Received: 12 January 2022 | Revised: 16 May 2022 | Accepted: 4 June 2022

DOI: 10.1002/ejp.1990

ORIGINAL ARTICLE

Pain-autonomic interaction is a reliable measure of pain habituation in healthy subjects

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Abstract

Background: Habituation is a response decrement resulting from repeated stimuli. Reduced habituation to noxious stimuli is considered to be a proxy for central sensitization in subjects with chronic pain. Despite numerous investigations of pain habituation in relation to central sensitization, there is no consensus on the most sensitive and reliable readout, as well as analysis approach. Therefore, this study compared the usability and reliability of different readouts and habituation analysis approaches to measure pain habituation in response to repetitive heat simulation.

Methods: Three blocks of 20 contact heat stimuli were applied on the volar forearm of 20 healthy subjects on two separate visits. Habituation was assessed by three different readouts: pain ratings, contact heat evoked potentials (CHEPs) and heat-induced sympathetic skin responses (SSRs). In addition, two different habituation analysis approaches were used: between the three stimulation blocks (between-block) and within the first stimulation block (within-block).

Results: Significant between-block habituation for SSRs ($p < 0.001$), but not for pain ratings ($p = 1.000$) and CHEPs ($p = 0.078$) was found. There was significant within-block habituation for pain ratings ($p = 0.012$) and SSRs ($p < 0.001$), but not for CHEPs ($p = 0.246$). Only the between-block habituation of heat-induced SSR was reliable between the two visits (first to second block: intraclass correlation coefficient [ICC] = 0.58, $p = 0.030$; first to third block: ICC = 0.64, $p = 0.015$).

Conclusion: Heat-induced SSR as a measure of pain-autonomic interaction revealed the strongest pain habituation and showed the highest test–retest reliability.

1 | INTRODUCTION

Chronic pain is a growing global problem, with an estimated prevalence of up to 20% in the worldwide population (Goldberg & McGee, 2011). Independent of the diverse clinical presentations of chronic pain conditions, a common underlying pathophysiological mechanism is ongoing sensitization along the nociceptive neuraxis (Arendt-Nielsen et al., 2017). The International Association for the Study of Pain (IASP) defines central sensitization as an 'increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input' (Loeser & Treede, 2008). Different experimental proxies are used to investigate this hyperexcitable state within the central nervous system. In particular, commonly used assessments are temporal summation, widespread hyperalgesia, conditioned pain modulation as well as experimental pain habituation (Arendt-Nielsen et al., 2017).

Habituation of experimentally induced pain is a widely used approach in pain research and is reflected as a response decrement that results from repeated stimulation (Rankin et al., 2009). Such a response decrement is not limited to subjective (e.g. pain ratings), but can also include more objective pain-related readouts. The latter ones are less biased by the individual interpretation of the pain rating scale for instances and applicable in non-compliant subjects due to cognitive or physical impairments. An important mechanism of pain habituation seems to be endogenous pain modulation. This was demonstrated by reduced activation of the thalamus and somatosensory cortex as well as increased activation of the periaqueductal grey and subgenual anterior cingulate cortex during the application of repetitive noxious stimuli (Bingel et al., 2007; Rennefeld et al., 2010). Consequently, dysfunctional experimental pain habituation, and thereby hyper- or hypoactivity of certain structures in the central nervous system were shown to be a proxy for central sensitization (Arendt-Nielsen et al., 2017). Numerous studies including different chronic pain cohorts have demonstrated the phenomenon of reduced experimental pain habituation of either pain ratings or pain-related evoked potentials (De Tommaso et al., 2005; Hüllemann et al., 2017; Kumru et al., 2012; Valeriani et al., 2003). In addition to the somatosensory system, also the sympathetic nervous system gets activated by painful stimuli (Benarroch, 2001). For example, sympathetic skin responses (SSR) have been suggested to be objective and reliable responses to noxious stimuli (Cervera et al., 2002; Garcia-Larrea & Hagiwara, 2019; Rossi et al., 2002). Similar to the habituation of pain-related evoked potentials, reduced habituation of SSR was shown to be a proxy for central sensitization in patients with chronic pain (Schestatsky et al., 2007) and experimentally induced central sensitization (Scheuren et al., 2020).

Despite the number of investigations studying habituation of pain ratings, pain-related evoked potentials, and heat-induced SSRs, there is no gold standard in terms of stimulation protocol and analysis approach. This is reflected in the variety of methods used in investigations of chronic pain patients, experimentally induced central sensitization or healthy subjects; some examine the effects of habituation between multiple stimulation blocks (between-block analysis) (De Tommaso et al., 2011; Flor et al., 2004; Ozkul & Ay, 2007; Valeriani et al., 2003), whilst others only focus on one stimulation block (within-block analysis) (De Tommaso et al., 2017; Donadio et al., 2005; Kumru et al., 2012; Scheuren et al., 2020; Shunzo et al., 1997; van den Broeke et al., 2019). It is, therefore, of utmost importance to investigate which analysis approach and habituation readout (pain ratings, pain-related evoked potentials, SSRs) are the most sensitive and reliable to first enable meaningful comparison between different studies and second emphasize experimental pain habituation as a complementary proxy for central sensitization. Hence, the primary goal of this study was to systematically compare the usability and reliability of different readouts and habituation analysis approaches to assess pain habituation in healthy young subjects. We hypothesized that objective readouts (evoked potentials and SSRs) are superior to subjective readouts (pain rating) with regard to the assessment of pain habituation because they are less dependent on the subject's active participation and less biased by the subjective percept of pain. Also, we hypothesized that recordings composed of more averaged trials (between-block analysis) are more reliable because they are less affected by potential outliers of single trials/stimuli.

