Scheuren Paulina Simonne (Orcid ID: 0000-0001-7568-0133)

Title:

Pain-autonomic measures reveal nociceptive sensitization in complex regional pain syndrome

Short Title (Running Head):

Pain-autonomic readouts of sensitization

Authors:

P.S. Scheuren[1,2], I. De Schoenmacker[1], J. Rosner[1,2], F. Brunner[3], A. Curt[1], M. Hubli[1]

[1] Spinal Cord Injury Center, Balgrist University Hospital, University of Zurich, Zurich, Switzerland

[2] Department of Neurology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

[3] Department of Physical Medicine and Rheumatology, Balgrist University Hospital, University of Zurich, Zurich, Switzerland

Corresponding author:

Paulina Simonne Scheuren

Address: Forchstrasse 340, CH-8008 Zurich, Switzerland

Telephone: +41 (44) 510 7411

E-mail: paulina.scheuren@balgrist.ch

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What's already known about this topic?

- Autonomic responses to noxious stimulation represent potential objective readouts of signs and symptoms in patients with chronic pain.
- Pain-related sympathetic skin responses are able to detect experimentally-induced secondary hyperalgesia in healthy individuals.

What does this study add?

- This is the first clinical study providing evidence that autonomic measures can depict pain hypersensitivities in patients with complex regional pain syndrome (CRPS).
- Reduced habituation of pinprick-induced sympathetic skin responses in both the affected and control area in CRPS may indicate widespread neuronal hyperexcitability.
- Pain-autonomic readouts may help explore pathophysiological mechanisms in a variety of pain patients and may represent useful outcome measures for clinical trials on novel mechanism-based therapeutic interventions.

Abstract

Background: Allodynia and hyperalgesia are common signs in individuals with complex regional pain syndrome (CRPS), mainly attributed to sensitization of the nociceptive system. Appropriate diagnostic tools for the objective assessment of such hypersensitivities are still lacking, which are essential for the development of mechanism-based treatment strategies.

Objectives: This study investigated the use of pain-autonomic readouts to objectively detect sensitization processes in CRPS.

Methods: Twenty individuals with chronic CRPS were recruited for the study alongside 16 age- and sex-matched healthy controls (HC). All individuals underwent quantitative sensory testing and neurophysiological assessments. Sympathetic skin responses (SSRs) were recorded in response to 15 pinprick and 15 noxious heat stimuli of the affected (CRPS hand/foot) and a control area (contralateral shoulder/hand).

Results: Individuals with CRPS showed increased mechanical pain sensitivity and increased SSR amplitudes compared to HC in response to pinprick and heat stimulation of the affected ($p<.001$), but not the control area ($p>.05$). Habituation of pinprick-induced SSRs was reduced in CRPS compared to HC in both the affected ($p=.018$) and slightly in the control area ($p=.048$). Habituation of heat-induced SSR was reduced in CRPS in the affected ($p=0.008$), but not the control area ($p=0.053$).

Conclusions: This is the first study demonstrating clinical evidence that pain-related autonomic responses may represent objective tools to quantify sensitization processes along the nociceptive neuraxis in CRPS (e.g., widespread hyperexcitability). Pain-autonomic readouts could help scrutinize mechanisms underlying the development and maintenance of chronic pain in CRPS and provide valuable metrics to detect mechanism-based treatment responses in clinical trials.

Significance: This study provides clinical evidence that autonomic measures to noxious stimuli can objectively detect sensitization processes along the nociceptive neuraxis in complex regional pain syndrome (CRPS) (e.g., widespread hyperaxcitability). Pain-autonomic readouts may represent valuable tools to explore pathophysiological mechanisms in a variety of pain patients and offer novel avenues to help guide mechanism-based therapeutic strategies.

1 Introduction

Complex regional pain syndrome (CRPS) is a multifactorial disease, including sensory, vasomotor, sudomotor, trophic, and motor dysfunction in the affected limb (Birklein et al., 2018; Marinus et al., 2011). Based on the International Association for the Study of Pain (IASP), CRPS is classified as chronic primary pain (Nicholas et al., 2019) and the most prominent factor for the diagnosis based on the Budapest criteria (Harden et al., 2010) is the presence of "continuing pain, which is disproportionate to any inciting event", such as a fracture, sprain, or elective surgical intervention. In particular, hyperalgesia and allodynia to mechanical stimuli are hallmark signs in individuals with CRPS. A major contributing factor to such pain hypersensitivities is sensitization of the nociceptive system (Gierthmühlen et al., 2012; Jensen and Finnerup, 2014; Latremoliere and Woolf, 2009; Reimer et al., 2016).

Previous studies employing quantitative sensory testing (QST) have demonstrated widespread hypersensitivities in previously unaffected areas, suggesting generalized sensitization (Drummond et al., 2018; Reimer et al., 2016). While QST allows the investigation of sensory loss (i.e., hypoesthesia/hypoalgesia) and gain (i.e., hyperalgesia and allodynia), the method relies heavily on patient reports and remains purely subjective in its nature (Hansson et al., 2007). Beyond psychophysical measures, objective readouts of sensitization are essential to detect meaningful alterations in somatosensory function (Garcia-Larrea and Hagiwara, 2019). Such objective readouts may represent useful outcome measures for clinical trials on novel therapeutic interventions targeting sensitization processes. In this regard, autonomic readouts have been proposed as candidate measures as enhanced pain-related autonomic responses, such as increased sympathetic skin responses (SSR) and reduced SSR habituation, have been reported in patients with chronic pain (Garcia-Larrea and Hagiwara, 2019; Ozkul and Ay, 2007; Schestatsky et al., 2007; De Tommaso et al., 2017). Other autonomic measures, such as pupil dilation, blood pressure, and heart rate variability have been related to pain in healthy individuals (Koenig et al., 2014; Nickel et al., 2017; Treister et al., 2012; Wildemeersch et al., 2018).

More recently, studies employing experimental pain models in healthy individuals have demonstrated candidate autonomic surrogate markers of experimentally-induced central sensitization (van den Broeke et al., 2019; Scheuren et al., 2020). Alongside

the development of mechanical hyperalgesia, pinprick-induced SSRs were increased and their habituation was reduced in the area of experimentally induced central sensitization (Scheuren et al., 2020). These findings suggest that altered pain-autonomic interaction (i.e., enhanced autonomic responses to nociceptive input), which can occur at multiple levels of the neuraxis (Benarroch, 2001), might be utilized as an objective proxy of sensitization in pain patients.

The objective of the current study was to investigate the value of pain-autonomic readouts as an objective assessment of sensitization along the nociceptive neuraxis in individuals with CRPS presenting with marked signs of mechanical hyperalgesia. We hypothesized that 1) individuals with CPRS show increased pain-related SSRs compared to HC and 2) that SSRs are enhanced in both the affected and a remote, control area in CRPS. The latter implying a potential contribution of widespread neuronal hyperexcitability, i.e., central sensitization, to the pain phenotype.

2 Methods

2.1 Individuals

Individuals with CRPS were recruited at the Department of Physical Medicine and Rheumatology of the Balgrist University Hospital in Zurich, Switzerland. Individuals between 18-80 years old with a clear diagnosis of chronic CRPS based on state of the art diagnostic criteria including the Budapest criteria (Harden et al., 2010) and the IASP classification of CRPS (Nicholas et al., 2019) were included in the study. Exclusion criteria comprised any neurological disorder (e.g., polyneuropathy, radiculopathy, entrapment neuropathy, central nervous system disorder), history of chronic pain prior to the development of CRPS, or history of psychiatric disorder.

Age- and sex- matched healthy control individuals (HC) with no history of or current neurological disorder or psychiatric disorder were recruited for the study. Additional exclusion criteria for healthy control individuals were acute or chronic pain, and intake of medication (i.e., antidepressants, opioids, anticonvulsants, benzodiazepines).

All individuals provided written informed consent prior to study participation. The study was approved by the local ethics committee Kantonale Ethikkommission Zürich (EK-04/2006, PB_2016-02051) and in accordance with the Declaration of Helsinki.

