Subjective salience ratings are a reliable proxy for physiological measures of arousal

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Running title (45 characters including space): Salience ratings and SCR

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Abstract

Pain is an inherently salient multidimensional experience that warns us of bodily threat and promotes escape behaviours. Stimuli of any sensory modalities can be salient depending on stimulus features and context. This poses a challenge in delineating pain-specific processes in the brain, rather than salience-driven activity. It is thus essential to salience match control (innocuous) stimuli and noxious stimuli, in order to remove salience effects, and identify pain-specific activity. Previous studies have salience-matched either through subjective salience ratings or the skin conductance response (SCR), the latter of which serves as a physiological measure of arousal. Though an objective measure that overcomes the nebulous construct of salience, SCR cannot be used to salience-match in real-time (i.e., during an experiment) and assumes an association between salience and physiological arousal elicited by painful and non-painful stimuli, but this has not been explicitly tested. To determine whether salience and physiological arousal are associated, sixteen healthy adults experienced 30 heat pain and 30 non-painful electric stimuli of varying intensities. Stimuli were subjectively matched for salience and physiologically matched for arousal using SCR. A linear mixed model found no differences in SCR between salience-matched heat and electric stimuli. A mediation analysis showed that salience fully mediated the relationship between stimulus intensity and SCR (**proportion mediated=84%**). In conclusion, salience and physiological arousal are associated, and subjective salience ratings are a suitable for salience-matching pain with non-painful stimuli. Future work can thus use subjective salience ratings to delineate pain-specific processes.

Introduction

Pain is inherently salient [21], as it signals potential or actual bodily threat [31]. This salience of pain drives behaviour, first reorienting toward the stimulus [25], allowing the evaluation of the threat to mount an appropriate behavioural response. However, many stimulus modalities can be salient, and elicit the same cascade of events. This was highlighted in a series of studies that showed no differences in brain activity in response to salience-matched painful heat and non-painful electric stimuli [13,27,28]. Therefore, controlling for salience is essential to identify pain-specific processes. One approach is to use salience-matched stimuli across modalities to isolate modality-specific processes. Salience can be defined as the extent to which a stimulus differs from perceptual features of existing stimuli in the environment, making it stand out and grab our attention [37]. Given the subjective nature of salience, it would be reasonable to rely on self-report ratings, as is the case with other subjective experiences (e.g., pain). However, cross-modal salience matching is not straightforward: a bright light may not grab the same level of attention as a noxious stimulus, given differences in the sensory qualia [2]. Thus, using an objective measure to match physiological arousal as a proxy for salience across modalities could overcome this challenge.

The skin conductance response (SCR) is a phasic change in electrical conductivity of the skin in response to an external stimulus [26]. SCR has previously been used to physiologically match salience between various levels of nociceptive and auditory modalities [12], demonstrating that stimulus intensity and intensity ratings are correlated, and increases in intensity ratings lead to increases in SCR magnitude. This study assumed that SCR magnitude can be used to approximate the extent of a stimulus' salience. However, it remains unknown whether there is an association between physiological arousal and salience in response to

noxious and innocuous somatosensory stimuli, and whether increases in stimulus intensity leading to increases in SCR magnitude are mediated by salience.

Although desirable to have objective proxy measures of salience, salience-matching with these methods is determined after data collection during post-processing—as opposed to matching in real-time using subjective salience ratings. This is particularly important in cases where salience matching was suboptimal, thus not allowing to parse out salience effects from effects of interest—i.e., pain-specific activity. However, if self-report salience ratings are tightly related to SCR magnitude across various stimulus intensities and modalities, this would allow for real-time confirmation of matching.

Here, we aim to determine whether different intensities of subjectively salience-matched stimuli elicit similar levels of physiological arousal, as measured by SCR. Healthy participants experienced various intensities of two stimulus modalities—heat pain and non-painful electric stimuli—calibrated to elicit six salience ratings, while SCRs were recorded. We hypothesized that SCR magnitude would increase as a function of self-reported salience, and the SCR magnitudes for salience-matched heat pain and electric stimuli would not differ. We hypothesize that increases in stimulus intensity will lead to increases in SCR, and that salience would mediate this effect.

Methods

Participants

All study procedures were approved by the University of Toronto Human Research Ethics Board (Protocol #33465). Sixteen participants (8 female) aged 18-45 years were recruited from the University of Toronto environment for a single study visit. Exclusion criteria were having current or past chronic pain, pain in the last two months, being an SCR non-responder (participants do not have an SCR peak to control stimuli, see below), having neurological or psychiatric conditions, and clinically relevant levels of depression (> 21 on the Beck Depression Inventory; BDI), or reporting suicidal ideation (>0 on item 9 of the BDI). Additional questionnaires collected for exploratory analyses of individual differences included the State Trait Anxiety Inventory [32] and Pain Catastrophizing Scale [34].