2 | METHODS

2.1 | Subject cohort

For this study, healthy young subjects (18–30 years) were recruited. Subjects with diagnosed neurological conditions (e.g. epilepsy, polyneuropathy, or herniated disc), systematic diseases (e.g. diabetes), pregnancy or psychological conditions (e.g. depression or anxiety disorder) were excluded. Furthermore, episodes of chronic pain (>3 months) in the last year, as well as acute pain or intake of pain medication on the day of examination, led to an exclusion of the subject. All subjects signed a written informed consent before participation. The study was approved by the local ethics board 'Kantonale Ethikkommission Zürich' (reference number: EK-04/2006, PB_2016–02051) and was in accordance with the Declaration of Helsinki.

2.2 | Study design

Upon arrival, subjects filled out a questionnaire related to their medical history. Further, all subjects completed the pain catastrophizing scale (PCS) (Sullivan & Bishop, 1995) and the hospital anxiety and depression scale (HADS) (Zigmond & Snaith, 1983). Sensory integrity of the tested area, i.e. volar forearm, was assessed using thermal quantitative sensory testing, vibration detection threshold as well as a pinprick and light touch testing. Pain habituation was assessed using three different readouts following contact heat stimulation: pain ratings, CHEPs and SSRs (Figure 1a). Regarding the pain ratings, subjects had to rate every heat stimulus in terms of perceived pain intensity on a numeric rating scale (NRS) from 0 to 10 (0: no pain and 10: worst pain imaginable) and thereby we

attempted to draw the subjects' attention towards every single stimulus. The same procedure was repeated after 1–2 weeks to investigate the reliability of pain habituation.

2.3 | Contact heat stimulation

Subjects were in a comfortable supine position in a quiet and temperature-controlled room (22°C) whilst three blocks of 20 contact heat stimuli were applied on the volar forearm of the dominant hand. The blocks were separated by a 2-min break. Stimuli were delivered using a contact heat stimulator (Pathway, Medoc, Ramat Yishai, Israel) with a thermode heating rate of 70°/s, a cooling rate of 40°/s and a surface diameter of 27 mm. The baseline and destination temperatures of the heat stimuli were 42 and 52°C, respectively (Jutzeler et al., 2016; Rosner et al., 2018). If the stimulation was not tolerated by the subject, a baseline temperature of 35°C with the same destination temperature was applied. The stimulation duration was 393 ms (calculated based on heating/cooling ramp and temperature delta) and the inter-stimulus interval was set at 13–17 s (Lütolf et al., 2021). The thermode was slightly repositioned after every heat stimulus to prevent peripheral adaptation (Greffrath et al., 2007).

2.4 | CHEP acquisition

CHEPs were recorded in accordance with the international 10–20 system using 9 mm Ag/AgCl electrodes filled with conductive adhesive gel. The N2P2 waveform was

