magnitude differences.

RSA by Trial

Because RSA analysis encompassed cue and response periods (Wait1 to Wait2; see Figure 1B), we could only evaluate effects by comparing whole trials. To evaluate whether the type of stimulus (warm or painful) affected RSA, we used a 2 × (2) mixed-model ANOVA with one between-group factor with two levels (yogis, controls) and one repeated measure with two levels (stimulus: certain warm, certain painful).

RESULTS

Baseline Physiological Recordings

During the 5-minute baseline, there were no differences between yogis and controls for mean HR, SBP, DBP, tonic SCL, or measures of HRV and RSA (Table 1). However, yogis had significantly slower respiration rates ($p = .047$) and significantly deeper abdominal respiration than did controls ($p = .014$), as well as a tendency for breathing deeper at the level of the thorax ($p = .087$; Table 1). These findings show that the yogis had in fact practiced enough yoga to engage yoga-like breathing without explicit instruction to do so at rest.

Thermal Task

Intensity and Hedonic Ratings

Intensity and unpleasantness ratings did not differ between yogis and controls (Figure 2; all p values ≥.297; see figure captions for details and specific p values). For both groups, however, the uncertain context significantly increased the intensity ratings of warm, but not of painful stimuli.

Dynamic HR Response

Although mean HR traces suggest that yogis and controls may respond differently during the cue period (Figure 3A), there were no significant differences in HR responses to the different cues in either group, or any differences between groups (Figure 3B, statistical details in legend).

As shown in Figure 3C, HR was not significantly affected by certainty context for either yogis or controls. However, controls showed a higher HR during pain than during warm stimuli, whereas yogis' HR was not different during painful and nonpainful stimuli (statistics in legend).

TABLE 1. Demographics and Physiological Measures at Baseline

	Yogis (*n* = 19)	Controls (*n* = 15)
Age, y	43.63 (11.57)	42.73 (11.79)
Sex (M/F)	4/15	4/11
BMI, kg/m^2	22.78 (2.66)	24.57 (2.86)
Systolic BP, mm Hg	117.31 (12.49)	117.48 (11.75)
Diastolic BP, mm Hg	77.38 (9.33)	76.88 (10.98)
Tonic SCL, μS	4.00 (2.65)	3.44 (2.63)
Respiration rate, cpm	11.15 (4.68)*	14.03 (3.00)*
Thoracic amplitude, V	4.19 (3.17)†	2.59 (2.11)†
Abdominal amplitude, V	2.98 (2.58)*	1.26 (1.07)*
Heart rate, bpm	66.35 (6.35)	67.81 (14.82)
RSA, ms	96.20 (54.45)	74.81 (28.80)
SDRR, ms	49.1 (21.9)	52.3 (18.1)
RMSSD, ms	37.6 (17.6)	45.4 (22.6)
pNN50, %	14.43 (15.82)	23.43 (19.00)
Total power (frequency domain), ms^2	1076.2 (1174.0)	926.7 (667.7)

SD = standard deviation; M/F = male/female; BMI = body mass index; BP = blood pressure; SCL = skin conductance level; RSA = respiratory sinus arrhythmia; SDRR = standard deviation of RR intervals; RMSSD = root mean square of successive differences; pNN50 = the proportion of NN50 divided by the total number of NN (R-R) intervals.

Values are mean (SD), unless otherwise indicated.

* Significant differences between groups for *t* test (*p* < .05).

† Tendency for difference between groups (.05 < *p* < 1).

Phasic SCR
Both yogis and controls showed higher SCR during the certain-pain cue than during certain-warm or uncertainty cues, with no difference between the certain-warm and uncertain cues. However, yogis had higher SCR responses than did controls to all cues (Figure 4A, statistics in legend).

Regardless of group, SCR was significantly higher for painful than warm stimuli. Furthermore, yogis had larger phasic SCRs in response to both warm and painful stimuli when they were presented in uncertain contexts. Finally, yoga practitioners were more responsive than controls for phasic SCRs to each condition (Figure 4B, statistics in legend).

HR Variability
Yogis and controls had different responses when comparing RSA in response to certain-pain versus certain-warm trials. Yoga practitioners maintained the same level of RSA for both warm and pain trials, whereas there was a tendency for controls to have lower RSA during pain than during warm trials (Figure 5, statistics in legend).