Study visit

Upon arrival to the study visit, participants provided informed e-consent and completed questionnaires. E-Consent and questionnaire data were collected and managed using REDCap electronic data capture tools hosted at the University of Toronto [9,10]. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.

Our study was designed based on previous work by Horing and colleagues [12], with the key difference that we compared two somatosensory stimuli (noxious heat and innocuous electric stimuli). Participants passively received 8s heat or electric stimuli, each delivered at temperatures and currents predicted to elicit six different salience ratings. Participants had their SCR recorded (see below) and provided a self-report salience rating after each stimulus.

Sensory Thresholds

To ensure sensation of each stimulus modality was within a normal range, sensory thresholds to thermal heat stimuli (warm detection, heat pain), and electric stimuli were measured. A TCS thermal stimulator T-11 probe (QST.Lab, Strasbourg, France) was attached to the lateral right calf using a Velcro strap and the method of limits was performed to determine the warm detection and heat pain thresholds. Participants pressed a stop button when they felt a rise in temperature (1˚C/second) from 32˚C to warm and painful hot temperatures over four trials each. The sensory threshold was calculated as the average temperature across all four trials for warm and across the last three trials for heat pain. The last three trials were used to calculate the heat pain threshold due to anticipatory effects during the first trial that lead to pressing the button at a lower temperature. Electric thresholds were determined by applying two surface electrodes 2cm apart along the sural nerve trunk on the right ankle, and stimulating the area (DS7A Digitimer, Welwyn Garden City, UK) with single 200µs pulses. We first delivered a 1mA pulse, and steadily increased the stimulus intensity by 1mA steps until the participant reported a light tapping sensation. Then, the current was lowered by 0.5mA until it was imperceptible. Next, current was increased by 0.25mA until another response was recorded, and then down and up by 0.05 until 5 positive responses were recorded.

Familiarization and Stimulus Intensity Determination

To familiarize participants with the various stimulus intensities used in the experiment, a familiarization procedure was performed. Participants passively received two blocks of 6 trials each comprising six different intensities above the detection thresholds of both heat and electric stimuli. Across the two blocks, participants will have experienced all 6 stimulus intensities for each modality. The intensity of each modality occurred in an ascending fashion, with no more than four stimuli of the same modality in a block, and no more than two consecutive stimuli of the same modality.

A train delay generator (DG2A, Digitimer, Welwyn Garden City, UK) triggered the DS7A to deliver an 8s train of electric stimuli (200 µs pulses) at 100Hz. The lowest intensity was at threshold, and the following five intensities each increased by 1mA. In cases where the higher intensities were perceived as painful, the stimulus intensity increments were adjusted to deliver six non-painful electric stimuli, maintaining an equal distance between stimuli. Thermal heat was delivered using the TCS device with a T-11 probe. The minimum stimulus temperature delivered was above the warm detection threshold, but below the heat pain threshold (ramp rate: 100˚C/second), such that the first three stimuli were in the innocuous range, and the higher three stimuli were above the heat pain threshold. Temperatures were subsequently increased by 1˚C each time until a maximum of 48˚C was reached, for safety reasons.

After each stimulus, participants were asked to report whether the stimulus they received was painful (yes/no). Participants were instructed of the IASP definition of pain, followed by “If the stimulus hurts or feels like it is or could potentially be damaging the skin, that would be

considered painful." This is to ensure that non-painful salient and aversive stimuli are not labelled as painful.

The thermode was moved 5cm along the lateral calf to prevent habituation or sensitization. A second familiarization procedure was performed, with temperatures and currents presented once more, but this time in a pseudorandom order of both intensity and modality to reflect the experimental design (see below). Participants were asked to rate each stimulus along three rating scales: perceived intensity, perceived salience, and perceived unpleasantness. Intensity was described as "the strength or magnitude of the stimulus." Salience was defined as "the extent to which a stimulus is likely to grab and direct attention." Unpleasantness was defined as "the extent to which a stimulus elicits a negative emotional experience." To distinguish the definitions of the three rating scales from one another, a script was read to the participant (see Appendix A). Based on a pilot experiment (data not shown), participants could not adequately discriminate salience at only six unique values using a visual analog scale, as was previously done [12], despite verbally reporting perceptual differences. We trialed various scales and found that a Likert-like scale provided greater discrimination matching participants' perceptual experience. This scale had 6 response items: 25, 35, 45, 55, 65, 75, each labelled as follows: lowest imaginable, mild, moderate, strong, very strong, strongest imaginable, respectively. Ratings of 0 and 100 were omitted, as stimuli were always above threshold and below tolerance. Based on the obtained ratings, a linear regression was performed for each participant to calibrate stimulus intensities (temperatures and currents) needed to elicit subjective salience ratings at each of the specified anchors during the experiment.