2.2 Study design

An extensive pain phenotyping test battery was performed in all individuals and separated into two study visits. Both visits were performed in a quiet room with constant temperature (22°C). We performed a focused clinical neurological examination (visit 1), quantitative pain assessments (visit 1), quantitative sensory testing (visit 1) and SSR recordings in response to noxious heat and pinprick stimulation randomized to either visit 1 or 2). All of the assessments were performed in the affected area (i.e., CRPS-affected limb with the highest pain intensity) and a remote, control area (i.e., clinically unaffected area) defined according to the location of the affected area. The control area was 1) the contralateral shoulder if the affected area was the hand, as previous studies have demonstrated that CRPS can spread to the contralateral extremity over the course of the disease (Reimer et al., 2016; Rommel et al., 2001; Van Rooijen et al., 2013) and 2) the contralateral hand if the affected area was the foot. This followed the protocol of a larger multi-cohort study, which the present study was part of. The affected and control areas were matched in all HCs and the terms “affected” and “control” area will be used to describe the matched areas in HCs throughout the manuscript and figures. All participants, individuals with CRPS and HC, completed online questionnaires including the Hospital Anxiety and Depression Questionnaires (HADS) (Stern, 2014) and Pain Catastrophizing Scale (PCS) (Sullivan et al., 1995).

2.3 Clinical examination and pain assessments

All individuals with CRPS underwent a clinical examination by a physician specialized in CRPS (FB) to assess all signs and symptoms including vasomotor, sudomotor, trophic changes, motor changes, and neglect-like symptoms. They were asked to rate their current and average pain intensity over the last seven days on a numeric rating scale from 0 (no pain) to 10 (worst pain imaginable) with 1/10 as the subjective pain threshold. Semi-quantitative bedside sensory testing was performed in all HCs to exclude any overt sensory impairments. This included the assessment of vibration detection using a tuning fork, light touch using a cotton swab, pinprick using a safety pin and thermal testing using a cold and warm thermoroller (Rolltemp II, Somedic SenseLab AB, Sweden). The bedside sensory examination was also performed in the control area of all individuals with CRPS to exclude any clinical signs of sensory dysfunction in this area.

2.4 Quantitative Sensory Testing

All individuals underwent a subset of the QST battery according to the German Research Network on Neuropathic Pain (Rolke et al., 2006) to assess gain in large (Aβ-) and small (Aδ- and C-) fiber function or corresponding central pathways. Heat pain thresholds (HPT), mechanical pain thresholds (MPT), mechanical pain sensitivity (MPS), and dynamic mechanical allodynia (DMA) were assessed by a QST certified experimenter. QST was performed in the affected and control area to investigate potential signs of localized and widespread sensitization, respectively. A familiarization procedure was conducted in an unaffected area other than the affected and control area prior to the actual testing. QST values were normalized to reference values from the German Research Network on Neuropathic Pain (Rolke et al., 2006) (specific to body region, age, and sex) and presented as z-scores.

2.5 Pain-autonomic readouts: Stimulation paradigm

Fifteen contact heat and fifteen pinprick stimuli were applied to the affected and control area in a randomized order with a two-minute break between stimulation areas (Fig. 1). The two modalities (pinprick and heat) were chosen to investigate modality specific SSR alterations in relation to psychophysical readouts (i.e., pain ratings). The stimulus modality (heat or pinprick) was randomized between two study visits across all individuals. Contact heat stimuli (baseline temperature 42°C; destination temperature 52°C; ramp 70°C/s) (Kramer et al., 2012) were applied to the skin with a 27mm diameter CHEPs thermode (PATHWAY Pain and Sensory Evaluation System, Medoc Ltd., Ramat Yishai, Israel). Weighted pinprick stimuli (256mN) were applied to the testing sites with the modified pinprick stimulator with an integrated contact trigger (MRC Systems, Heidelberg, Germany). The interstimulus interval was 13-17s for both stimulus modalities.

2.6 Sympathetic skin response recording set-up

All recordings were performed in a supine position. Time-locked SSRs were recorded in response to contact heat and pinprick stimulation of the affected and control area. Surface electrodes (Ambu BlueSensor NF, Ballerup, Denmark) were attached to the recording site, which consisted of the hand contralateral to the affected area. The skin

temperature of the recording and stimulation sites was kept constant (>=32°C) with heating lamps throughout all measurements (Deltombe et al., 1998). The recording site was prepared with skin prep sandpaper tape (Red Dot™ Trace Prep, 3M, United States) and alcohol. The active electrode was attached to the hand palm and the reference electrode was attached to the hand dorsum. SSRs were measured as the voltage difference between the active and the reference electrode (mV). SSRs were sampled at 2000Hz with a preamplifier and a 0.1-12kHz frequency filter. The recording window was set to 1s pre-trigger and 9s post-trigger in a customized program based on LabView (V2.6.1. CHEP, ALEA30 Solutions, Zurich, Switzerland). Signals contaminated with movement artefacts or non-time locked responses were excluded offline. SSR latencies defined as the first deflection point of the signal and SSR amplitudes (i.e., peak-to-peak responses) were detected using a customized algorithm in R statistical software for MacOS Mojave 10.14.6, version 4.1.0. (Scheuren et al., 2020).

2.7 Statistical analyses

All statistical analyses were performed in R statistical software (version 4.1.0. macOS Mojave 10.14.6) and chosen according to the data distribution, which was tested by means of histograms and quantile–quantile plots. The statistical significance was set at 0.05. Bonferroni correction was performed to adjust for multiple comparisons.

Differences between cohorts (HC and CRPS) in terms of age, questionnaire scores, and QST parameters (z-scores) were assessed with two-sample t-tests. We used linear mixed effect models ("lmer" function from R package "lme4") with post-hoc repeated measures (R package "emmeans") to test the difference in the stimulus-response function (i.e., MPS) between both cohorts. For each area (affected and control), we examined the effect of "cohort" (CRPS, HC) and stimulus "intensity" (8mN, 16mN, 32mN, 64mN, 128mN, 256mN, and 512mN) on pain ratings (NRS 0-100) with "individual" as random effect. The interaction effect "cohort x intensity" was included in the model.

Moreover, we used linear mixed effect models ("lmer" function from R package "lme4") to test the effect of "cohort" and "area" on a) pinprick pain ratings, b) heat pain ratings, c) pinprick-induced SSR, and d) heat-induced SSR with post-hoc multiple comparisons (R package "emmeans"). The interaction effect "cohort x area" was

included in all models and "individual" was added as a random effect. Habituation of pain ratings and SSRs was assessed as 1) absolute reductions in SSR amplitudes over time and 2) the percent change (normalized values) over time within each individual. The latter was performed s absolute SSR amplitudes can be variable and SSR habituation has been shown to be a reliable measure(De Schoenmacker et al., 2022). For this, we separated the 15 stimuli into three consecutive stimulation "blocks" (first, middle, last) and calculated the mean ratings and SSRs for each stimulation block. First, habituation was calculated as the remaining percent (%) of the last compared to the first block (last/first*100). Again, we used linear mixed effect models (R package "lme4") to test the effect of "cohort" (CRPS, HC) and "area" (affected, control) on the habituation (%) of a) pinprick pain ratings, b) heat pain ratings, c) pinprick-induced SSRs, and d) heat-induced SSRs. The interaction effect "cohort x area" was included in all models and "individual" was added as a random effect. Post-hoc multiple comparisons were performed for each model using the R package "emmeans". Second, we investigated habituation in terms of absolute reductions in pain ratings and amplitudes across all three blocks. We used linear mixed effect models (R package "lme4") to test the effect of "cohort" (CRPS, HC) and stimulation "block" (first, middle, last) on a) pinprick pain ratings, b) heat pain ratings, c) pinprick-induced SSR, and d) heat-induced SSR for both the affected and control area separately. The interaction effect "cohort x block" was included in all models and "individual" was added as a random effect. Post-hoc multiple comparisons were performed for each model using the R package "emmeans".

Lastly, spearman correlation analyses were performed to test the correlation between a) pinprick pain ratings and pinprick-induced SSR and b) heat pain ratings and heat-induced SSRs to test the effect of pain appraisal on autonomic readouts (Mischkowski et al., 2019).

