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Sympathetic Skin Response as an Objective Tool to Estimate Stimulus-associated Arousal in a Human Model of Hyperalgesia

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Running title: Online detection of pain using skin responses

Abstract

Background: Pain is a private experience, whose assessment relies on subjective self-reporting. Inaccurate communication renders pain evaluation unreliable in individuals with alteration of consciousness, lack of verbal interaction, cognitive dysfunction or simple malingering, hence the importance of developing reliable objective assessment tools. **Objectives:** Since pain is associated with autonomic arousal, here we used readouts of autonomic activity to assess objectively the arousing effect of somatic stimuli in a human model of hyperalgesia. **Methods:** We used topical capsaicin to induce cutaneous hypersensitivity in the right arm of 20 healthy volunteers, and recorded sympathetic skin responses (SSR) and numerical perceptive ratings (NRS) to stimulation of the sensitized region and its homologous contralateral site, using brush ($A\beta$), pinprick ($A\delta$) and laser (C-Warmth) stimuli. **Results:** Both subjective ratings and SSRs were significantly enhanced to stimulation of the sensitized region, and their respective ratios of maximal enhancement were positively correlated. At individual level, a significant association was observed between SSR and NRS behavior ($\chi^2(1)= 11.03$; $p<0.001$), with a positive predictive value of 87% (CI95 [77-97%]) for SSR increase predicting enhancement of subjective reports. A “lie experiment” asking subjects to simulate elevated NRS failed to enhance SSRs. Significant habituation of SSRs appeared when stimuli were repeated at ~15s intervals, hence decreasing their negative predictive value when several consecutive stimuli were averaged (NPV=46%; CI95[30-62%]). **Conclusion:** The SSR may represent a rapid and reliable procedure to assess cutaneous hypersensitivity, simple to use in clinical practice and resistant to simulation. Rapid habituation is a drawback that can be countered by using few repetitions and low stimulus rates.

Keywords: Sympathetic skin response, autonomic nervous system, subjective perception of nociception, human model of pain, capsaicin

Introduction

Current 'gold-standard' pain assessment tools rely on subjective self-reporting via more or less sophisticated tools, always requiring an individual ~~both~~ to process external information and to communicate this personal experience. To overcome the subjectivity of self-reported pain, it seems important to develop reliable objective tools. Pain at rest and pain evoked by external stimuli are thought to have distinct pathophysiological mechanisms[47]. Whereas assessing spontaneous pain remains elusive, provoked pain may be more accessible to 'on-line' objectivation. While some objective assessment tools such as neuroimaging, microneurography or blood biomarkers are not yet relevant for use in routine clinical practice, 'on-line' and easy clinically implementable tools such as EEG, EMG and autonomic nervous system recordings are promising techniques. By entailing arousal, noxious stimuli increase sympathetic and baroreceptor responses and decrease parasympathetic activity[5]. Consequently, objective readouts of autonomic activity such as heart rate variability, blood pressure responses, pupillary changes or skin sweating can be applied to assess quantitatively the effect of pain on the autonomic nervous system[9, 25]. These measures are of importance to assess painful procedures in situations where communication is compromised, such as in infants and newborns[7,10,20,22], critically ill adults in intensive care settings[18,19,24] or patients under general anesthesia, in whom autonomic readouts were found to outclass cortical responses to detect pain-related changes[41,42]. Taking advantage of pain-autonomic interactions [3,39] might be also useful to unmask malingering, the prevalence of which in chronic pain patients has been estimated at 20-50%[16].