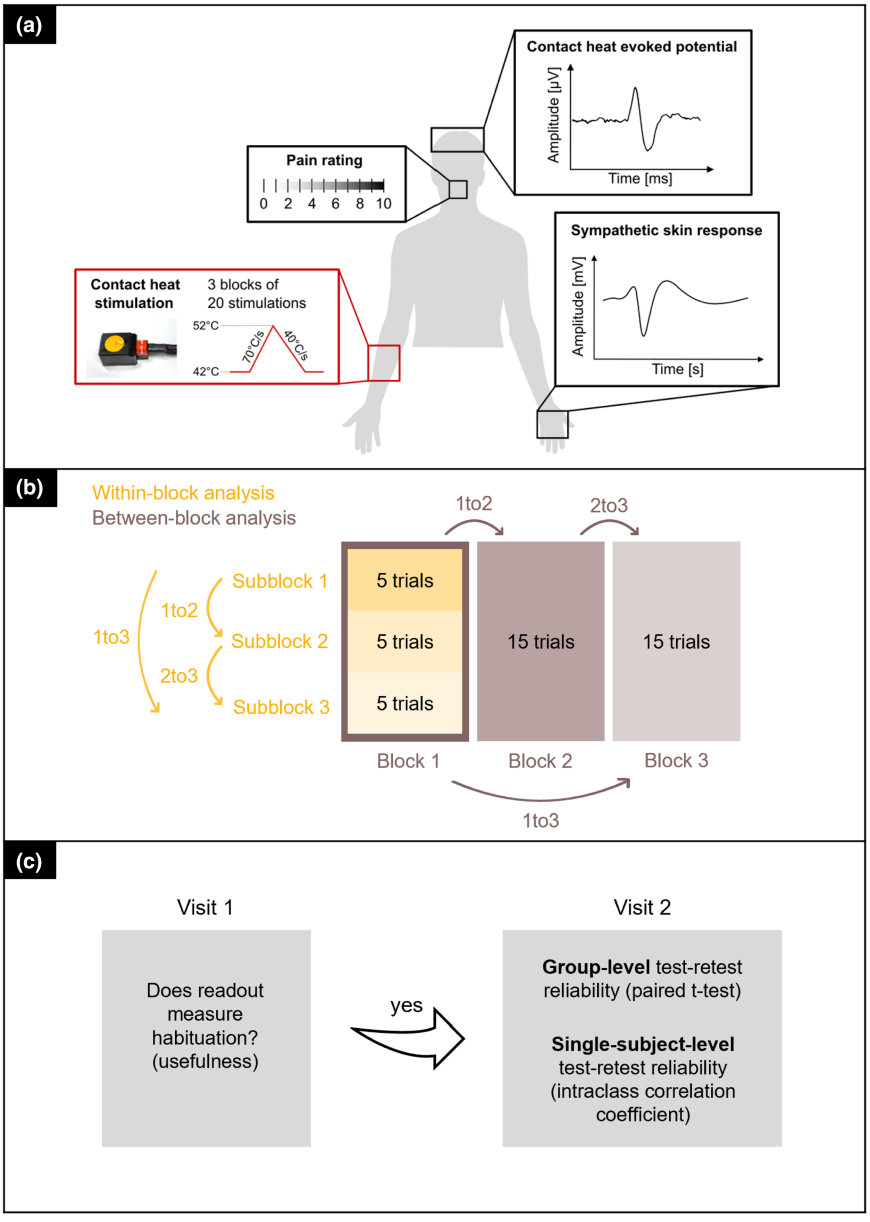

FIGURE 1 Study design and statistical analysis. (a) Illustrated is the contact heat stimulation (red) and the three different readouts (black) to assess pain habituation. (b) The brown boxes illustrate the between-block analysis using 3 blocks of 15 stimuli. The beige boxes illustrate the within-block analysis for the first stimulation block only. (c) Schematic illustration of the statistical analysis procedure.

measured from the vertex position (Cz) with reference linked to both earlobes (A1-A2). The electrode placement was done in a reduced set-up since consistent negative and positive potentials, i.e. N2 and P2, have been shown to be reliably detectable at Cz (Kramer et al., 2012). The sample rate was set at 2000 Hz, with a bandpass filter of 0.5-30 Hz. The window of recording was set to 0.5 s pre- and 1 s post-stimulus. Data were recorded using a customized program on LabView (V2.6.1. CHEP, ALEA Solutions, Zurich, Switzerland). Electrooculography was measured to remove blink artefacts contaminating EEG trials. Additionally, trials superimposed with alpha waves were excluded from further analysis. Out of the 20 recorded CHEPs, the first 15 artefact-free trials were considered for analysis. Correct identification of artefacts and peak detection was ensured by inspection through two independent investigators. Absent CHEPs were further assigned a missing latency (N/A) and an amplitude of 0 μV.

2.5 | SSR acquisition

SSR was recorded from the non-dominant hand with the active electrode attached to the palm and the reference electrode to the dorsum of the hand using standard electrocardiogram electrodes (Ambu BlueSensor NF, Ambu A/S, Ballerup, Denmark). As skin temperature might influence SSR amplitudes (Deltombe et al., 1998), it was ensured that during the measurement the skin temperature was kept constant around 32°C. The contralateral side for the recording of SSRs was chosen to reduce possible movement artefacts due to startle reflexes. The sampling rate was set at 2000 Hz and recorded within a 0.5 s pre- to 9 s post-stimulus time window. A moving average filter of 50 Hz was applied after the recording. SSR amplitudes of the first 15 trials were identified by a custom-made algorithm in R Studio (Version 1.2.1335, R Studio, Inc.) whereas trials superimposed with motion artefacts were excluded from further analysis. Absent SSR amplitudes (flat lines) were assigned a missing latency (N/A) and an amplitude of 0 μV. SSR responses with an artefact (not time-locked SSRs) were assigned a missing latency and amplitude (N/A). Single-trial amplitude correction was performed by two independent investigators to ensure accurate peak labelling.

2.6 | Pain habituation analysis and statistics of pain ratings, CHEPs and SSRs

Subject demographics and raw data were assessed using descriptive statistics (mean and standard deviation). The average of all 15 trials per stimulation block for each habituation readout (pain ratings, CHEPs and SSRs) was used for a between-block analysis (three blocks, Figure 1b). For the within-block analysis, only the 15 trials of the first stimulation block were considered and divided into three subblocks (average of 5 trials each, Figure 1b). The normality of the readouts was tested by a Shapiro Wilk test.

A two-way repeated-measures ANOVA was conducted to evaluate if there was significant habituation in the first visit only (Figure 1c). Pain rating or amplitude (CHEPs and SSRs) was included as a dependent variable and block/subblock and analysis approach (between- or within-block) as independent variables. A paired *t*-test corrected for multiple comparisons (Bonferroni correction) served as a post hoc test. The amount of habituation was compared between the three readouts (pain rating, CHEPs and SSRs) to investigate which readout shows the most pronounced habituation. For this purpose, the relative change in readout (pain rating, CHEP or SSR amplitude) was calculated and termed as 'habituation index'. A negative index indicated habituation, whilst a positive index indicated an increase in the respective readout. A two-way repeated measure ANOVA was conducted to compare the habituation index between the three readouts. The habituation index was included as a dependent variable and block/subblock and readout as independent variables. Paired t-tests corrected for multiple comparisons (Bonferroni correction) served as post hoc tests. Readouts with significant habituation in the first visit were then compared to the second visit to investigate test–retest reliability (Figure 1c). To test for reliability of habituation on a group level, the habituation index was compared between the two visits by a paired t-test corrected for multiple comparisons (Bonferroni correction). Finally, the reliability of the habituation index on a single-subject level was tested using an intraclass correlation coefficient (ICC, two-way mixed model, absolute agreement, Figure 1c). ICC <0.5 was considered 'poor', 0.5 < ICC <0.75 'moderate', 0.75 < ICC <0.9 'good' and ICC >0.9 'excellent' (Koo & Li, 2016). Differences in start ratings or amplitudes between the two visits were compared using a paired t-test. All statistical tests were performed at an α level of 0.05 in R Studio statistical software (R version 4.0.5 for Windows).