3 Results

3.1 Clinical and pain characteristics

A total of 20 individuals with CRPS (17 females, 3 males) and 16 HC (14 females, 2 males) participated in the study. The two cohorts did not differ in age (CRPS: 44.9 ± 12.8 years; HC: 41.8 ± 13.3years; t=0.7; p=0.45). The time between visit 1 and 2 was 6.6 ± 6.8 days in individuals with CRPS and 11.7 ± 24.8 days in HC. Individuals with

CRPS presented with a pain duration of 38 ± 35.6 months (range 6-144 months), a mean current pain intensity of NRS 4.9 ± 2.3, and a 7-day average pain intensity of NRS 5.4 ± 2.5. Moreover, individuals with CRPS presented with higher pain catastrophizing scores (CRPS: 22.9 ± 12; HC: 5.9 ± 7.9; t=5.1; p<.001) and higher anxiety (CRPS: 8.1 ± 3.9; HC: 3.6 ± 2.8; t=4.0; p<.001) and depression scores (CRPS: 6.8 ± 5.0; HC: 1.5 ± 1.7; t=4.41; p<.001) than healthy controls. All clinical and pain characteristics are presented in Table 1. Individual characteristics can be found in supplementary table 1.

3.2 Quantitative Sensory Testing

In the affected area, individuals with CRPS showed higher MPS z-scores (hyperalgesia to mechanical pinprick stimuli) compared to the HC group (t=2.52, p=0.02) (Table 2A). In the control area, MPS did not differ between CRPS and HC (t=-0.16, p=0.87). Individuals with CRPS presented with a leftward shift in the stimulus-response function (MPS) compared to HC in the affected area for the 64-512mN pinprick intensity (F=13.7; p<.001) (Fig. 2C) and in the control area (F=2.2; p=0.045) for the 128mN (t=2.46, p=0.02) and 512mN (t=2.35, p=0.02) pinprick intensity (Fig. 2D). All post-hoc comparisons for each stimulus intensity can be found in the supplementary table 2. There was no difference in MPT between CRPS and HC in both the affected (t=1.03, p=0.31) and control area (t=-0.57, p=0.57). There was also no difference in HPT between CRPS and HC in both the affected (t=1.52, p=0.14) and control area (t=1.43, p=0.16). When comparing the two areas within the CRPS group, the MPT was lower in the affected compared to the control area (t=-2.47, p=0.03), but there was no difference in HPT (t=-1.17, p=0.26) and MPS (=-1.48, p=0.16) between the affected and control area. Individuals with CRPS presented with pathological QST z-scores (±1.96) in both the affected (up to 65%) (Figure 2A) and control area (up to 41%) (Figure 2B). Some HC also presented with gain of function in the affected (HPT: 13% (n=2); MPT: 13 % (n=2); MPS: 25% (n=4)) and control area (HPT: 0%; MPT: 13% (n=2); MPS: 19% (n=3).

3.3 Pain ratings

3.3.1 Pinprick pain ratings and habituation

Individuals with CRPS presented with higher mean pinprick pain ratings compared to HC after stimulation of the affected area, but not the control area (Fig. 3A). In individuals with CRPS, pain ratings were higher in the affected (NRS 4.0 ± 1.8) compared to the control area (NRS 2.4 ± 2.1). In the HC group, pain ratings did not differ between the affected (NRS 2.1 ± 1.6) and control area (NRS 2.0 ± 1.4). Pinprick pain ratings did not habituate in both the affected and control area for both CRPS and HC ($F=0.10$, $p=0.75$) (Table 3a). In both cohorts (CRPS and HC), pinprick pain ratings did not differ across the three stimulation blocks in the affected and control area. All model statistics and post-hoc comparisons can be found in the supplementary tables 3-6).

3.3.2 Heat pain ratings and habituation

Mean heat pain ratings did not differ between CRPS and HC after stimulation of the affected (CRPS: 5.1 ± 2.6; HC: 3.6 ± 2.0) and control area (CRPS: 3.9 ± 2.2; HC: 3.9 ± 2.6) (Fig. 3C). Moreover, there was no difference in the habituation of heat pain ratings in individuals with CRPS compared to HC in both the affected and control area ($F=1.46$, $p=0.24$) (Table 3b). In both cohorts (CRPS and HC), heat pain ratings did not differ across all three stimulation blocks (i.e., first, middle, and last) in both the affected and control area. All model statistics and post-hoc comparisons can be found in the supplementary tables 3b, 4b, 5b, and 6b).

3.4 Pain-related SSR

3.4.1 Pinprick-induced SSR amplitudes and habituation

Fig. 4 shows an illustrative example of pinprick-induced SSRs in CRPS and HC. A total of 1.6 ± 1.7 pinprick-induced SSR traces had to be excluded due to artefacts. Due to technical issues during the recording of pinprick-induced SSRs in one individual, the 15 traces had to be excluded from the analysis. Overall, pinprick-induced SSRs were higher in individuals with CRPS compared to HC after stimulation of the affected, but not the control area (Fig. 5A). Individuals with CRPS presented with higher pinprick-induced SSRs in the affected (2.7 ± 2.9mV) compared to the control area (1.4 ± 1.4mV). In HCs, pinprick-induced SSRs did not differ between the affected (0.8 ± 1.0mV) and control area (0.9 ± 1.1mV). In addition, individuals with CRPS showed reduced habituation of pinprick-induced SSRs compared to HC in both the affected and control area ($F=6.19$, $p=0.02$; observed statistical power 69.7%)

(Table 3c). In detail, pinprick-induced SSRs did not differ across all three stimulation blocks in the affected area in CRPS (Fig. 5B, left). In the control area of CRPS, however, pinprick-induced SSRs were higher in the first compared to the last block (Fig. 5B, right). In HC, pinprick-induced SSRs were higher in the first compared to the last block in the affected area. In the control area of HCs, pinprick-induced SSRs were higher in the first compared to both the middle and last block (Fig. 5B, right). Model statistics and post-hoc comparisons can be found in supplementary table 3c, 4c, 5c, 6c.

3.4.2 Heat-induced SSR amplitudes and habituation

A total of 1.7 ± 1.7 heat-induced SSR traces had to be excluded due to artefacts. Heat-induced SSRs were higher in individuals with CRPS compared to HC after stimulation of the affected, but not the control area (Fig. 5C). Heat-induced SSRs did not differ between the affected vs. control area in both individuals with CRPS (5.4 ± 3.6 mV vs. 5.1 ± 3.6 mV, respectively) and in HC (2.5 ± 3.2 mV vs. 3.2 ± 2.9 mV, respectively) Moreover, individuals with CRPS showed reduced habituation of heat-induced SSRs in the affected, but not the control ($F=10.93$, $p=0.003$) (Table 3d). In detail, both cohorts (CRPS and HC) showed higher heat-induced SSRs in the first compared to both the middle and the last stimulation block in both the affected (Fig. 5D, left) and control area (Fig. 5D, right). All model statistics and post-hoc comparisons can be found in supplementary table 3d, 4d, 5d, 6d.

3.4.3 Correlation between pain-related SSRs and pain ratings

In both CRPS and HC, there was no correlation between the amplitude of pinprick-induced SSRs and pinprick pain ratings (HC: $R=0.07$, $p=0.72$; CRPS: $R=-0.01$, $p=0.96$) (Fig. 6A). The amplitude of heat-induced SSRs was, however, correlated with heat pain ratings in HC ($R=0.5$, $p=0.004$), but not CRPS ($R=0.13$, $p=0.45$) (Fig. 6B).

4 Discussion

The present findings provide compelling clinical evidence that pain-autonomic interaction represents a surrogate marker of sensitization of the nociceptive system in CRPS. Individuals with CRPS presented with enhanced pinprick-induced autonomic responses alongside marked signs of mechanical hyperalgesia. Moreover, pinprick-induced SSR habituation was reduced in CRPS compared to HC. These findings further corroborate results from experimental pain models in healthy individuals (van

den Broeke et al., 2019; Scheuren et al., 2020) and demonstrate that autonomic responses can objectively detect nociceptive sensitization.