Experimental Task

Participants experienced heat pain and non-painful electric stimuli at temperatures and currents calibrated to elicit six different salience ratings (Figure 1). Stimuli were presented in a pseudorandom order with the goal of applying six stimulus intensities five times, resulting in five trials of each salience rating for each stimulus modality, for a total of 60 stimuli (30 electric, 30 heat). The stimuli were presented in a block design, with 6 trials per block, for a total of 10 blocks. Due to habituation throughout the experimental task, there was not always a 1:1 relationship between stimulus intensity applied and the corresponding predicted salience rating. In these cases, stimulus intensities were modified between task blocks, meaning there were more than six temperatures or levels of current applied to obtain 5 trials of each salience rating. The pseudorandom order of modality and stimulus intensity were presented following the same constraints as done previously [12]: two consecutive stimulus intensities had to elicit salience within three rating values of each other; the block could not begin with intensities that elicited the highest two salience ratings; and no more than two consecutive stimuli could be from the same modality. In each trial, participants viewed a fixation cross on a laptop (Macbook Pro, 2015, macOS High Sierra, running Windows 10 using BootCamp, Apple, Cupertino, CA) running E-Prime 3.0 software (Psychology Software Tools, Pittsburgh, PA) during stimulus delivery, followed by horizontal Likert-like rating scales of intensity, salience, and unpleasantness, and a pain rating (yes/no). Participants responded by moving a slider left or right using designated keys.