3 | RESULTS

3.1 | Subject demographics and questionnaire outcomes

A total of 20 healthy young subjects (9 female and 11 male, 23.6 ± 2.1 [19–27 years]) were recruited for this study. One subject had to be excluded because the subject did not tolerate the contact heat stimuli even when decreasing the baseline temperature to 35°C. In two other subjects, the

intensity of the contact heat stimuli had to be lowered to 35°C baseline temperature. The remaining 19 subjects showed intact somatosensory function based on the initial sensory testing. The time between the two visits was on average 10.4 ± 2.6 days. The PCS and HADS scores were 14.4 ± 9.5 and 5.1 ± 1.7, respectively and therefore in a range of no clinical relevance for all subjects (i.e. PCS <30 points and HADS <8).

3.2 | Habituation of pain ratings, CHEPs and SSRs

There was no missing data regarding pain rating for the within- or between-block analysis approach. CHEPs had to be excluded for one subject because there were not 15 artefact-free trials for every stimulation block due to blink artefacts. The remaining 18 subjects had 15 artefact-free CHEP trials in every stimulation block. Regarding SSR, out of 15 trials per block on average 2 ± 2 trials had to be excluded across all subjects due to artefacts.

3.2.1 | Between-block analysis

Figure 2 shows the three stimulation blocks for pain ratings, CHEPs and SSRs of the first visit. There was no habituation between the stimulation blocks for pain ratings (Figure 2a, $p = 1.000$, $F = 0.6$). Also, CHEPs N2P2 amplitude did not show an overall decrease over the three stimulation blocks (Figure 2b, $p = 0.078$, $F = 3.6$). The SSR amplitude, however, decreased over the three stimulation blocks (Figure 2c, $p < 0.001$, $F = 16.5$). Here, pronounced habituation along the three blocks could be observed (see Figure 2c for post hoc comparisons). Compared to the other readouts, SSR habituation between blocks was most pronounced (pain rating-SSR: $p < 0.001$; CHEP-SSR: $p < 0.001$; pain rating-CHEP: $p = 1.000$).

3.2.2 | Within-block analysis

Figure 3 illustrates habituation within the first stimulation block of the first visit. There was pronounced habituation of pain ratings within 15 heat stimuli (Figure 3a, $p = 0.012$, $F = 7.2$). Lower pain ratings from the first to the second and third subblocks were observed (see Figure 3a for post hoc comparisons). The CHEP N2P2 amplitude, however, did not habituate over the three subblocks (Figure 3b, $p = 0.246$, $F = 2.2$). Importantly, habituation was significant (Figure 3c, $p < 0.001$, $F = 36.5$) and most pronounced for the SSR amplitude (pain rating-SSR: $p < 0.001$; CHEP-SSR: $p = 0.005$; pain rating-CHEP: $p = 0.447$). There was a steady decrease in SSR amplitude observable between the three subblocks (see Figure 3c for post hoc comparison).

3.3 | Reliability of habituation on a group-level

3.3.1 | Between-block analysis

When comparing the starting values (first stimulation block) between the two visits, we saw lower values

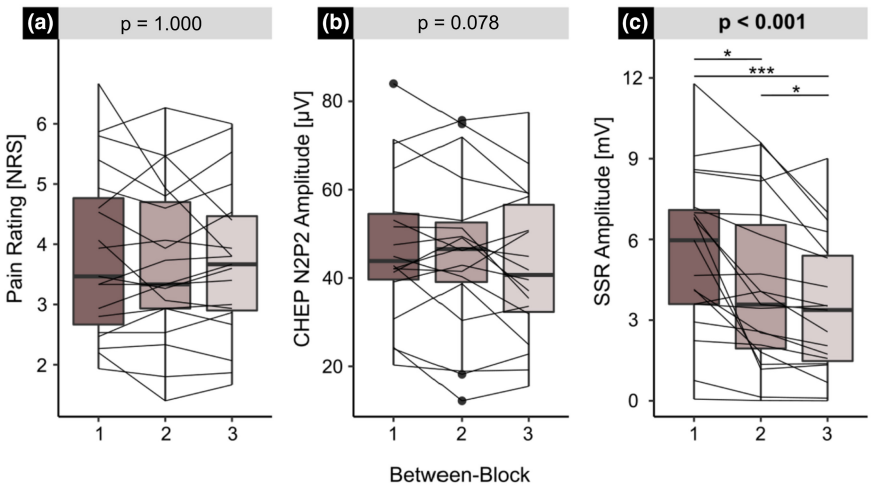

FIGURE 2 Between-block habituation of the measured pain-related readouts of the first visit. Boxplots with additional single subject values (black lines) illustrate the change in response (y axis) between three stimulation blocks (x axis) for (a) pain rating, (b) CHEP and (c) SSR amplitude. The result of the repeated measures ANOVA is illustrated in the grey bars above each plot. The asterisks illustrate the results from the post hoc analysis. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. CHEP, contact heat evoked potential; NRS, numeric rating scale; SSR, sympathetic skin response.