4.1 Pain-autonomic interaction depicts mechanical hypersensitivities

Individuals with CRPS presented with pronounced mechanical hyperalgesia, which could be demonstrated by a leftward shift in their stimulus-response function (i.e., increased MPS) as well as increased pinprick pain ratings in the affected area compared to HC. Alongside these psychophysical changes in the affected area, pinprick-induced SSRs were also increased and SSR habituation was reduced in CRPS compared to HC. These findings demonstrate the clinical utility of pain-autonomic interaction with respect to objectively substantiating the clinical observation of mechanical hyperalgesia. Building up on our previous study showing increased pinprick-induced SSRs and reduced SSR habituation after experimentally-induced central sensitization (Scheuren et al., 2020), these results indicate that pain-autonomic interaction may be a potential surrogate marker of nociceptive sensitization. In addition, there was no difference in the habituation of pinprick pain ratings in CRPS compared to HC. This can be explained by the low pinprick ratings in HC (i.e., floor effect), which limits the analysis of pain rating habituation in this cohort. Hence, the objective recording of autonomic readouts compared to only pain ratings offers a great advantage to characterize habituation (or the lack thereof) to repetitive mechanical stimuli.

In the present study, individuals with CRPS presented with mechanical, but not heat hyperalgesia. Moreover, heat pain ratings did not differ between CRPS and HC. Nevertheless, heat-induced SSRs were increased and SSR habituation was reduced in CRPS compared to HC in the affected area. Heat-induced SSRs did, however, show a stronger reduction over time (38%) compared to pinprick-induced SSRs (19%). As such, investigating SSR habituation provided added value to detect modality specific hypersensitivities (i.e., mechanical vs. heat) in this cohort. It is, however, important to note that pinprick- and heat-induced SSRs were recorded at two different days and thus differentially influenced by mental and emotional factors that may vary between visits. As such, future studies should assess mental and emotional factors prior to each testing session as well as each testing area to enable direct comparisons between modalities and areas for both psychophysical and autonomic readouts.

Overall, these findings are in line with previous studies demonstrating increased autonomic responses in individuals with chronic pain (Ozkul and Ay, 2007; Schestatsky et al., 2007) as well as human experimental pain models (van den Broeke et al., 2019; Scheuren et al., 2020). As nociceptive and autonomic pathways intersect at multiple levels of the central nervous system, it is highly conceivable that autonomic responses, such as SSRs induced by noxious stimuli, can indirectly reflect the existence of provoked pain. A variety of alterations in autonomic responses to noxious stimuli have been reported, such as changes in heart rate, pupil dilation, blood pressure, and electrodermal activity (Kyle and McNeil, 2014). Previous investigations have demonstrated that sympathetic responses to noxious stimuli result from nociceptive processes rather than the subjective perception of pain (Loggia and Napadow, 2012; Nickel et al., 2017; Treister et al., 2012). This emphasizes the potential use of autonomic responses (i.e., reduced SSR habituation) as an indirect objective readout of nociceptive sensitization in pain patients.

4.2 Dissociation between subjective pain ratings and autonomic responses

In the present study, we observed a dissociation between subjective heat pain ratings and objective autonomic responses. Autonomic responses to noxious stimuli have previously been related to pain appraisal, indicating that stimuli that are perceived as more painful will in turn generate increased sympathetic and/or decreased parasympathetic outflow (Mischkowski et al., 2019). Moreover, reduced or absent SSR amplitudes have been reported in patients with documented hypoesthesia and reduced afferent integrity (Veciana et al., 2007). Although individuals with CRPS and HC presented with similar heat pain ratings, heat-induced SSRs were increased in CRPS compared to HC. Moreover, heat-induced SSRs did not correlate with the respective heat pain ratings in CRPS. This is intriguing as it indicates that the enhanced autonomic responses were not only driven by stimulus-induced arousal due to the perceived pain intensity. Notably, the modulation of autonomic responses to noxious input may be partially independent from perceptual processes. SSRs may reflect a nociceptive-specific response driven through direct spinal somato-sympathetic reflexes or brainstem mechanisms involved in both nociceptive and autonomic function (Benarroch, 2001; Kyle and McNeil, 2014; Sato and Schmidt, 1973). Subjective perception of pain is likely more dependent on secondary evaluative

(affective/emotional) influences. In contrast, in HC the magnitude of the autonomic responses was associated with heat-pain intensities. The dissociation between psychophysical and autonomic responses in CRPS but not HC implies that physiological mediating effects of pain appraisal on autonomic responses (as seen in HC) may undergo pathological changes in individuals with chronic pain. One can thus postulate a state of generalized hyperexcitability in individuals with CRPS, possibly rendering the autonomic nervous system more susceptible to noxious input, irrespective of the perceived stimulus intensity. This may be mediated by sensitization processes involving key substrates implicated in both nociceptive and autonomic processes at spinal and/or supraspinal levels (Benarroch, 2001; Schestatsky et al., 2007). While the present data negates a simple linear relationship between perceived pain intensity and the observed autonomic responses in CRPS, SSRs may still be influenced, at least in part, by higher order cognitive and affective processes (Barnes et al., 2021; Colagiuri and Quinn, 2018; Rainville et al., 2005). Individuals with CRPS may present with increased attention and/or negative expectation towards noxious input compared to HC, which may lead to higher autonomic responses in CRPS compared to HC. To our knowledge, the influence of cognitive and affective variables on pain-related autonomic responses has only been investigated in a few studies (Kyle and McNeil, 2014). In particular, future investigations are warranted to assess the influence of attention and expectation on pain-related autonomic responses in patients with chronic pain.

4.3 Disentangling peripheral and central sensitization

In the present study, the most prominent sign in individuals with CRPS was increased mechanical hyperalgesia in the affected area compared to HC, which reproduces findings of previous studies investigating QST profiles in individuals with CRPS (Gierthmühlen et al., 2012; Reimer et al., 2016). In addition, 41 % of individuals with CRPS presented with pathological MPS scores and increased MPS compared to HC in the unaffected, control area, especially for the 128mN and 512mN pinprick intensity. These findings demonstrate signs of generalized sensitization of the nociceptive system. This is also in line with previous studies demonstrating hypersensitivities at the unaffected contralateral limb over the course of the disease (Huge et al., 2008; Reimer et al., 2016; Van Rooijen et al., 2013). In addition to widespread sensitivities

upon QST, pinprick-induced SSR habituation was reduced in CRPS compared to HC not only in the affected, but also the control area. These findings highlight that autonomic responses may be able to detect widespread signs of hyperexcitability (i.e., central sensitization). In conjunction with psychophysical readouts (i.e., pain ratings and QST), the observed enhanced autonomic responses offer a novel objective proxy of nociceptive sensitization in the present CRPS cohort.

In individuals with CRPS, it still remains challenging to localize pathological processes along the neuraxis, as alterations in somatosensory function can be driven by peripheral, spinal, and/or supraspinal mechanisms (Birklein et al., 2018; Marinus et al., 2011). However, widespread somatosensory dysfunction has been related to central sensitization of the nociceptive system (Arendt-Nielsen et al., 2017). In particular, mechanical hyperalgesia is a hallmark sign of central sensitization (Baron et al., 2017; Vollert et al., 2018) and was frequently documented in the current study. Moreover, the initial local inflammatory response in acute CRPS (i.e., keratinocyte proliferation, release of inflammatory mediators, growth factors, and mast cell accumulation) is known to normalize over time in the majority of patients, but not all. In such patients with reduced inflammatory responses over time, peripheral sensitization can no longer adequately explain pain hypersensitivities in chronic stages (Birklein et al., 2018). The exaggerated post-traumatic inflammatory response may lead to sensitization of peripheral nociceptors and second order neurons in the spinal dorsal horn (Birklein and Schlereth, 2015), which can persist in chronic CRPS. This pathophysiological shift from acute to chronic CRPS has been related to the ipsilateral spread of hyperalgesia or even the spread to contralateral and remote areas in chronic CRPS (Drummond et al., 2018; Marinus et al., 2011; Reimer et al., 2016; Van Rijn et al., 2011). Nevertheless, some patients in the present study still presented with heat hyperalgesia in the affected area, which may be due to ongoing peripheral sensitization and represent an important contributor to their pain phenotype. In addition, the finding that individuals with CRPS present with lower pain thresholds to mechanical stimuli in the affected compared to the control area highlights that both peripheral and central sensitization may be contributing in a cumulative manner to the pain phenotype in chronic CRPS. As such, alterations in both psychophysical and objective autonomic responses after stimulation of the affected and the control area in CRPS suggest alterations beyond, but not excluding, the peripheral nervous system, possibly due to hyperexcitability at the level of the central nervous system. Taken

together, pain-autonomic interaction holds the potential to objectively characterize sensitization processes that may occur at different levels of the nociceptive neuraxis. To further disentangle between peripheral and central processes, additional autonomic measures such as pupil dilation and heart rate variability may allow a more precise investigation of pain-autonomic interaction, encompassing both sympathetic activation and parasympathetic withdrawal (Loggia and Napadow, 2012; Möltner et al., 1990; Tousignant-Laflamme et al., 2005).