Systolic Blood Pressure
SBP was not different between yogis and controls during the cue or stimulus period (cue: $F_{(1,32)}$ = 0.009, *p* = .925; stimulus: $F_{(1,32)}$ = 0.026, *p* = .872). In contrast to other measures (ratings, HR, SCRs, and RSA), SBP was not increased during pain ($F_{(1,32)}$ = 0.158, *p* = .693).

DISCUSSION
These findings show that despite comparable perceptual ratings, yogis and controls in this study had different autonomic responses during the anticipation and experience of painful and nonpainful stimuli. Yogis had higher phasic SCRs in anticipation of and in response to all stimuli, suggesting heightened sympathetic arousal in response to cues and stimuli during the experimental trials. Furthermore, control subjects, but not the yogis, showed a higher
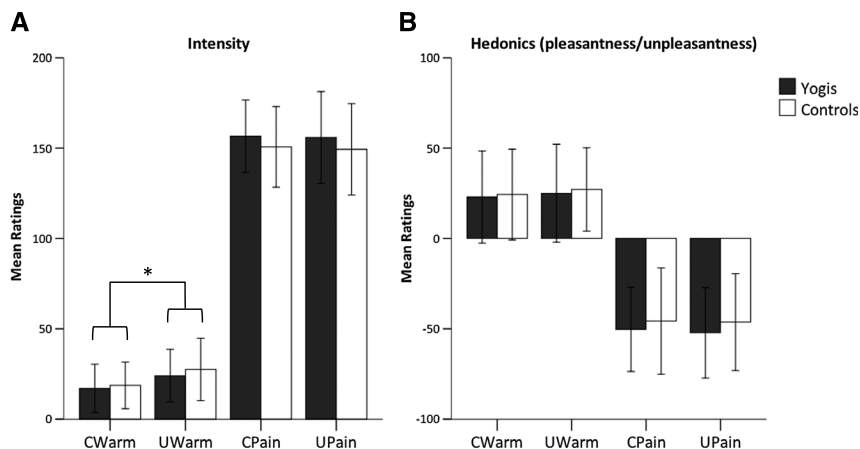
FIGURE 2. Intensity and pleasantness/unpleasantness ratings. There were no differences in either intensity or hedonic ratings between yogis and controls, that is, no group main effect (yogis versus controls intensity: $F_{(1,32)}$ = 0.135, *p* = .716; hedonics: $F_{(1,32)}$ = 0.806, *p* = .376) or any group interactions (INTENSITY: group by stimulus $F_{(1,32)}$ = 1.124, *p* = .297; group by context: $F_{(1,32)}$ = .067, *p* = .798; group by stimulus by context: $F_{(1,32)}$ = .225, *p* = .639 | HEDONICS: group by stimulus: $F_{(1,32)}$ = .052, *p* = .821; group by context: $F_{(1,32)}$ = 0.105, *p* = .748; group by stimulus by context: $F_{(1,32)}$ = .019, *p* = .890). Painful stimuli were significantly more intense ($F_{(1,32)}$ = 991.677, *p* < .001) and unpleasant ($F_{(1,32)}$ = 89.936, *p* < .001) than warm stimuli. A, For intensity, there was a significant