Among these methods, the sympathetic skin response (SSR) combines rapidity of response (allowing on-line monitoring) and technical simplicity (allowing clinical application) [4] unlike other autonomic tests which may require specific equipment or complicated off-line analysis. SSRs reflect changes in skin conductance following sudden, unexpected or stressful stimuli –and in general any stimulus inducing sudden arousal [8,49]. They have been used to objectify stress reactions in premature infants[20], to identify arousal reactions to pain in both animals[35] and humans[6,36], and to detect hypoalgesia due to neural lesions[4,48]. In contrast with other autonomic signals, the SSR is thought to be largely independent of adrenergic agents, haemodynamic variability and respiratory rate [41]. While SSR

has shown its ability to respond to noxious stimuli such as laser or thermode pulses[6,26,28] and to detect hypalgesia due to neural lesions such as syringomyelia[48], its ability to detect abnormal subjective perception, including pain, in states of pathological or experimental hypersensitivity consistent with routine clinical practice has scarcely been explored [31,39].

The main objective of this study was to assess the ability of SSR to detect abnormally enhanced sensations in a validated human model of hyperalgesia (enhanced pain to a painful stimulus) and allodynia (pain elicited by non-noxious stimuli) provoked by the topical application of capsaicin[21,24,32,34]. The secondary aims were to assess whether the magnitude of SSR was parametrically correlated with the magnitude of subjective noxious sensation. Since the magnitude of SSR is extremely sensitive to habituation, we also assessed its decay within short sequences of 3-5 consecutive stimuli to develop a recording methodology robust enough to be applied in patients with suspected allodynia or hyperalgesia.

Methods

Participants

The study was performed in 20 healthy volunteers (mean age 38.1 ± 16.7 years, 8 women). None of them was on use of medication or under health conditions known to affect autonomic nervous system function or pain perception. All subjects gave written informed consent for the study, which was designed in accordance to the Helsinki Declaration and approved by the Local Ethics Committee (Comité de Protection des Personnes Sud-Est, n°2013-43 13/02/2017). Participants were remunerated for their contribution to the experiment.

Experimental setting: Hyperalgesia model and cutaneous stimuli

The experiments were performed in a quiet, semi-darkened room, at a temperature between 23.0°C and 24.0°C. The protocol had two parts: (a) application of topical capsaicin to develop a model of cutaneous hyperalgesia, and (b) recording of physiological and psychophysical responses to different cutaneous stimuli. During the period of capsaicin patch application, participants were installed in a comfortable semi-reclined position on a coach and kept in a calm atmosphere while experimenters installed the electrodes and finalized the preparations for the

experiment. During the experimental recordings, subjects were instructed to remain as calm as possible (refrain from coughing, sighing, laughing or breathing deeply) and to concentrate on the sensations in the stimulated territories during the procedure.

Set-up of the hyperalgesia model

A 5 x 5 cm patch of capsaicin 8% (Qutenza®) was applied to the anterior aspect of the right forearm, 7 cm distal to the elbow crease, for 30 minutes. After removal of the patch the experimenter verified the flare-up of the skin at the site of application (Fig. 1) as well as the existence of spontaneous burning sensations, their quality and intensity using a numeric rating scale (see description below). Whenever spontaneous sensations abated along the experiment, rekindling of the skin receiving the patch was performed using a thermode maintained at 40.0°C for 3 minutes. This procedure improves the stability and reproducibility of hyperalgesia[34] and was performed via a circular thermode embedded with micro-Peltier elements and allowing a contact with the skin of 700 mm² (TSII system®, QST lab, Strasbourg, France).

Stimulation modalities used in hyperalgesia assessment

Thermal and mechanical stimuli of different types were applied to the hyperalgesic primary area in the right arm, and to the homologous control (untreated) area in the left arm. The modalities were selected to allow selective activation of A β , A δ and C-fibers through brush, pinprick and laser stimulation respectively.

- Laser radiant innocuous heat (stimulation of C-warmth fibers)

Warm stimuli of non-nociceptive nature were used to test heat hypersensitivity by stimulating C-warmth skin terminals. They were delivered via laser pulses of 10 ms duration applied to a 15-mm diameter spot (~177 mm²) using a solid-state Nd:YAP laser stimulator (1.34 μ m wavelength, ElEn®, Florence, Italy). A red helium-neon (He-Ne) laser confocal with the infrared beam was used to visually indicate the irradiated area. Stimulus energy was fixed at 2 times the perception threshold of warmth on the control side, determined by the method of levels[23], and remained between 34 mJ/mm² and 39.6 mJ/mm², which correspond to a stimulation energy of

6 to 7 Joules, applied to a skin circle of 15 mm diameter. These parameters allow activating C-warm fibers without concomitant excitation of A δ axons, and elicit a subjective sensation of 'slow warmth' on normal skin[11,46].