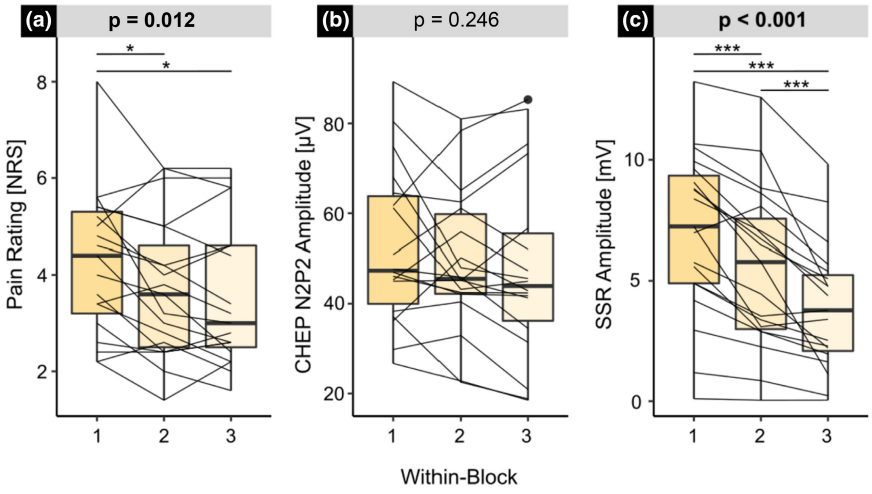

FIGURE 3 Within-block habituation of the measured pain-related readouts of the first visit and stimulation block. Boxplots with additional single subject values (black lines) illustrate the change in response (y axis) between three subblocks (x axis) for (a) pain rating, (b) CHEP and (c) SSR amplitude. The result of the repeated measures ANOVA is illustrated in the grey bars above each plot. The asterisks illustrate the results from the post hoc analysis. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. CHEP, contact heat evoked potential; NRS, numeric rating scale; SSR, sympathetic skin response.

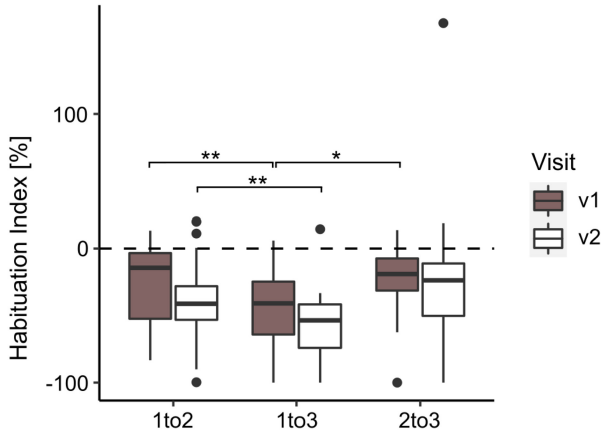

FIGURE 4 Comparison of between-block SSR habituation between visits 1 (brown) and 2 (white). The x axis illustrates the different comparisons between the three stimulation blocks. The habituation index of SSR amplitude is illustrated on the y axis. The asterisks illustrate whether one analysis approach (1 to 2, 1 to 3 or 2 to 3) manifests more habituation compared to the others for the first and second visits separate. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. SSR, sympathetic skin response.

in terms of pain rating ($p < 0.001$) and SSR amplitude ($p < 0.001$) from the first to the second visit. However, the starting value of CHEP amplitude did not differ between the two visits ($p = 0.443$). All raw values of the first and the second visit (between-block analysis) can be found in the Table S1 and S3.

Considering the data of the first visit (Section 3.2.1), we could only observe significant between-block habituation for SSR amplitudes, but not for pain ratings or CHEP amplitudes. Therefore, only the habituation index for SSR amplitudes was compared between visits 1 and 2. There was no significant difference in SSR habituation between the two visits (Figure 4).

3.3.2 | Within-block analysis

The starting values (first 5 trials) between the two visits were lower in terms of pain rating ($p = 0.007$) and SSR amplitude ($p = 0.011$). The starting values of CHEP amplitude did not differ between the two visits ($p = 0.597$). All raw values of the first and the second visit (within-block analysis) can be found in Table S2 and S4.

Considering the data of the first visit (Section 3.2.2), significant within-block habituation was observed for pain ratings and SSRs, but not for CHEPs. Therefore, only the habituation index of pain ratings (i.e. 1–2 and 1–3) and SSR (all subblock comparisons) were compared between visits one and two. There was no significant difference in habituation of pain ratings between the two visits (Figure 5a). The reduction of SSR amplitude was larger in the second compared to the first visit when comparing the subblocks 1–3 (Figure 5b, beige asterisks). However, the habituation index seen between the subgroups 1to2 and 2to3 were similar in both visits.