While the present study offers novel insights into the potential use of autonomic readouts of sensitization in CRPS, there are some limitations that warrant a discussion. First, the small sample size limits the generalizability of the current findings to a broader cohort of individuals with CRPS. That said, the female/male ratio was slightly higher than suggested in epidemiological studies (Sandroni et al., 2003). Nevertheless, the primary aim of this study was to investigate differences in autonomic responses between CRPS and HC. As all HC were matched in terms of age, sex, and areas tested, the overall gender distribution is not as relevant to the current study. Moreover, it would have been beneficial to have used the same control area across all individuals, as SSRs may be different after stimulation of the shoulder or hand. Lastly, our analysis did not take the intake of medication into account as subgrouping based on medication was not feasible due to the small sample size, as was withholding medication.

4.4 Conclusion

This study demonstrates that pain-autonomic readouts may represent objective tools to assess widespread (i.e., peripheral and central) sensitization of the nociceptive system in individuals with CRPS with evoked signs of mechanical hyperalgesia. Future studies are warranted to assess the applicability of pain-autonomic measures in a broader CRPS cohort, across different pain conditions and evaluate their clinical application at an individual level. In this sense, newly introduced pain-autonomic readouts may offer objective novel avenues to explore pathophysiological mechanisms in a wide variety of pain patients and could help guide mechanism-based treatment strategies.

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6 Author contributions

P.S.S. contributed substantially to the study conception and design, data acquisition, analysis, data interpretation and drafted the manuscript. I.DS. contributed to study design, data acquisition, data interpretation and revised the manuscript. J.R. contributed to the study conception and design, interpretation of results and revised the manuscript. F.B. contributed to the study conception, interpretation and revised the manuscript. A.C. contributed to the study conception and design, data interpretation and revised the research article for important intellectual content. M.H. made substantial contributions to study conception and design, data acquisition, analysis and interpretation and revised the research article critically for important intellectual content. All authors gave their final approval of the version to be published.

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8 Figure Legends

Fig. 1. Pain-autonomic interaction. Recordings of sympathetic skin responses (SSRs) in response to noxious heat or pinprick stimulation of the affected and control area.

Fig. 2. Quantitative Sensory Testing. The frequencies of pathological QST z-scores (±1.96 SD) in CRPS are shown for the affected (A) and control area (B). The shift in the stimulus-response function in terms of mechanical pain sensitivity (MPS) is shown for the CRPS (red) compared to HC (blue) in both the affected (C) and control area (D). *p<.05; ***p<.001. *DMA, dynamic mechanical allodynia; HPT, heat pain threshold; MPT, mechanical pain threshold*

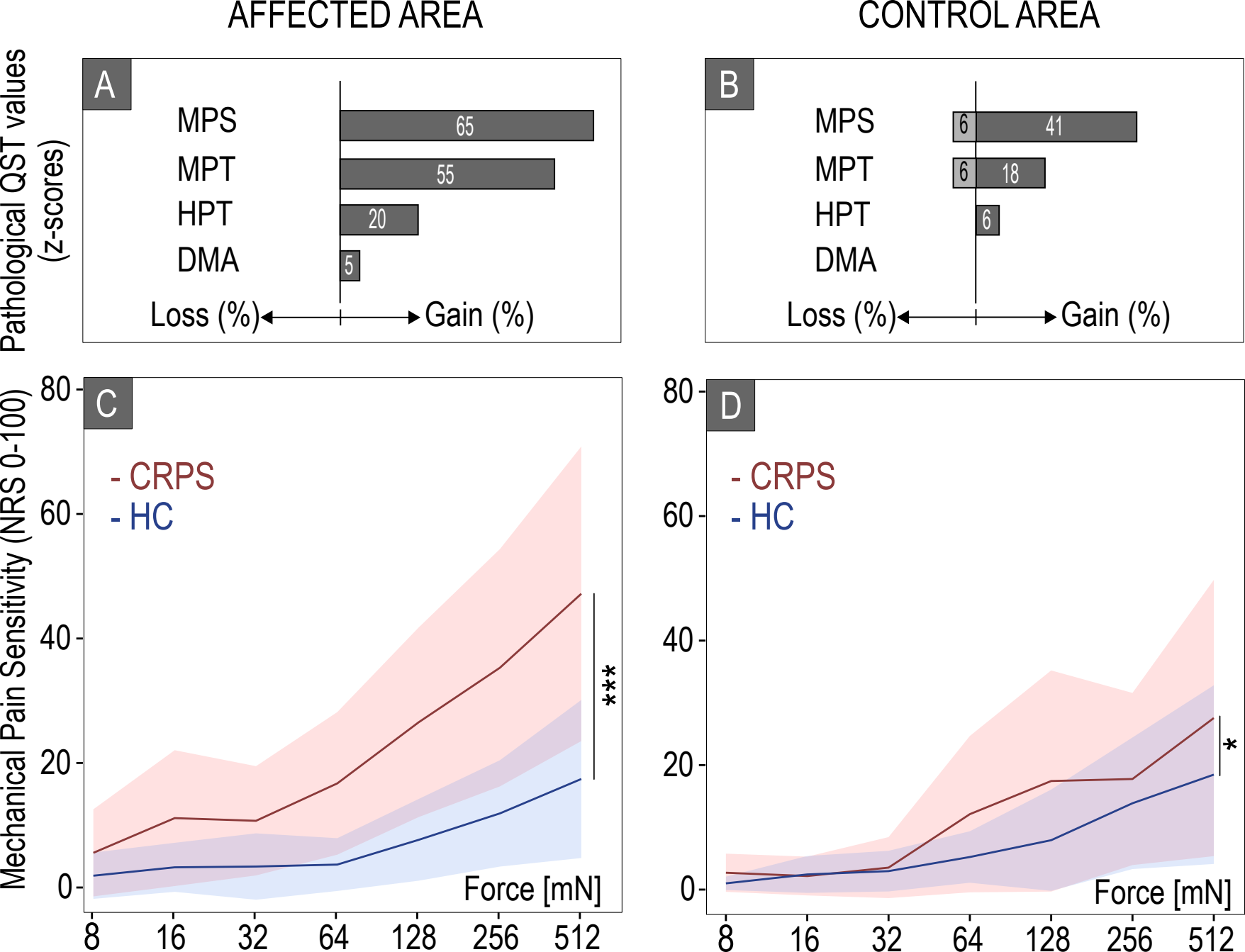
Fig. 3. Pinprick and heat pain ratings. Mean pinprick pain ratings (A) and habituation of pinprick ratings (B) from the "first" "middle" and "last" stimulation blocks are shown in the top panel. Mean heat pain ratings (C) and habituation of heat pain ratings (D) area shown in the bottom panel. Pain ratings are shown after stimulation of the control (CON) and affected area (MP) in patients with complex regional pain syndrome (CRPS - red) and healthy controls (HC - blue). Significances are shown for comparisons between areas: *p<.05; **p<.01; ***p<.001; and between cohorts: $^{\#}$p<.05; $^{\#\#}$p<.01; $^{\#\#\#}$p<.001