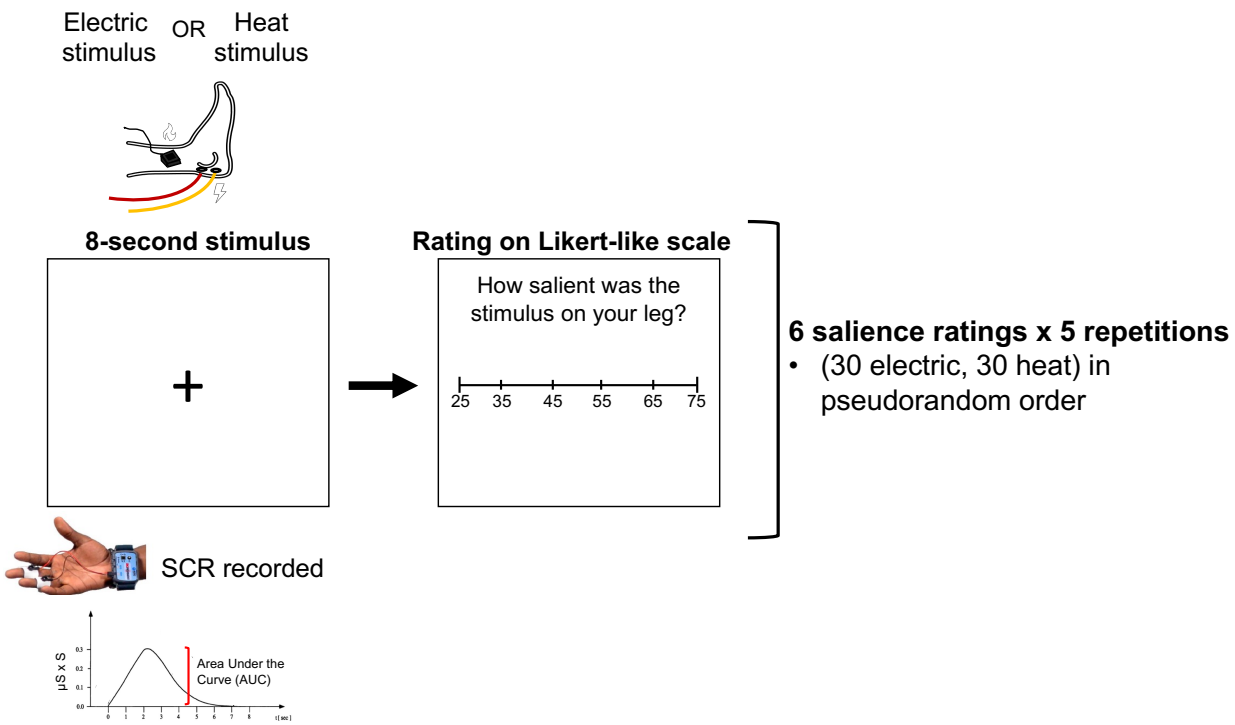

Figure 1. Experimental task design. As done previously, 8s stimuli were presented while their SCR was recorded, followed by a period where participants rated the perceived intensity, salience, and unpleasantness of the stimulus on a Likert-like scale (intensity and unpleasantness not shown). Temperatures and currents that corresponded to six salience ratings were applied 5 times for each stimulus modality (electric and heat), resulting in 60 total trials.

SCR Recording Setup

SCRs were recorded using the MP160 Data Acquisition System and Bionomadix wireless PPG/EDA amplifier (BIOPAC Systems Inc., Goleta, CA). Disposable electrodermal activity (EDA: EL507) isotonic gel electrodes were applied with isotonic electrode gel to the index and middle fingertips of the left hand. A wireless Bionomadix transmitter was worn around the wrist and connected to the electrodes using EDA electrode leads. The transmitter sent a signal to the amplifier which was recorded at a sampling rate of 62.5 Hz on a separate testing laptop (XPS 15-9570, Dell Technologies, Round Rock, Texas) on BIOPAC's Acqknowledge 5.0 software. Once connected, we performed a final screen before the experiment to ensure that participants were

SCR responders. To do so, participants were instructed to take a deep breath, which generates a visible SCR. All participants who consented to procedures were SCR responders.

The E-Prime program triggered the devices (TCS II or DS7A) to deliver the specified stimulus modality and intensity for each trial by sending a TTL pulse via a Multifunction Input/Output Device (NI USB-6343, National Instruments Corp., Austin TX, USA). Given the manual control of stimulus intensity of the DS7A, the experimenter would set the next stimulus current to be applied during the rating screen. The stimulation devices each sent an analog TTL pulse to the MP160 unit such that each stimulus modality had its own recording channel in Acqknowledge, marking the onset and offset of each stimulus.

SCR Analysis

To analyze the SCR data, we used Acqknowledge v5.0 software (BIOPAC, Goleta, CA). First, we performed a median smoothing and low-pass filter at 1Hz to remove noise and high frequency signals in the waveforms. We then visually inspected data for artefacts, such as participant movement, and performed a manual artefact correction. The stimulus event was used to estimate event-related SCRs, which were defined as the SCRs occurring 0.5-12 seconds after the stimulus onset. Event-related SCRs with amplitudes below 0.02μs were considered non-responses and trials with no event-related SCRs were assigned an amplitude of 0 but were still included in analyses [23]. We measured the area under the curve (AUC) [23] of event-related SCR amplitudes for every trial and organized them across stimulus modality and salience rating per participant. In some cases, SCRs within the time window were not captured by the software,

and we commissioned a script from BIOPAC to address this issue. We z-scored the AUC on each trial across participants within each modality to account for outliers.

Stimulus Intensity and Salience Ratings

To determine whether stimulus intensity elicited a parametric increase in self-report salience, we performed a Friedman ANOVA for each stimulus modality given that data were not normally distributed. Significance was set at $p < 0.05$.

Linear Mixed Models

To determine whether salience ratings and SCR AUC were related, and whether there were differences in this relationship between modalities, we fit a random slope and intercept linear mixed model using the lmerTest package in RStudio (RStudio, Boston MA, USA) [16]. Specifically, the dependent variable was z-scored AUCs and independent variables were stimulus modality and salience rating. Two further linear mixed models were performed, one for intensity ratings and one for unpleasantness ratings. The fixed effects for each model were stimulus modality and rating, and participant was set as a random effect. Ratings were coded as numeric to reflect their ordinal nature and mean-centered within modality to make the model interpretations generalizable. Significance was set at $p < 0.05$.