3.4 | Reliability of habituation on a single-subject-level

Based on the calculated ICCs, moderate reliability for between-block SSR habituation between the two visits was

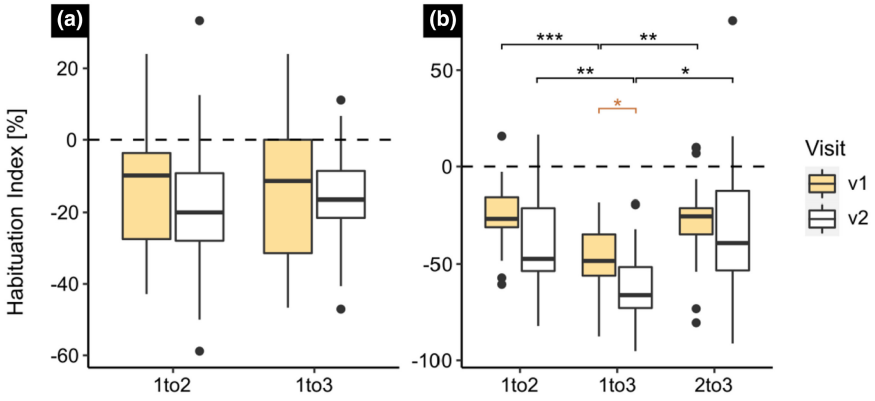

FIGURE 5 Comparison of within-block habituation of pain rating and SSR between visits 1 (beige) and 2 (white). The x axis illustrates the different comparisons between the three subblocks of the first stimulation block. The habituation index of (a) pain rating and (b) SSR amplitude is illustrated on the y axis. The asterisks illustrate whether one analysis approach (1 to 2, 1 to 3 or 2 to 3) manifests more habituation compared to the others for the first and second visits separate. Beige asterisks indicate significant differences in habituation between the two visits. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. SSR, sympathetic skin response.

TABLE 1 ICCs of pain rating and SSR habituation for both analysis approaches

	Between-block		Within-block			
	SSR		Pain rating		SSR	
	ICC	*p*	ICC	*p*	ICC	*p*
1–2	0.58	0.030	−0.18	0.631	0.34	0.164
1–3	0.64	0.015	−0.09	0.567	0.34	0.157
2–3	0.39	0.160	N/A	N/A	0.97	0.965

Abbreviations: ICC, intraclass correlation coefficient; N/A, no habituation in the first visit; SSR, sympathetic skin response.

found (Table 1). However, the within-block habituation of pain ratings and SSRs was not reliable on a single-subject level regardless of which subblocks were compared.

4 | DISCUSSION

The goal of this study was to compare the usefulness of different readouts to measure pain habituation and evaluate their reliability in healthy young subjects. Pain habituation was assessed with subjective (pain ratings) as well as objective readouts (CHEPs and SSRs). We applied three blocks of 20 contact heat stimuli to the volar forearm and simultaneously recorded subjects' pain ratings, CHEPs and SSRs. The same protocol was repeated 1–2 weeks later. The three readouts were analysed using a between- and within-block approach. Heat-induced SSRs as a measure of pain-autonomic interaction were the strongest measure of pain habituation. In addition, the between-block analysis approach was the most reliable one for SSR habituation.

4.1 | Differences in pain habituation and neural substrates

We reported differences in habituation of the three readouts, i.e. pain ratings, CHEPs and SSRs. In contrast to CHEPs and pain ratings, SSR amplitudes decreased between stimulation blocks. Furthermore, for the within-block analysis, pain ratings and SSRs decreased, whilst CHEPs did not. Generally, SSR showed the most pronounced habituation for both the within- and between-block analysis. Although pain ratings, CHEPs and SSRs were all previously used as readouts to investigate habituation to noxious stimuli, they do not necessarily share identical neural substrates. This might be an explanation for the observed differences in habituation for the three different readouts. In this regard, a recent study from our group (Lütolf et al., 2021) also reported diverging results when investigating the integrity of ascending nociceptive projections in subjects with spinal cord injury employing the same three readouts. There a potential contribution of different neural structures in the generation of pain ratings, CHEPs and SSRs were discussed to explain the diverging results. For example, the conscious perception of noxious stimuli is divided into the sensory-discriminative and affective-motivational aspects of pain (Schaible, 2007). On the one hand, pain ratings reflect the sensory-discriminative aspect of pain generated in the primary and secondary somatosensory cortices and posterior parietal cortex. On the other hand, aversive emotional responses reflect more the affective-motivational aspect of pain generated in the medial thalamocortical system, e.g. the anterior cingulate cortex (ACC), the insula and the prefrontal cortex (Schaible, 2007). Pain-related evoked potentials are likely conveyed within the latter pain system (Treede et al., 1999; Vogt, 2005). Thus, different brain

regions might be involved in generating pain ratings and CHEPs, potentially explaining differences in habituation when investigating the two readouts. Nevertheless, a relation between CHEP amplitudes and pain ratings were previously shown in healthy subjects (Chen et al., 2001; Granovsky et al., 2008; Roberts et al., 2008).