- Pinprick stimuli (stimulation of A δ fibers)

Pinprick stimuli were used to test mechanical hyperalgesia. They were applied using a 24 g (235 mN) von Frey / Semmes-Weinstein nylon filament with a flat contact area of 0.65 mm. The pressure application was made slowly (~ 1 s) until bending of the filament, so as to elicit a pricking sensation attributed to activation of A δ fibers. A digital trigger was sent to the computer by a second experimenter concomitantly to each pinprick stimulus, when the filament came into contact with the skin of the participant.

- Brush stimuli (stimulation of A β fibers)

Brush stimuli were used to test dynamic mechanical allodynia (DMA). They were applied using a soft brush, by stroking tangentially the skin at a constant speed of about 2 cm/s, hence activating A β fibers. A digital trigger was sent to the computer by a second experimenter concomitantly to each pinprick stimulus, when the brush came into contact with the skin of the participant.

Brush modality was discarded in 5 participants in order to increase stimulation trials of the other modalities without increasing experiment duration.

Stimulation procedures

All stimuli were applied to the anterior part of the forearm, either to the sensitized region of the right forearm where capsaicin had been applied (primary hyperalgesic region) or to its homologous zone on the left forearm (control region). An exception to this scheme was introduced during the complementary "lie experiment" (see below). For each modality, stimulations were delivered alternatively to the control and sensitized sides, always starting on the control region. Each subject was tested with 3 to 5 stimuli of each modality per side. To stabilize attention and minimize the "1st stimulus effect", participants were forewarned about the onset of the procedure, before the first stimulus of each modality and stimulation series, but no warning was

given for the rest of stimuli in the series, and this was true for all types of stimuli. Stimuli were applied at irregular intervals of 15-20 seconds, to minimize habituation. The sequence of stimulation modalities was pseudo-randomized, with the constraint that the first sequence was always radiant heat (warm stimulation of C-fibers).

Simulation followed by exaggerated perception: a “lie experiment”

This condition, performed in 10 participants, was introduced to investigate possible effects on SSRs of deliberate manipulation of pain ratings (malingering) on the stimulated limb. In this condition participants were asked to simulate an exaggerated perception when laser and pinprick stimuli were applied to a non-sensitized rectangle-shaped area of about 25 cm² on the right forearm, outside and faraway (at least 10 cm) from the hyperalgesic zone. The area was delineated by a marker to help the participants focusing on it. Responses were contrasted with those obtained when stimuli were applied to the contralateral (control) zone on the left forearm.

Quantitative assessment of subjective sensations

Subjective perceptions were rated using a verbal numerical rating scale (NRS), where “0” equaled “no sensation”; “4” was defined as the pain threshold and “10” as the maximal imaginable pain. Subjects were free to use fractions if they felt that these better described their sensations. Further to reporting intensity, participants were instructed to distinguish the perception of sharp, pricking or burning sensations from touch or pressure, and to declare when several types of sensation could be distinguished following a single stimulus. They were also instructed to wait at least 10 seconds before rating the intensity and quality of their perception, so as to minimize muscle artifacts on the physiological recordings.