Mediation Analysis

Next, to further elucidate the association between subjective salience and SCR, a mediation analysis was performed using the mediation package in RStudio [36] to determine whether and

how much salience mediates the relationship between stimulus intensity (temperature and current administered) and SCR AUCs. The temperature and current applied on respective heat and electric trials was z-scored to transform the data between modalities into comparable units. The independent variable, *X*, was the z-scored stimulus intensity (temperature/current) applied on a given trial, the mediator, *M*, was the subjective salience rating in that trial, and the dependent variable, *Y*, was the z-scored SCR AUCs elicited by the stimulus applied in that trial. First, three linear regressions were performed to ensure that *X* predicts *Y* (Path *c*) and *X* predicts *M* (Path *a*)—prerequisites for a mediation analysis. Next, the indirect mediation effect of *M* on *Y* while controlling for X (Path *b*) was assessed, which also allowed us to compute the indirect effect (Path a*b, mediation) and the direct effect (c', effect of X on Y while controlling for the mediator). Statistical significance of mediation was computed using bootstrapping with 1000 permutations with the mediate function in the RStudio mediation package [36]. The same procedures were performed for each rating type, separately, i.e., a model for intensity ratings and another model for unpleasantness ratings. In all mediation analyses, ratings were mean-centered. Significance was set at $p < 0.05$.

Results

Salience ratings increase as a function of stimulus intensity

Participants did not ever rate stimuli that elicited a salience of 25/100 for either modality as painful. Similarly, heat stimuli that elicited a salience of 75/100 were perceived as painful but this was not the case for the electric stimulus. As shown in Figure 2, a Friedman ANOVA revealed, similar to Horing et al. [12], that as stimulus intensity increases, self-report salience

ratings increase (electric: $\chi^2(5) = 55$, $p < 0.001$, Kendall's $w = 0.85$; heat: $\chi^2(5) = 42.83$, $p < 0.001$, Kendall's $w = 0.78$).

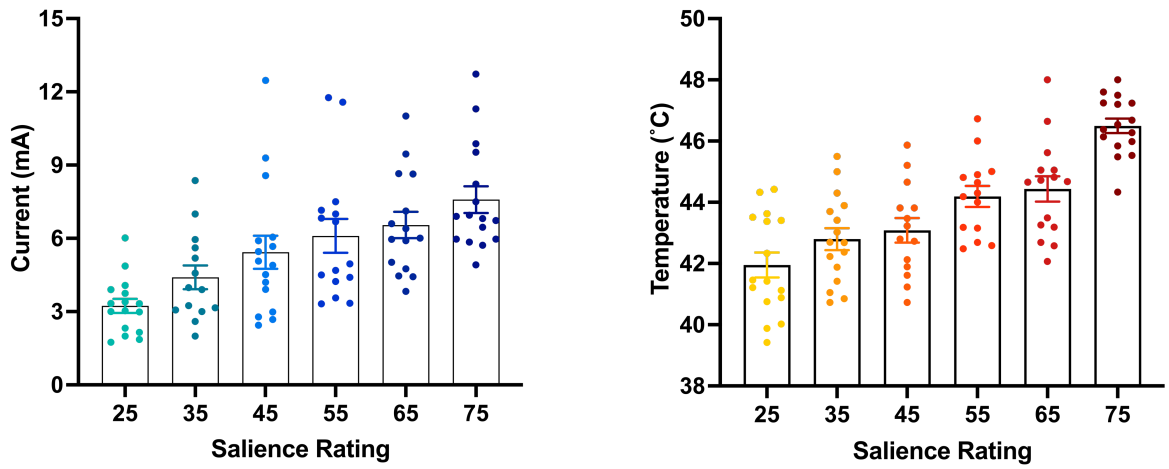

Figure 2. Stimulus intensities (A) electric current (mA) and (B) temperature (˚C) applied to elicit self-report salience ratings at six different levels. Stimulus intensities are presented as individual values around the mean ± SEM of each salience rating.

Arousal is associated with salience ratings

A linear mixed model comparing the relationship between salience and stimulus across modalities (i.e., heat vs. electric) revealed a significant main effect of salience ($t = 6.00$, $p = 0.000024$, $\eta^2 = 0.75$), but no main effect of stimulus modality ($t = 0.008$, $p = 0.99$) or stimulus-by-salience interaction ($t = 1.96$, $p = 0.069$). These results demonstrate that as self-report salience increases, there is a corresponding increase in SCR, a measure of physiological arousal, indicating that salience and arousal are associated (Figure 3). In addition, we show that subjectively salience-matched electric and heat stimuli can in fact be matched physiologically, as the SCR AUCs between stimulus modalities do not differ as salience increases. Linear Mixed Model results for intensity and unpleasantness ratings, which were also associated with physiological arousal, can be found in the Supplementary Materials.