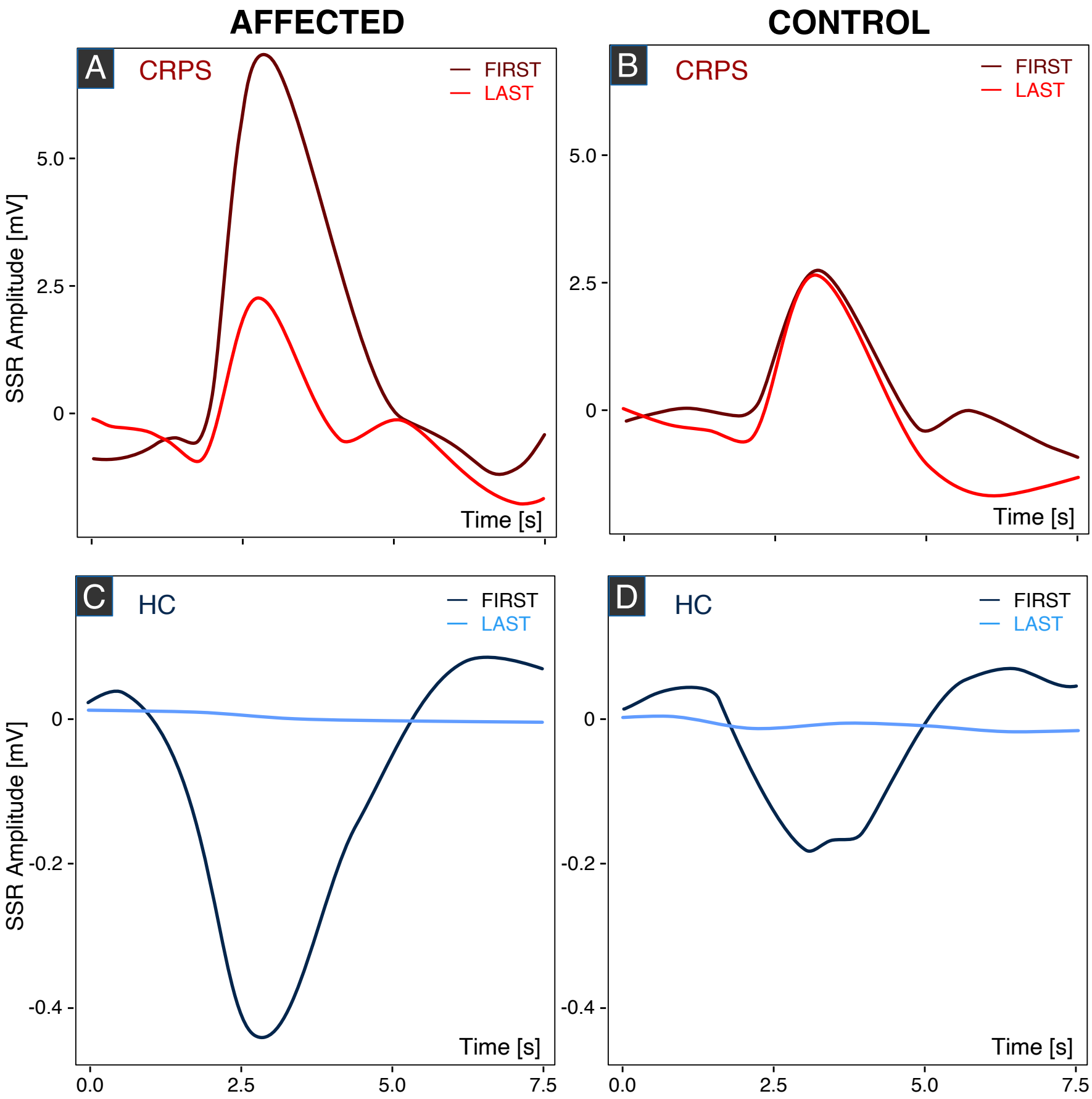
Fig. 4. Illustrative example of pinprick-induced sympathetic skin responses (SSRs) in CRPS and HC. Pinprick-induced SSRs are higher in the individual with CRPS (A-B) compared to the HC (C-D). In the patient with CRPS, pinprick-induced SSRs are still prominent in the last stimulation block (i.e., reduced habituation) in both the affected (A) and control area (B). In the HC, pinprick-induced SSRs are no longer present in the last stimulation block (i.e., normal habituation). Please note the different y-axis scales for CRPS and HC.

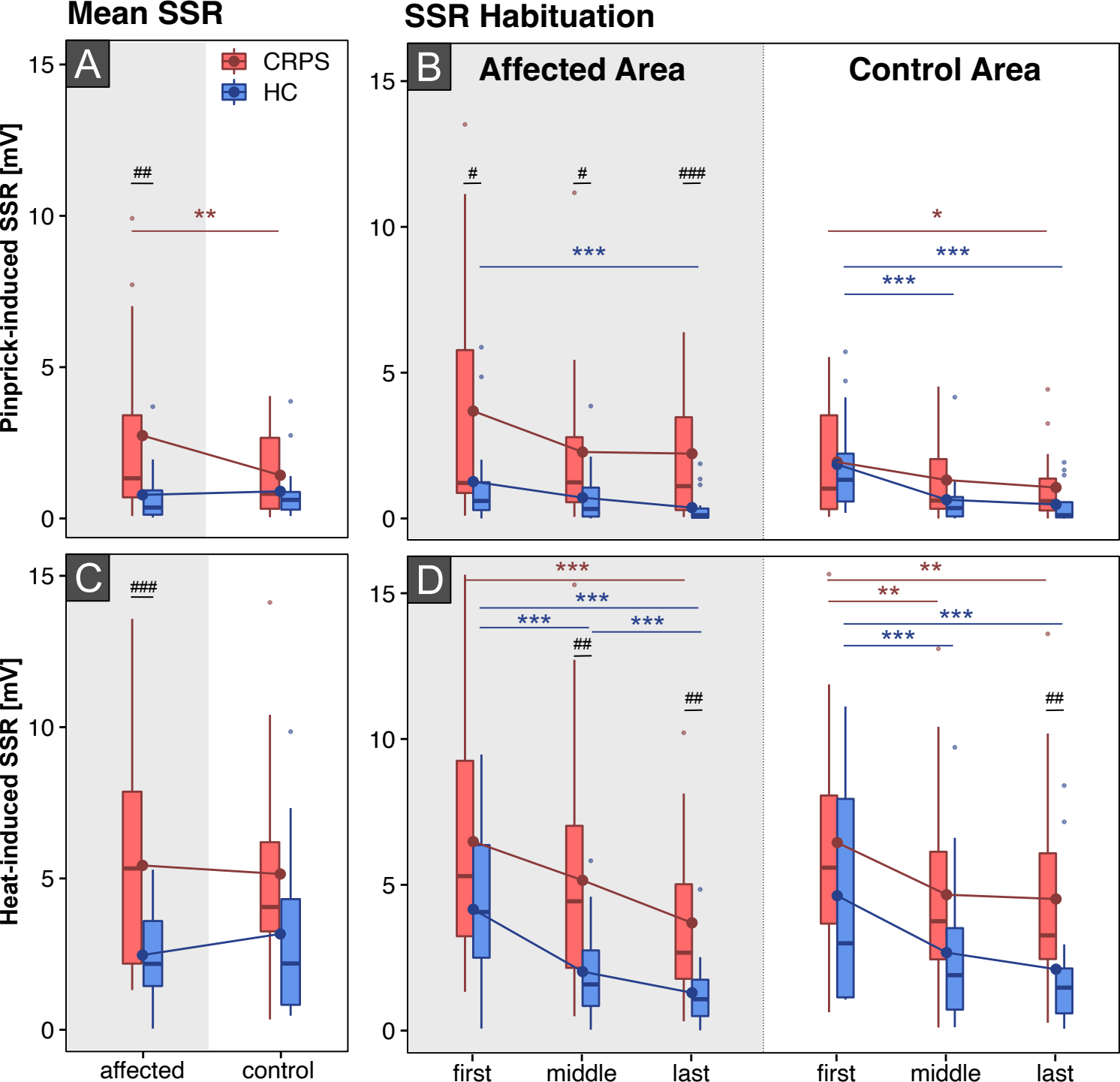
Fig. 5. Pinprick- and heat-induced sympathetic skin responses (SSRs). Mean pinprick-induced SSRs (A) and SSR habituation (B) from the "first", "middle", and "last" stimulation blocks are shown in the top panel. Mean heat-induced SSRs (C) and SSR habituation (D) are shown in the bottom panel. SSR amplitudes are shown in response