main effect of context ($F_{(1,32)}$ = 8.529, *p* = .006) and stimulus by context interaction ($F_{(1,32)}$ = 11.927, *p* = .002), with greater intensity ratings in the uncertain than certain warm condition (certain: 17.86 [13.04], uncertain: 25.74 [15.67]; *t*(33) = 5.211, *p* < .001). However, there was no context effect for painful stimuli (*t*(33) = 0.540, *p* = .593). B, There were no effects of context nor interactions for hedonic ratings (context: $F_{(1,32)}$ = 0.162, *p* = .690; stimulus by context interaction: $F_{(1,32)}$ = 2.819, *p* = .103). *x* Axis labels: CWarm = certain warm; UWarm = uncertain warm; CPain = certain pain; UPain = uncertain pain. All data are mean (SD).
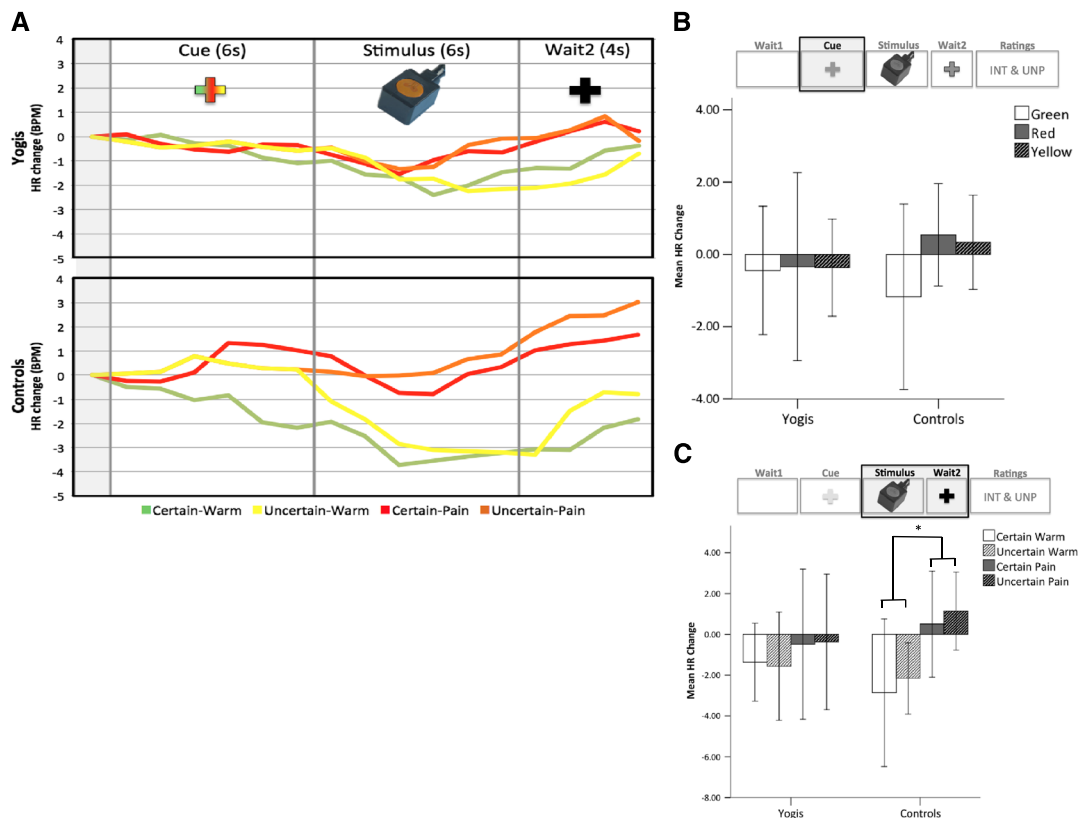
FIGURE 3. HR responses throughout trial. A, Second-by-second mean HR for yogis and controls (relative to the last second of the Wait1 period). B, Cue period: for mean HR during the cue period, there was no main effect of group ($F(1,31) = 0.443$, $p = .511$), cue by group interaction ($F(1,31) = 1.909$, $p = .157$), or main effect of cue ($F(1,31) = 2.361$, $p = .103$). C, Response period: control subjects showed a decrease in HR to warm stimuli (-2.51 [2.17]bpm) versus painful stimuli (0.83 [1.63]bpm; $t(13) = 5.212$, $p < .001$), whereas yogis did not (warm: -1.46 [1.79]bpm, pain: -0.42 [2.91]bpm; $t(18) = 1.513$, $p = .148$). There was a significant main effect of stimulus ($F(1,31) = 20.286$, $p < .001$) and a group by stimulus effect for HR response to the stimulus ($F(1,31) = 5.595$, $p = .024$), but no other significant effects (all $F(1,31) \leq 0.621$, $p \geq .437$). B and C graphs show mean (SD). HR = heart rate; bpm = beats per minute.