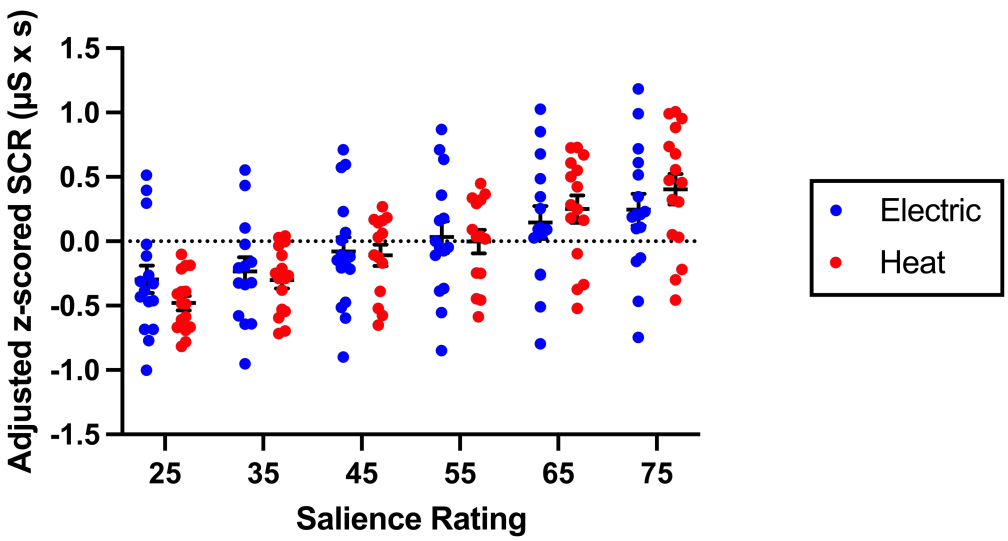

Figure 3. Area under the curve (AUC) of the skin conductance in response to heat (red) and electric (blue) stimuli across the six self-report salience ratings. AUCs are presented as mean ± SEM in µSiemens-by-second.

Salience Mediates the Relationship between Stimulus Intensity and Arousal

The mediation analysis (Figure 4) revealed that stimulus intensity had a significant effect on salience (a = 15.72, p < $2e^{-16}$), and the total effect of stimulus intensity on SCRs was significant (c = 0.25, p < $2.72e^{-16}$). However, when accounting for salience as a mediator, salience had a significant effect on SCRs (b = 0.013, p = $2.03e^{-10}$), but stimulus intensity no longer predicted SCR (c' = 0.041, p = 0.36). This was confirmed by causal mediation analysis, where we found that salience completely mediated the relationship between stimulus intensity and SCR (ab = 0.21, p < $2e^{-16}$; proportion mediated=84%), as there was no direct effect of stimulus intensity on SCRs. These findings are evidence that salience is directly associated with and contributes to the increase in physiological arousal that occurs as stimulus intensity increases. Mediation results for intensity and unpleasantness ratings, which showed full and partial mediation respectively, are reported in the Supplementary Materials (Figure S1).

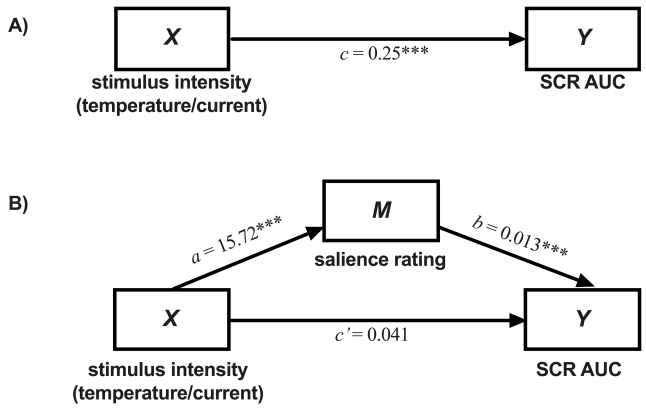

Figure 4. Salience mediates the relationship between stimulus intensity and SCR AUC. A) The total effect of stimulus intensity (*X*) on SCR AUC (*Y*). B) Regression coefficients between each factor, where *a* and *b* represent the indirect effect (mediation), and *c'* represents the direct effect of stimulus intensity on SCR AUC accounting for the mediator (*M*), salience.

Discussion

This study aimed to determine whether subjective salience ratings are associated with a physiological measure of arousal, SCR, in response to somatosensory stimuli of different intensities. As expected, we found that as stimulus intensity increased, salience ratings increased. Furthermore, increases in salience ratings were associated with increases in SCR. A causal mediation analysis revealed that increases in stimulus intensity resulting in increases in SCRs were completely mediated by salience. These increases were not different between modalities, allowing for salience-matching. These findings indicate that self-report salience ratings are a suitable metric to track physiological arousal, and can be used to perform reliable, real-time salience-matching in research studies employing multiple modalities where salience-matching is required.