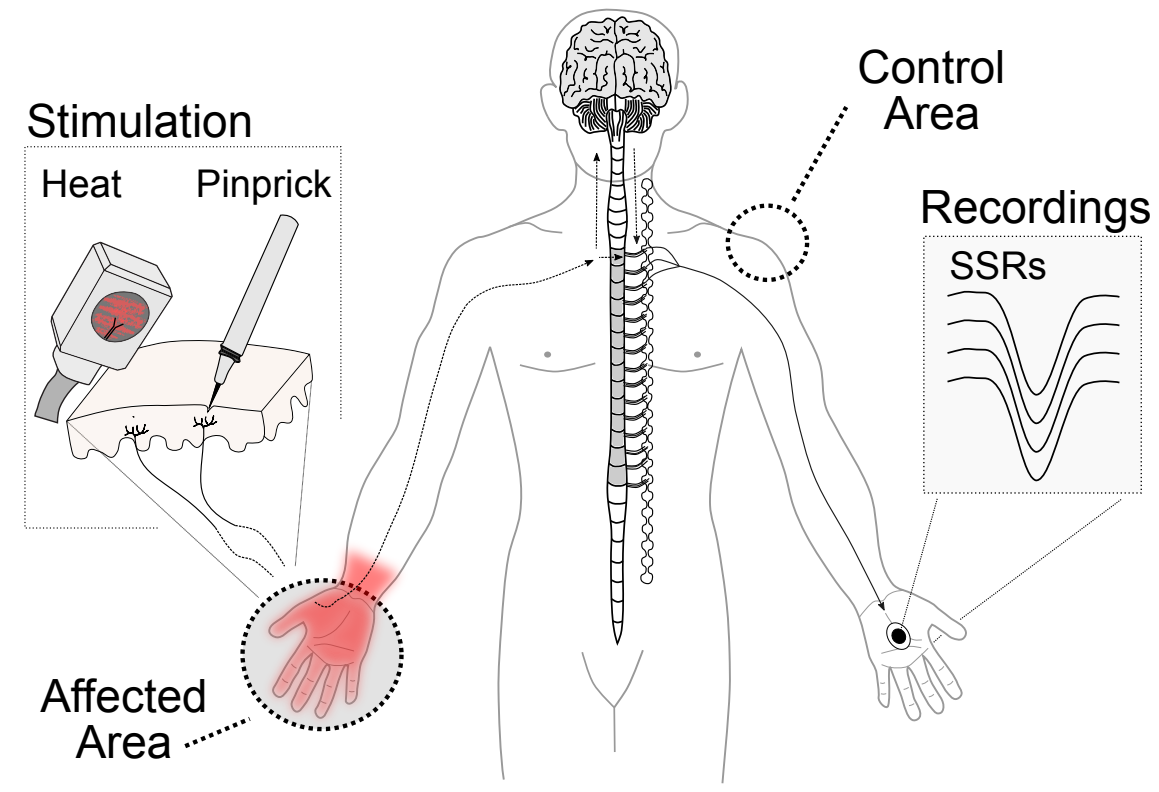
to stimulation of the affected and control area in individuals with complex regional pain syndrome (CRPS - red) and healthy controls (HC - blue). Significances are shown for comparisons between areas: *p<.05; **p<.01; ***p<.001; and between cohorts: #p<.05; ##p<.01; ###p<.001

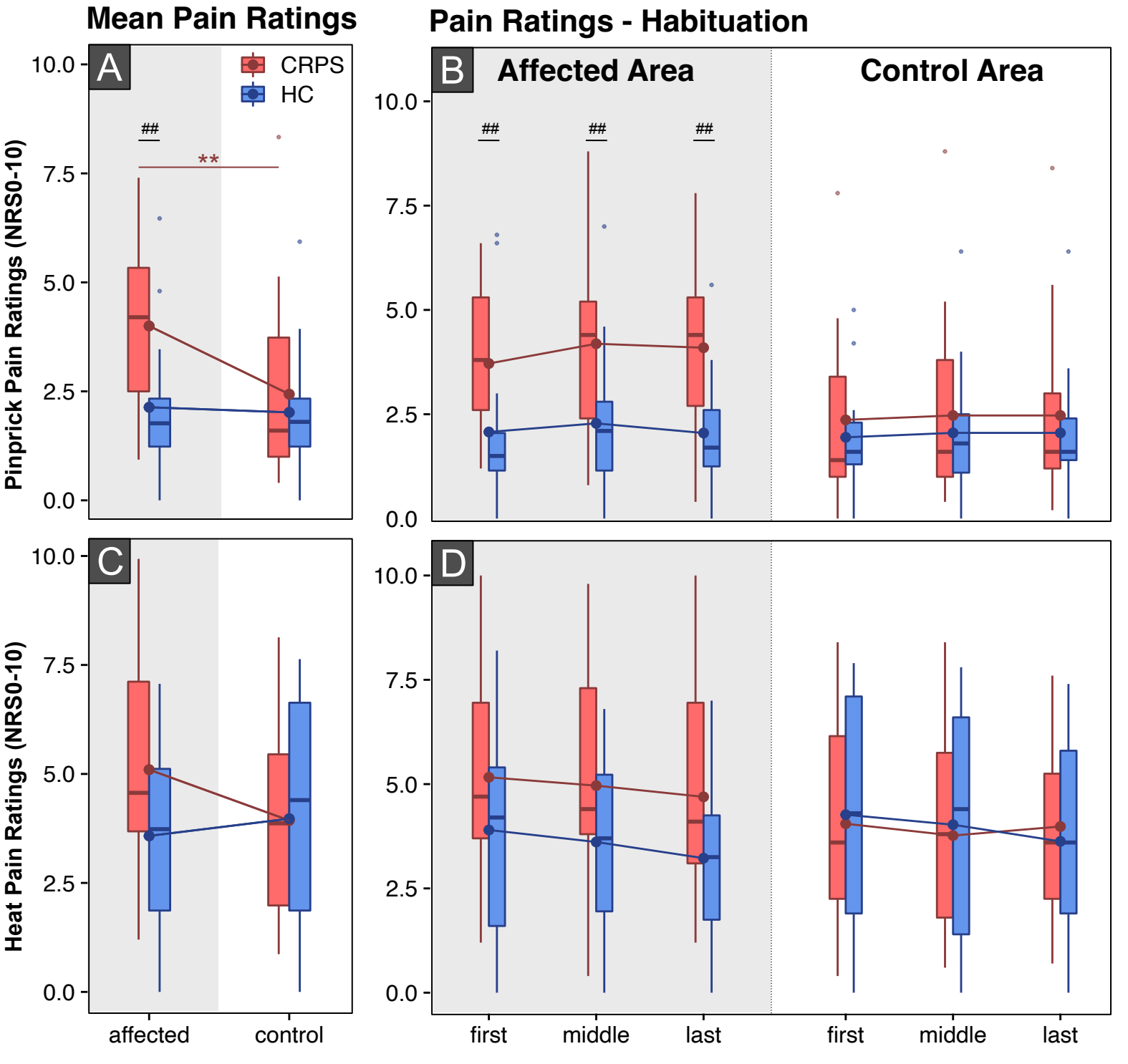
Fig. 6. Association between sympathetic skin response (SSR) amplitudes and pain ratings in response to noxious heat (A), and pinprick stimulation (B) for CRPS (red) and HC (blue).


AFFECTED AREA
CONTROL AREA
Pathological QST values (z-scores)
A
MPS
MPT
HPT
DMA
65
55
20
5
Loss (%)
Gain (%)
B
MPS
MPT
HPT
DMA
6
41
6
18
6
Loss (%)
Gain (%)
Mechanical Pain Sensitivity (NRS 0-100)
C
- CRPS
- HC
***
Force [mN]
8
16
32
64
128
256
512
0
20
40
60
80
D
- CRPS
- HC
*
Force [mN]
8
16
32
64
128
256
512
0
20
40
60
80


AFFECTED
CONTROL
A
CRPS
FIRST
LAST
B
CRPS
FIRST
LAST
C
HC
FIRST
LAST
D
HC
FIRST
LAST
SSR Amplitude [mV]
5.0
2.5
0
-0.2
-0.4
Time [s]
0.0
2.5
5.0
7.5


Mean SSR
SSR Habituation
A
B
C
D
CRPS
HC
Affected Area
Control Area
Pinprick-induced SSR [mV]
Heat-induced SSR [mV]
15
10
5
0
##
**
#
#
###
*
***
***
***
***
***
***
**
**
***
***
##
##
##
affected
control
first
middle
last
first
middle
last


Stimulation
Heat
Pinprick
Control
Area
Recordings
SSRs
Affected
Area


Mean Pain Ratings
Pain Ratings - Habituation
A
B
C
D
CRPS
HC
Affected Area
Control Area
Pinprick Pain Ratings (NRS0-10)
Heat Pain Ratings (NRS0-10)
10.0
7.5
5.0
2.5
0.0
##
**
affected
control
first
middle
last