In this study, the habituation of SSR was the most pronounced of all measured habituation readouts. Similar to CHEPs, the thalamo-limbic circuit was shown to be involved in controlling the degree of arousal and central habituation of SSR in response to noxious stimuli, i.e. palmar (emotional) sweating (Neafsey, 1990). Many cortical regions were associated with sympathetic outflow including the ACC, insula, amygdala, angular gyrus, supramarginal gyrus and orbitofrontal cortex (Beissner et al., 2013; Critchley et al., 2000). However, the difference in CHEPs and SSR habituation might be explained by the fact that the generation of pain-induced SSRs additionally includes spinal and bulbar processes (Wang, 1958). As an example, SSRs were previously shown to be mediated merely through spinal circuits (Reitz et al., 2002). In addition, multiple ascending spinal tracts, e.g. spinothalamic and spinoreticular tracts, are involved in transmitting action potentials following noxious stimuli (Rousseaux et al., 1999). The relative contribution of these tracts to generate an SSR or CHEP might be different which could be reflected in differences in habituation. More specifically, CHEPs might be primarily generated by the activation of the monosynaptic neospinothalamic pathway, whilst SSRs might be primarily generated by the activation of the multi-synaptic paleospinothalamic and archispinothalamic pathways (Fenton et al., 2015). In addition to differences in central processing, efferent peripheral components such as the lack of complete metabolic recovery of sweat glands may be involved in the progressive reduction of SSR to repeated noxious stimuli (Vetrugno et al., 2003). Although such a peripheral mechanism contributing to SSR habituation would reduce its usability as a proxy for central sensitization in chronic pain, we showed that no such SSR habituation was observed in healthy controls with experimentally induced central sensitization (Scheuren et al., 2020).

4.2 | Stimulation properties influencing pain habituation

In general, less intense and more frequent repetitive stimuli, i.e. shorter inter-stimulus-interval, were shown to result in more pronounced habituation (Rankin et al., 2009; Von Dincklage et al., 2013). Previously, intense stimuli showed no significant pain rating decrement (Hüllemann et al., 2013). We found on average pain ratings of NRS 4 explaining why we might not have seen significant habituation of pain ratings and CHEPs. By reducing the baseline temperature of contact heat stimuli from 42°C to 35°C, less intense contact heat stimuli can be delivered (Jutzeler et al., 2016; Rosner et al., 2018) which might lead to significant habituation of pain ratings and CHEPs.

As mentioned above, another factor diminishing habituation is the inter-stimulus-interval (Baumgärtner et al., 2012). The application of more frequent stimuli, but still below the critical wind-up frequency of 0.3 Hz (Herrero et al., 2000), was shown to result in more pronounced habituation (Thompson & Spencer, 1966). Compared to other studies investigating the habituation of evoked potentials, the application frequency of noxious stimuli was lower in our study because we concomitantly measured SSR. Palmar SSRs were slower (1.44 ± 0.21 s) compared to CHEPs (304 ± 30 ms), and, therefore, the inter-stimulus-interval had to be increased. Additionally, fixed inter-stimulus intervals were shown to result in increased habituation than random inter-stimulus intervals (Baumgärtner et al., 2012). Our random application of heat stimuli was, however, necessary to reduce the predictability of noxious stimuli and its potential influence on physiological readouts (Oka et al., 2010). In conclusion, the rather low frequency of contact heat application (~0.07 Hz) and the random time intervals between the stimuli might explain the observed lack of habituation, especially in pain ratings and CHEPs.

4.3 | Pain habituation analysis approaches

We found that both analysis approaches (between- and within-block) revealed habituation, but only for heat-induced SSR. Therefore, the choice of habituation readout seems to be more important to study habituation than the analysis approach. So far, different approaches to analyse habituation to noxious stimuli were used. Depending on the habituation readout, predominantly between-block (for evoked potentials) (De Tommaso et al., 2011; Hüllemann et al., 2017; Valeriani et al., 2003) or within-block (for SSR) (Schestatsky et al., 2007) analyses were used. The choice of analysis approach might have been mainly influenced by the difference in the signal-to-noise ratio of single trials between CHEPs and SSRs. In particular, amplitudes of SSR are much larger than the ones of CHEPs enabling the investigation of single-trial habituation for SSRs. In general, the signal-to-noise ratio of CHEPs is increased by averaging multiple time-locked single trials. Thus, CHEPs are commonly investigated by averaging trials of multiple, e.g. 15–25, stimuli, which renders the between-block analysis more appropriate than

the within-block one. Nevertheless, habituation of pain-related evoked potentials was previously also analysed using a within-block design (De Tommaso et al., 2017; Kumru et al., 2012).

The question of how many stimulation blocks or single stimuli are needed to measure significant habituation was not the focus of this study. Nevertheless, for the between-block analysis, we saw that two stimulation blocks are sufficient to report significant SSR habituation (Figure 2). However, whether also less than 15 stimuli within one stimulation block would have been enough to induce significant between-block habituation remains open. Regarding the within-block analysis, we saw significant habituation of pain ratings and SSRs including only 10 stimuli (the first two subblocks) (Figure 3). Whether even less stimuli would have been enough for the within-block analyses can also not be answered with our analysis approach. So far, De Tommaso et al., 2017 reported that only five electrical stimuli are not enough to show significant SSR habituation in healthy subjects, whilst Shunzo et al., 1997 reported that using seven electrical stimuli are enough. Given the different stimulation parameters (e.g. stimulation intensity and frequency) and modality (i.e. electrical vs. contact heat) used in these two studies, it is almost impossible to relate these findings to our study.