Recording and analysis of sympathetic skin responses (SSRs)

To allow time-locked averaging of the electrophysiological responses, digital triggers were sent to the computer simultaneously with each of the control or test stimuli. SSRs were continuously monitored through standard surface recording electrodes of 50 mm-diameter (E127 IMMED Europe) attached to the skin of the

palm (active) and dorsum (reference) of the subject's left hand, after cleaning the skin surface with pumice. For each subject, side and modality of stimulation, series of 3 to 5 stimuli were delivered at irregular intervals, with an interstimulus time of 15-20 seconds, to minimize habituation. SSRs latencies were measured trial by trial from the trigger signal to the beginning of the first deflection exceeding baseline amplitude by at least 3 standard deviations. The sudomotor signals (SSRs) were continuously recorded at 512 Hz sampling rate, amplified $\times 50,000$, and written to hard disk to be processed off-line (Advanced Neuro Technologies, ANT®). Epoching, band-pass filtering, selective averaging and analysis were performed off-line using the Brain Vision® system (Brain Products®, Munich, Germany).

Data pre-processing included bandpass filtering at 0.1–10 Hz (non-phase shift -3 dB cutoff at 0.1–10 Hz; 12 dB rolloff slope), segmentation over an analysis time of 10 seconds, including 1-second pre-stimulus baseline, linear detrending based on the first and last 1000 ms periods, baseline correction based on the 1000 ms pre-stimulus baseline, averaging and artifact rejection (pre-trigger and clear artifacts based on SSR shape during the 9 s post-trigger). The magnitude of the SSR was measured as the peak-to-peak amplitude of the main biphasic deflection. Amplitudes of the SSR peaks were measured trial by trial, then averaged across trials in each subject to obtain mean amplitudes per modality (brush A β , pinprick A δ , laser-C) and condition (control, sensitized). For illustration purposes, grand-averages of SSR of all subjects were computed for each stimulation type; however, statistical analysis was performed on individual (i.e., not grand-averaged) data.

Statistical analysis

Descriptive statistics of SSR values and NRS as well as graphic representation of data were performed using JASP 0.10.2 version and Microsoft Excel 2010, V14.0.0. Null hypothesis (H_0) for this experiment was set as subjective perception/pain scores (NRS) and vegetative responses (SSRs) not being different between control and sensitized sides. Assessment of statistical significance between NRS and SSRs values on sensitized and control sides was done with a 2- way repeated-measures ANOVA with condition (control vs. sensitized) and stimulation modality (laser C, pinprick and brush) as within factors. The habituation of NRS and SSRs between the first, 2nd and 3rd stimulations was assessed with a two-factor repeated-measures ANOVA

with order of stimulation (1st vs. 2nd vs. 3rd) and condition (control vs. sensitized) as within factors. For post-hoc comparisons, Bonferroni-adjusted t-tests were applied with a level of significance at $p < 0.05$ after correction. Correlation between SSR amplitude and NRS was assessed using linear Pearson-product-moment regression.

Chi-square test for independence was used to assess the strength of association at individual level between the behavior of SSR and NRS following capsaicin sensitization. To this aim, SSRs were considered as significantly enhanced if (i) they were at least 30 % greater in peak-to-peak amplitude than those to stimulation of the control side, and (ii) they exceeded by at least 3-fold the maximal peak-to-peak amplitude of the pre-stimulus baseline, calculated over 1 second before stimulation delivery.

Results

Spontaneous sensations

None of the participants reported any spontaneous unpleasant sensation on the control (non-sensitized) limb throughout the whole experiment, which lasted 105 ± 17 minutes on average. Conversely, all of the subjects reported spontaneous pain/discomfort (mean NRS = 3.55 ± 1.28) and unpleasantness (mean NRS = 3.94 ± 1.33), and showed local flare of the skin at the site of capsaicin application. Discomfort over the capsaicin-treated area reached or exceeded the threshold of pain (NRS=4) in 10 participants and unpleasantness in 11 subjects. Both the intensity and unpleasantness of spontaneous perceptions tended to abate progressively and were of 2.00 ± 1.54 (Student; $t(16)=3.04; p=0.004$) and 1.81 ± 1.95 (Student; $t(15)= 4.03; p=0.001$) respectively at the end of the experiment. Rekindling at 40 °C for 3 minutes was applied to 5 participants who reported a rapid decrease of discomfort.