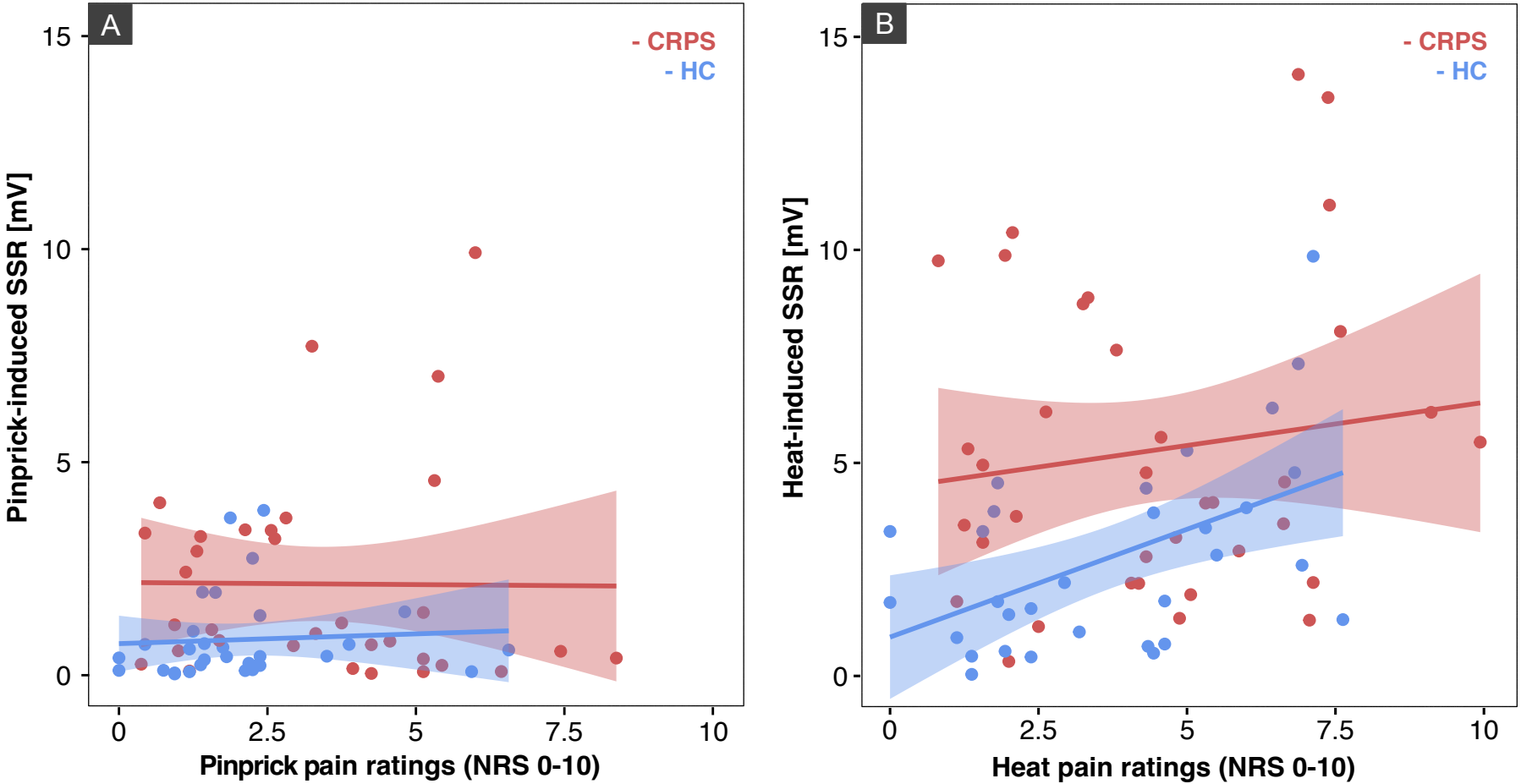

A
- CRPS
- HC
Pinprick-induced SSR [mV]
15
10
5
0
0
2.5
5
7.5
10
Pinprick pain ratings (NRS 0-10)
B
- CRPS
- HC
Heat-induced SSR [mV]
15
10
5
0
0
2.5
5
7.5
10
Heat pain ratings (NRS 0-10)

Table 1. Clinical and Pain Characteristics

CRPS characteristics	
CRPS limb, n (%)	
Upper limb	14 (70)
Lower limb	6 (30)
Multiple limbs	3 (15)
Inciting event, n (%)	
Bone fracture	10 (50)
Sprain	4 (20)
Surgical intervention	5 (25)
Bruising trauma	1 (5)
Other signs and symptoms, n (%)	
Trophic changes	7 (35)
Edema	10 (50)
Sudomotor changes	8 (40)
Vasomotor changes	10 (50)
Motor changes	15 (75)
Neglect-like symptoms	3 (15)
Medication, n (%)	
NSAID	8 (40)
SSNRI	4 (20)
Opioids	5 (25)
Anticonvulsants	
Gabapentin	3 (15)
Pregabalin	3 (15)
Lidocaine	1 (5)
Antidepressants	5 (25)

Abbreviations: NSAID, non-steroidal anti-inflammatory drugs; SSNRI, selective serotonin reuptake inhibitors

Table 2. Quantitative Sensory Testing Parameters

	HPT (mean ± SD)	**MPT** (mean ± SD)	**MPS** (mean ± SD)
A. Affected Area	n.s	n.s	*
CRPS	0.79 ± 1.35	1.66 ± 1.66	2.17 ± 1.35
HC	0.16 ± 1.15	1.30 ± 0.85	1.19 ± 0.97
B. Control Area	n.s	n.s	n.s
CRPS	0.07 ± 0.94	0.80 ± 1.30	1.47 ± 1.87
HC	-0.37 ± 0.82	1.06 ± 1.30	1.55 ± 0.87

Mean and standard deviation (SD) are shown for z-scores of heat pain thresholds (HPT), mechanical pain thresholds (MPT), and mechanical pain sensitivity (MPS) in a) the affected and b) control area. Significant differences between groups (CRPS vs. HC) are labelled as *p<.05.

Table 3. Habituation of pain ratings (a-b) and sympathetic skin responses (c-d)

	CRPS			HC			
	First block	**Last block**	**Habituation (%)**	**First block**	**Last block**	**Habituation (%)**	CRPS vs. HC
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	p-value
a) Pinprick pain rating (NRS)							
i. Affected	3.7 ± 1.6	4.1 ± 1.9	119 ± 49	2.1 ± 1.9	1.9 ± 1.2	120 ± 48	n.s
ii. Control	2.4 ± 2.1	2.5 ± 2.1	116 ± 61	2.1 ± 1.4	2.0 ± 1.6	109 ± 44	n.s
b) Heat pain rating (NRS)							
i. Affected	5.1 ± 2.5	4.7 ± 2.7	96 ± 20	3.9 ± 2.4	3.2 ± 1.8	94 ± 31	n.s
ii. Control	4.0 ± 2.4	3.9 ± 2.6	141 ± 169	4.3 ± 2.7	6.3 ± 2.4	87 ± 18	n.s
c) Pinprick-induced SSR (mV)							
i. Affected	3.7 ± 4.2	2.2 ± 2.2	81 ± 69	1.3 ± 1.7	0.4 ± 0.6	33 ± 32	*
ii. Control	1.9 ± 2.0	1.1 ± 1.2	67 ± 82	1.9 ± 1.7	0.5 ± 0.7	22 ± 23	*
d) Heat-induced SSR (mV)							
i. Affected	6.5 ± 4.1	3.8 ± 2.2	62 ± 28	4.2 ± 2.7	1.3 ± 1.1	36 ± 25	**
ii. Control	6.4 ± 4.0	4.6 ± 3.7	69 ± 26	4.6 ± 3.8	2.1 ± 2.5	49 ± 34	n.s

Habituation was computed as the percent (%) of the last compared to the first block (last/first*100) for both pain ratings and sympathetic skin response (SSR). The comparison of pain rating and sympathetic skin response (SSR) habituation (%) between both cohorts (CRPS vs. HC) is shown in the last column. Significance levels: *<.05; **<.01
NRS: numeric rating scale