When investigating pain-specific brain processes, and their association with behaviour, researchers face the challenge of parsing pain-specific activity versus the salience content of the painful stimulus [6,19-21]. Salience alone is sufficient to drive behaviour—first reorienting toward the stimulus [3,25], allowing the evaluation of the potential threat, and to further mount an appropriate behavioural response. To identify pain-specific processes, controlling for salience is essential. One approach to do so is to salience-match an innocuous stimulus and a noxious stimulus. This has previously been done in a series of studies that showed no differences in brain activity using mass-univariate statistics in response to salience-matched heat and electric stimuli [13,27,28]. In these studies, salience was loosely defined to participants as “the ability of the stimulus to capture attention.” However, there are significant limitations to this approach: the nebulous definition of salience, the unfamiliarity of the term for the average person [1], and the lack of validation of self-report salience ratings. It is not clear how an individual could assess the

extent to which a stimulus grabs their attention on a scale from 0 ("not salient") to 10 ("extremely salient"), without training or comparators.

One approach that has been utilized to overcome the limitation of subjective salience-matching is to record objective, physiological arousal in response to pain [24] and innocuous stimuli using the skin conductance response (SCR) [12]. In these studies, the SCR is considered a proxy measure of arousal [26]. In pain research, it is used as a measure of physiological arousal from noxious stimuli [5,7,8,33,35], and as an indicator of pain in individuals who cannot communicate, such as newborns [29], and general anesthesia and intensive care patients [33]. Indeed, Geuter et al showed that the temporal profile of SCR can be used to predict trial-by-trial pain ratings, but again, this does not allow for the isolation of pain-specific activity [8]—and, if anything, shows a physiological signature of pain ratings, but does not explicitly test the association between SCR and salience.

A significant limitation to SCRs is that salience-matching cannot be performed during the experiment, and requires later processing and analysis to determine whether matching was successful. This can lead to data or participants being excluded, which can be costly as well as resource- and time-intensive. Here we address this limitation by obtaining both SCRs and self-report salience to determine whether salience tracks with SCR, as a measure of physiological arousal. In addition, collecting self-report ratings allowed us to perform real-time salience-matching during the experiment, circumventing the data loss that can occur when salience-matching post-hoc using SCRs only. Our methods follow the procedures conducted by Horing et al. [12], with a few key differences: we compared the salience of pain to that of an innocuous electric stimulus, rather than an auditory stimulus, and the self-report rating of interest was perceived salience, rather than perceived intensity. Intensity and salience ratings, while often

correlated, do not assess the same sensory quality, and correlating intensity ratings with physiological arousal does not answer whether salience and arousal are associated [13,14,19,27,28]. In fact, our results suggest a differential influence of salience and intensity ratings on SCRs, where salience completely mediated the relationship between stimulus intensity administered and SCRs, and intensity ratings partially mediated the relationship (Figure S1).

To perform subjective salience-matching properly, it is essential to operationalize salience and provide a clear, understandable definition at the beginning of the experiment, as well as providing contextual examples. In our study, participants were provided an explanation with analogies to help understand how to interpret salience. We operationalized salience as "the extent to which the stimulus grabs and directs your attention" (see Supplementary Materials for precise script used). In other words, as they felt the stimulus on their leg, they were asked to think about how much of their attention they allocated to that sensation. We also differentiated salience from perceived intensity and unpleasantness by providing a simple thought exercise: participants were instructed to imagine that they are studying intently in a quiet library and suddenly experience a light tap on their shoulder from a friend trying to get their attention. This example highlights to participants that a stimulus can be salient without being intense or unpleasant. No difference in SCRs between stimuli across each rating scale indicates that the scales are related, which is expected in healthy adults. However, the finding that salience and unpleasantness completely mediated the relationship between stimulus intensity and SCRs, whereas perceived intensity partially mediated it (Figure S1), suggests that the concepts are distinguishable, and SCRs are most sensitive to increases in perceived salience and unpleasantness. This may be explained by arousal having a valence, meaning SCRs could reflect not only increases in general alertness driven by salience, but also negative affect driven by

stimulus unpleasantness. This is consistent with Horing et al. [12], who found a similar association between auditory unpleasantness and SCRs.

Much like any subjective experience, the salience of a stimulus is dynamic, and ratings are often inconsistent over time [4,11,15,22,30]. Therefore, it is important to consider the frequency of sampling of salience ratings when aiming to salience-match across stimuli. Asking for ratings too often can lead to the participant paying too much attention to the stimulus, and thus amplifying the experience. Conversely, sampling too sparsely can miss changes in salience, and adversely affect matching. Therefore, salience-matching may not be maintained over an experiment, and taking ratings only at the beginning or end of the experiment will miss these dynamic changes in the sensory experience. One way to overcome this is to parse an experiment into multiple blocks, and assess salience of stimuli after each block. This provides an opportunity to periodically adjust stimulus intensity to maintain the desired salience rating.