4.4 | Reliable acquisition of pain habituation

Habituation is measured by investigating the response decrement over a specific trait of stimuli (Rankin et al., 2009). Hence, the initial response as well as its decrease over time is of interest. We found lower initial pain ratings and SSR amplitudes in the second compared to the first visit. A reliable measure of pain rating is generally considered difficult (Rosier et al., 2002). Similar SSR amplitude decreases in the second visit were found by others (Shunzo et al., 1997; Toyokura & Murakami, 1996). Reduced physiological responses were previously discussed as a result of learning effects, changes in expectation and long-term habituation (Rankin et al., 2009; Rennefeld et al., 2010; Rosier et al., 2002). However, by investigating the reduction of responses in a relative manner, i.e. the initial response serves as a baseline for normalization of the following responses, the variability of initial pain rating and SSR amplitudes might be controlled for. In contrast, the initial CHEP amplitudes did not decrease from the first to the second visit potentially because other neural substrates are involved than in the generation of pain ratings and SSRs.

Regarding the test–retest reliability of habituation between two visits, one must distinguish between group- (Figures 4 and 5) and single-subject level reliability (Table 1). Whilst clinical studies comparing groups (e.g. healthy subjects and patients with chronic pain) might primarily rely on group-level reliability, a potential clinical application of habituation as a proxy for central sensitization would profit from single-subject level reliability analysis. On a group level we found similar habituation between the two visits when investigating the between-block SSR habituation (Figure 4). Additionally, for the within-block analysis approach, we found similar habituation in pain rating and SSR in visits one and two (Figure 5). However, this was only the case when comparing subsets of the first stimulation block (i.e. 1–2 and 2–3). When investigating the first to the last subblock (i.e. 1–3), we observed more pronounced SSR habituation in the second compared to the first visit. Here, a possible explanation might be that in the second visit the stimuli were generally perceived as less intense compared to the first visit. As mentioned in Section 4.2. less intense stimuli result in more pronounced habituation (Rankin et al., 2009).

On a single-subject level, we only found reliable SSR habituation between several stimulation blocks (between-block analysis) (Table 1). The moderate reliability seen for the between-block, but not the within-block analysis might be attributed to the fact that average values composed of a larger number of trials are less susceptible to small changes in amplitude or outliers. Such a between-block analysis approach for SSR habituation was previously employed by Ozkul & Ay, 2007 reporting reduced SSR habituation in migraine and tension-type headache patients compared to healthy subjects, potentially due to central sensitization.

4.5 | Limitations and future considerations

This study investigated the assessment of pain habituation in young healthy subjects. However, many clinical pain conditions include subjects of older age. Therefore, a reliable assessment of pain habituation needs to be investigated across different age categories including the elderly population. Additionally, usability and reliability of habituation readouts might differ in clinical conditions and should, therefore, be assessed as well. Furthermore, clinical examinations often take place at intervals of 3, 6 or 12 months. In our study, reliability was only examined at intervals of 2 weeks. Furthermore, in contrast to other studies (Greffrath et al., 2007; Kumru et al., 2012), pain ratings and CHEPs did not reveal pain habituation using our study protocol. This might be partially owed to the lack of an international standardized protocol for CHEPs acquisition, and we would argue that the application of heat stimuli with lower stimulation intensities

and a decreased inter-stimulus interval might result in significant habituation of pain ratings and CHEPs. Lastly, in this study, we measured time-locked SSRs in response to painful stimuli, but previous studies demonstrated that SSRs can also be elicited in response to non-painful stimuli (Elie & Guiheneuc, 1990; Lim et al., 2003; Vossel & Zimmer, 1992). Hence, a salient stimulus is enough to elicit an SSR and, therefore, caution with regard to potential overestimation of pain-related SSR should be applied. Moreover, baseline electrodermal activity and thereby also phasic SSRs can be affected by many other factors such as fatigue, arousal, attention or emotions. Such factors might be partially controlled for by stringent experimental protocols as well as additional recordings of concomitant background electrodermal activity which allows for statistical correction of its confounding effect on pain-related SSRs.

5 | CONCLUSION

Compared to pain ratings and CHEPs, heat-induced SSR was the strongest readout to measure pain habituation. The difference in habituation of these three readouts might reflect the involvement of distinct neural substrates. Regarding heat-induced SSR, additional spinal or bulbar mechanisms might augment habituation. The most reliable approach to measure SSR habituation between two visits, i.e. testing days, in young healthy subjects was the between-block analysis. Thus, employing two blocks of heat-induced SSR might serve as an easy and objective tool to investigate endogenous pain modulation and potentially unmask central sensitization in chronic pain patients.

AUTHOR CONTRIBUTIONS

I.D.S. substantially contributed to the study conception and design, data analysis, interpretation of results and she drafted the manuscript. C.L. was responsible for data acquisition and contributed significantly to data analysis and interpretation. Furthermore, she revised the manuscript. A.C. was substantially involved in data interpretation and revised the research article implementing his neurophysiological expertise. M.H. made significant contributions to the study conception and design, data analysis and interpretation and revised the manuscript.

ACKNOWLEDGEMENT

The authors would like to thank the subjects for their participation in this study. Open access funding provided by Universitat Zurich.

FUNDING INFORMATION

The study was supported by the Clinical Research Priority Program of the University of Zurich (CRPP Pain).

CONFLICT OF INTEREST

None declared.

SIGNIFICANCE

Pain habituation can be most reliably measured in young healthy subjects by means of pain-autonomic interaction, i.e. heat-induced SSR, and may serve as a complementary tool to investigate central sensitization.

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How to cite this article: De Schoenmacker, I., Leu, C., Curt, A., & Hubli, M. (2022). Pain-autonomic interaction is a reliable measure of pain habituation in healthy subjects. *European Journal of Pain*, *26*, 1679–1690. https://doi.org/10.1002/ejp.1990