Provoked sensations and SSRs

All types of stimulation produced enhanced perceptions when applied to the sensitized skin. (Fig. 2). Repeated measures two factor ANOVA showed significant effects of condition (sensitized vs. control) and stimulation modality (laser C-warmth vs. pinprick vs. brush) on NRS values (respectively $F(1,14)=47.07; p < 0.001$)

and $F(2,28)=18.39;p<0.001$). The interaction between condition and stimulus modality was also significant ($F(2,28)=13.25;p<0.001$), suggesting that the effect of sensitization on perception/pain ratings differed according to the modality of stimulus being applied. On post-hoc analysis, NRS to laser C-warmth, pinprick and brush stimulation were significantly higher on the sensitized limb compared to the control limb ($t(19)= 8.99; p<0.001$; $t(19)= 6.95; p<0.001$; $t(14)= 2.87; p=0.012$) respectively.

Repeated measures ANOVA on SSR amplitudes also showed a significant effect of condition (sensitized vs. control), responses to stimulation on the sensitized limb being significantly higher compared to the control limb ($F(1,14)=8.10;p=0.013$). There was also a significant effect of stimulation modality (laser C-warmth vs. pinprick vs. brush) ($F(2,28)=5.52;p=0.010$) but no significant interaction between the two factors ($F(2,28)=1.19;p=0.341$). On post-hoc analysis, SSRs to laser C-warmth stimulation were significantly higher compared to pinprick and brush stimulations ($t(39)= 3.90; p<0.001$ and $t(30)= 2.75; p=0.010$ respectively) while there was no statistically significant difference in SSRs to brush compared to pinprick ($t(30)= 1.40; p=0.17$). Grand-averages of SSR to different stimulation modalities across all participants are shown in (Fig. 2).

SSR latencies on the sensitized side were shorter than those on control side (mean \pm SE = 1.47s \pm 0.087s vs. 1.69s \pm 0.087s respectively). Repeated measures two-factor Anova showed a significant effect of stimulation modality (laser vs. pinprick vs. brush) and condition (sensitized vs. control)] on SSR latencies ($F(2,28)=30.7;p<0.001$ and $F(1,14)=7.93;p=0.014$ respectively). There was no interaction between the 2 factors ($F(2,28)=0.06;p=0.936$). On post-hoc analysis, SSR latencies to stimulation of the sensitized side were significantly shorter than to control side $t(19)= 2.82; p=0.014$ with a mean difference of 0.22s.

During the “lie experiment”, no significant SSR differences between control (SSR_c= 1.87 \pm 0.46 mV) and test (SSR_t= 1.83 \pm 0.58 mV) sides were found when the subjects were asked to simulate an exaggerated perception ($t(9)=0.26; p=0.80$) (Fig. 3).

No significant correlations were observed between the absolute values of SSR amplitude and NRS. Conversely, significant positive linear correlations existed for both C- laser and pinprick, between the *ratio* of maximal SSR values on sensitized versus control sides, and the corresponding NRS ratios. ($R=0.825$; $p<0.001$ and $R=0.52$; $p=0.018$ respectively for C-warmth and pinprick) (Fig. 4).

At the individual level, significant enhancements of NRS values and SSR amplitudes to stimulation of the sensitized territory were obtained in all subjects for at least one modality. The behavior of NRS and SSR was significantly associated ($\chi^2(1)= 11.03$; $p<0.001$). When all stimulus modalities were pooled, the positive predictive value (PPV) of an enhanced SSR toward an increase in subjective perception/pain ratings (NRS) was 87% (CI[77% - 97%]) while the negative predictive value of a non-enhanced SSR was 46 % (CI[30% - 62%]) (Fig. 5).