The present study is unique in that we compared the objective SCR to increasing levels of subjectively salience-matched noxious and innocuous stimuli to determine whether salience and physiological arousal are in fact associated. We found no differences in physiological arousal in response to heat and electric stimuli as the salience increased, represented by the AUC of the SCR. These findings are in line with previous work showing no difference in SCR between heat pain and auditory stimuli [12], and SCR increasing as a function of numeric rating scale reports of postoperative pain [17,18]. Importantly, we show that salience completely mediated the increase in physiological arousal that occurs because of increasing stimulus intensity, providing further evidence of the strong association between SCRs and salience. Here, we demonstrate that salience is associated with physiological arousal, and when instructed properly, salience can be used as a proxy of physiological arousal to investigate pain-specific processes.

Though our sample size is a limiting factor of the study, the effect sizes of our ANOVA and linear mixed model were very large. In addition, salience explained 84% of the mediation effect of stimulus intensity on SCRs. Next, while we had enough repeated trials to compare participants at the group level, some participants did not provide salience ratings for up to two of the six salience ratings, no matter how many times the predicted temperature/current was applied or re-calibrated. This could be due to rating bias or that the temperature/current applied was not easily discernable, as similar patterns were observed for the intensity ratings, but not unpleasantness. This meant that for those participants, at those missing salience ratings, no comparison could be made between the self-report salience and the corresponding SCR, however this meant there were more repetitions of the remaining salience ratings that were included in analyses. In addition, most participants did not ever rate electric stimuli as 75 for unpleasantness, likely because the stimuli were not painful, despite rating many trials as 75 for both intensity and salience. While the two-way interaction between SCRs and the respective salience and unpleasantness ratings was not statistically significant, it was approaching significance. Upon further inspection it appeared that SCR AUCs diverged at lower salience rating levels (25/100), whereas SCR AUCs diverged at higher levels of self-report unpleasantness (75/100). Given that the lowest salience ratings for heat were not painful and that SCR AUCs between stimuli diverged at the highest unpleasantness, salience is the better metric to associate with SCRs, as SCRs elicited by salience-matched heat and electric stimuli at higher levels where the heat is painful are not different.

In sum, we show that salience and physiological arousal are associated, where subjectively salience-matched heat and electric stimuli track with and strongly influence physiological arousal, measured by the SCR. Subjectively salience-matching pain with

innocuous stimuli, when instructed properly, can be used in future studies to control for salience while investigating pain-specific processes, as it poses a real-time metric that tracks with physiological arousal and can be adjusted to reflect the dynamic experience of pain.

Acknowledgements

M Moayedi is supported by a University of Toronto Centre for the Study of Pain — Pain Scientist Award, a Canada Research Chair (Tier 2) in Pain Neuroimaging, and the Bertha Rosenstadt Endowment Fund at the Faculty of Dentistry, University of Toronto. The authors have no conflicts to report. This study was funded through Moayedi's discretionary funds.

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FIGURE LEGENDS

Figure 1. Experimental task design. As done previously, 8s stimuli were presented while their SCR was recorded, followed by a period where participants rated the perceived intensity, salience, and unpleasantness of the stimulus on a Likert-like scale (intensity and unpleasantness not shown). Temperatures and currents that corresponded to six salience ratings were applied 5 times for each stimulus modality (electric and heat), resulting in 60 total trials.

Figure 2. Stimulus intensities (A) electric current (mA) and (B) temperature (˚C) applied to elicit self-report salience ratings at six different levels. Stimulus intensities are presented as individual values around the mean ± SEM of each salience rating.

Figure 3. Area under the curve (AUC) of the skin conductance in response to heat (red) and electric (blue) stimuli adjusted from the linear mixed model across the six self-report salience ratings. Each dot represents the average adjusted AUC per participant in μSiemens-by-second. The mean ± SEM AUCs are shown in black.

Figure 4. Mediation results where salience mediated the relationship between stimulus intensity and SCR AUC. A) The total effect of stimulus intensity (*X*) on SCR AUC (*Y*). B) Regression coefficients between each factor, where *a* and *b* represent the indirect effect (mediation), and *c'* represents the direct effect of stimulus intensity on SCR AUC accounting for and SCR AUC, respectively.