HR and a lower RSA during painful than nonpainful trials, suggesting increased arousal when pain is present, whereas the yogis maintained the same level of arousal in response to all stimuli. As a caveat to interpreting these findings, it should be noted that our sample size was not large enough to allow for a single multivariate analysis, so that these differences between yogis and controls could be inflated due to multiple comparison errors.

Skin conductance is a pure measure of sympathetic activity, without contamination from the parasympathetic nervous system (29). Although factors involved in poor health, such as anxiety and stress, increase skin conductance, so do several forms of potentially protective arousal, including attentional and cognitive demand in orienting to significant stimuli (8,30). In the current study, the finding that yogis had greater SCR than did controls during both the anticipation and experience of pain, without higher pain ratings, suggests that yogis may have developed an elevated attentional arousal system that potentially could improve attentional focus and cognitive function. Yogis use different mental techniques from those used by controls to regulate pain sensations, with these techniques involving increased focused attention and reinterpretation of the pain sensation (16). The finding that yogis, but not controls, have increased SCR during nonpainful warm stimuli, whereas both groups show increase SCR during pain, suggests that

the underlying cause of the increased sympathetic response may differ between groups, with the SCR increase in yogis being attention related and the SCR increase in controls being anxiety related. This interpretation is further reinforced by the finding that controls, but not yogis, show higher HR during pain than during warm stimuli.

We found that uncertainty about whether an upcoming stimulus was to be painful or not increased the perceived intensity of nonpainful, but not painful, stimuli. These results are similar to those reported by Sawamoto et al. (31), who reported that nonpainful warm stimuli were perceived as more unpleasant when the subjects expected pain. Thus, it seems that expectations of pain can have an important influence on the perception of nonpainful stimuli, and this phenomenon occurs in both yogis and active healthy subjects. Previous studies show that subjects rate pain higher when attending to a painful stimulus than when distracted (23,32). The current finding of differential warm ratings and similar pain ratings during certain and uncertain conditions suggests that during both the uncertain condition and the certain pain condition, subjects focused attention on the stimulus, whereas during the certain warm stimulus, subjects may have been less vigilant to the upcoming stimulus.
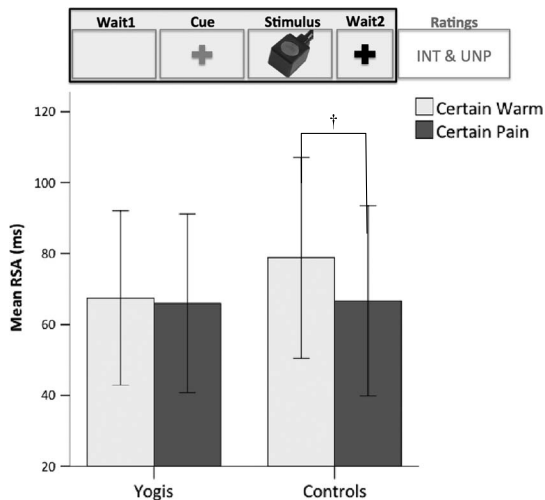
A question addressed in this study was whether autonomic activity could be used as a predictor of pain. Studies have shown that
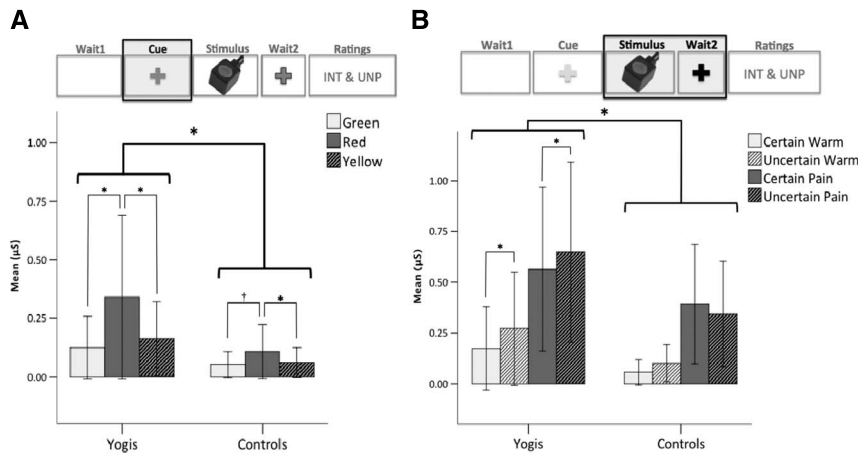
FIGURE 4. SCRs. A, Yogis showed a greater SCR magnitude during the cue period, as seen from an overall group effect ($F(1,32) = 6.298$, $p = .017$) and a cue by group interaction ($F(1.304) = 4.231$, $p = .036$). Red cues elicited a greater SCR compared with yellow cues in both groups (cue: $F(1.304) = 12.351$, $p < .001$) and compared with green cues in yogis ($t(18) = 3.506$, $p = .003$) and near significantly in controls ($t(14) = 1.842$, $p = .087$). Yellow and green cue responses did not differ within either group (yogis: $t(18) = 1.378$, $p = .185$; controls: $t(14) = 0.506$, $p = .621$). *Significance. †Tendency toward significance. B, Yogis also showed increased SCR magnitude during the stimulus/Wait2 period (group: $F(1,32) = 4.683$, $p = .038$). Across all subjects, painful stimuli elicited greater SCR magnitudes than nonpainful ones (stimulus: $F(1,31) = 20.286$, $p < .001$) and context (certain/uncertain) also affected responses (context: $F(1,32) = 8.366$, $p = .007$). Post hoc tests showed that uncertain conditions elicited a greater response in yogis (0.46 [0.34] μS, $t(18) = 3.962$, $p = .001$), but that this difference was not seen in controls (certain: 0.23 [0.17] μS, uncertain: 0.22 [0.17] μS; $t(18) = 0.131$, $p = .898$). All data are mean (SD). SCR = skin conductance response.