Habituation phenomena

Two-factor ANOVA showed a significant effect of the order of stimulation (1st versus 2nd versus 3rd) on SSR amplitude, for both the control ($F(2,38)=14.65$; $p<0.001$, 1st SSR(2.37 ± 0.48 mV), 2nd SSR(1.8 ± 0.38 mV) and 3rd SSR (1.64 ± 0.45 mV) and sensitized sides ($F(1,19)=8.50$; $p=0.009$, 1st SSR(3.58 ± 0.75 mV), 2nd SSR(2.26 ± 0.42 mV) and 3rd SSR(2.11 ± 0.45 mV), with no interaction ($F(2,38)=3.00$; $p=0.061$). On post-hoc analysis after Bonferroni adjustment, SSR amplitudes to the 1st stimulus were significantly higher than those to the 2nd and 3rd ($t(19)= 3.89$; $p=0.003$ and $t(19)= 4.06$; $p=0.002$) while no significant difference appeared between the 2nd and 3rd values ($t(19)= 1.34$; $p=0.585$).

On the other hand, two-factor ANOVA did not show any significant effect of the order of stimulation (1st versus 2nd versus 3rd) on NRS ($F(2,38)=0.67$; $p=0.52$) for both control and sensitized sides suggesting the absence of habituation of subjective perception during the first 3 repetitions (Fig. 6). Accordingly, no significant correlation was found between SSR and NRS 1st and 2nd values ratios or differences $R= [0.07; 0.08]$ and $p= [0.74; 0.73]$.

Discussion

The topical capsaicin model is a reliable and easy-to-use procedure to induce cutaneous hypersensitivity in humans[34]. Thirty minutes of 8% capsaicin patch application over the forearm entailed spontaneous heat, discomfort and/or burning perceptions restricted to the skin under the patch, which tended to decrease as the experiment progressed. The capsaicin model was also successful in inducing significant perceptive changes to thermal and mechanical stimulation during at least one hour following the removal of the patch.

The principal aim of this study was to assess the ability of vegetative skin responses (SSRs) to detect enhanced perceptions to a variety of thermal and mechanical stimuli. Therefore, although capsaicin is known to induce changes over both the territory of application (primary hyperalgesia) and the normal skin surrounding it (secondary hyperalgesia), all stimuli except for the “lie experiment” were applied exclusively over the patch application zone, hence on the territory of primary hyperalgesia, which is the only condition that consistently exhibits both mechanical and heat hypersensitivity after capsaicin application[33,45]. Both subjective sensations and vegetative responses were concomitantly enhanced upon stimulation of the sensitized limb, relative to the contralateral control limb. Statistical significance for subjective responses was reached for each stimulus modality ($A\beta$, $A\delta$ and C-warmth). SSRs were significantly enhanced on the sensitized side (Fig. 2) suggesting enhanced pain-autonomic interaction as a result of peripheral and central sensitization.

A well-known hindrance of SSR usage is intra- and inter-subject variability[1,44]. Inter-side ratios of SSRs have been advocated to overcome this variability[37], and were used in the present study. In accordance, while no direct correlation was observed between raw values of NRS and SSR, a significant relationship was obtained between the *ratios* of maximal values of both responses and their corresponding control values (Fig. 4). This suggests that the autonomic responses and the subjective perceptions are linked by their relative variation, rather than by their absolute values. The link between the enhancement of SSRs and NRSs was supported by their significant categorical association at the individual level (Chi-2). Indeed, SSR enhancements greater than 30% relative to the control side were

associated to subjective increases in perception and pain reports of at least 1 NRS point with 87% predictive value. A similar categorical relationship was reported by Mobascher et al.[28], who contrasted SSRs with the fMRI ‘pain matrix’ activity to laser stimuli. Enhancement of SSRs appears therefore as a robust indicator of hypersensitivity [12,39,40], which may be used as an ancillary test to objectify the existence of clinical hyperalgesia [17]. Hyperalgesic symptoms in neuropathic pain are typically of greater magnitude than those observed in this study. This is exemplified in Fig. 7, showing a clinical example of focal neuropathic allodynia secondary to peripheral nerve trauma, with parallel and significant enhancement of pain reports and SSRs to brush stimulation of the allodynic region, relative to the stimulation of a control non-affected region of the same foot.