painful stimuli cause an increase in sympathetic responses, particularly skin conductance in experimental pain situations (3,33–36). The current findings show an increase in SCR peak magnitude during painful stimuli, supporting the idea that this might be a surrogate measure for pain. Nevertheless, other factors also led to increased SCR. For both groups of subjects, the SCR increased

FIGURE 5. RSA. There was no difference between groups in RSA overall ($F(1,31) = 0.469$, $p = .498$), but a main effect of stimulus ($F(1,31) = 6.327$, $p = .017$), driven by lower RSA in controls in certain pain versus certain warm condition (group by stimulus interaction: $F(1,31) = 3.855$, $p = .059$; pain versus warm: $t(13) = 2.257$, $p = .042$). Yoga practitioners maintained a stable level of RSA across both warm and painful conditions ($t(18) = 0.607$, $p = .551$). Both groups showed the same RSA response to certain-warm trials ($t(31) = 1.226$, $p = .229$). †Tendency toward significance. Data are mean (SD). RSA = respiratory sinus arrhythmia.

during the anticipation of certain pain. Furthermore, although pain ratings did not differ between yogis and controls, the yogis had a higher SCR during the stimulation period. This suggests that SCR would not be useful to grade pain intensity among subjects. Both HR and skin conductance measures are known to differentiate pain compared with nonpainful stimuli (5,37–39). However, in our study, we did not find a significantly increased HR during pain in either group. Nevertheless, we did find that the nonyogis had a decrease in HR during the nonpainful condition, suggesting a relaxation response (40,41). This was supported by a higher RSA during warm than pain in controls, a measure of parasympathetic activity. In contrast, yogis' HR and RSA response was similar between pain and warmth, suggesting that, in certain groups of people, the expected decrease in parasympathetic activity in response to pain can be absent. Yogis seemed to have an increased sympathetic arousal throughout the trials, without decreased parasympathetic response to pain or expectation of pain. Because yogis and controls had different sympathetic and parasympathetic activity during a task involving pain, our findings support the conclusions of other studies that autonomic responses do not reliably correlate with pain perception (3,6,7), especially in individuals trained in disciplines that include activities that affect the ANS.

The question of how practicing yoga might change autonomic reactivity arises. ANS function is under cerebral cortical control, with the insular cortex having an important role in regulating autonomic function (42). Studying individuals with 6 through 16 years of yoga practice, Villemure et al. (18) found that yogis had increased gray matter in multiple cortical regions, with the number of years of yoga experience positively correlating with gray matter volume in the left mid-insula, left frontal operculum, right posterior middle temporal cortex, and left orbitofrontal cortex, suggesting that practicing yoga continues to bring positive changes to the

brain years after beginning practice. The mid-insula is particularly important for autonomic integration, so the finding that this region continues to increase in size with increased years of yoga practice suggests that autonomic control by yoga may be a long-term developmental process mediated through plastic changes in the cerebral cortex. Although pain perception did not differ between yogis and active controls in our study, we previously showed that yogis have elevated pain *tolerance,* which correlated with insular cortex volume and intrainsular white matter integrity (16). Together, these findings suggest that yoga may have top-down cortical influences on the ANS that can contribute to managing pain.

In conclusion, we found that autonomic responses to pain differed between people who had been practicing yoga on a regular basis for at least 6 years and healthy physically active volunteers, despite having similar pain ratings. These findings suggest that autonomic reactivity to pain may be altered by environmental, physical, and psychological factors throughout an individual's life.

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