Since sympathetic responses reflect the arousal value of external stimuli, they are susceptible to changes induced by attentional and motivational states[2,43]. We therefore devised a ‘lie experiment’ to assess whether, in our experimental setting, the SSR could still reflect the actual magnitude of somatic input in conditions when individuals would consciously simulate pain –thus probably increasing their attention to the stimulus. Desmedt and Tomberg [14] showed that stimuli identical to those in control regions could enhance cognitive brain responses when the subject is instructed to treat them like ‘targets’. Although it is theoretically possible to intentionally generate an arousal response, especially for individuals trained in deception techniques, our results showed an absence of significant changes in SSR during this experiment suggesting the robustness of the technique to withstand conscious manipulation of verbal reports –e.g., malingering– in clinical settings.

Of note, SSRs reflecting hyperalgesia can be detected even in patients with focal peripheral autonomic dysfunction. Indeed, even if the stimulus triggering abnormal sensations is applied over an autonomically-affected territory, the SSR reflecting hyperalgesia can be recorded from a distant and non-affected region (palm of the hand, forearm, front) where sympathetic efferents are preserved and can still reflect vegetative reactions of arousal[27]. Therefore, while severe central autonomic dysfunction, including major depression, may seriously disrupt SSR[2], useful

recordings can still be obtained if autonomic dysfunction is peripheral and not widespread.

The sensitivity of SSRs to physiological changes unrelated to pain, including deep inspiration[38], body temperature changes[13] or bursts of anticipatory anxiety[30], may seriously hamper their clinical use if recording conditions are uncontrolled, and therefore SSR must be recorded and interpreted according to certain rules in order to be reproducible and reliable in clinical situations. It is essential to ascertain that during SSR recording the subject is relaxed, in a temperate room, with the responses initiated exclusively by the somatic stimulus applied. Anticipatory responses can be minimized by having the subject's eyes closed to limit visual control of the stimulus; any SSR starting immediately before the stimulus should be disregarded as they may reflect anticipatory arousal. Stimulations can be alternatively applied on the affected zone and a control region –ideally the homologous contralateral area–on a trial-by-trial basis to decrease the effect of attention fluctuations and habituation phenomena. In addition, to overcome SSR variability, comparison between the affected and control zones is to be preferred to absolute values. Small averages of 2-3 SSRs can enhance the signal/noise ratio and allow verifying that the latency and morphology of consecutive responses are reproducible –on the condition that the interval between stimuli is sufficiently long.

Limitations

In this study we did not use familiarization stimuli, and cannot rule out some effects of expectation, in particular to the initial (control) stimulus. We did not report either psychological assessment of the participants (anxiety / depression scores) which might have some influence on both subjective and objective responses. Also, the spontaneous sensations at capsaicin application zone decreased rapidly, and abatement of spontaneous sensations throughout the experiment occurred regularly. Results could have been still more robust had we heat-rekindled the zone regularly in every subject [34]. Using a pseudo-randomized modality-stimulation procedure with laser stimulation always at first may have reduced the responses to pinprick and brush stimulations. Similarly, using von Frey filaments of greater grammage or smaller contact area (e.g., as in [39]) would have probably enhanced

nociceptive input and hence the differences between sensitized and control areas, hence future experiments should consider using higher pressure densities by decreasing the pinprick contact area. This, together with slower repetition rates might also reduce the habituation of sudomotor responses which, in the present study, was significant from the second stimulus on (Fig. 6). Such a rapid SSR habituation to repetitive stimuli is well known from previous literature [e.g. 8,15,29]. While habituation did not prevent the highly significant association between SSR and subjective responses in this study, it decreased the negative predictive value to only 46% when three consecutive SSRs were averaged. Interstimulus rates of at least 30 seconds have been advocated to prevent habituation [8,49] and should be used to assess hyperalgesia in the clinics.

Conclusions

The SSR demonstrated to be a useful procedure to reflect enhanced perception in experimentally induced hyperalgesia and appears robust enough, under optimal recording settings, to be applied in patients with suspected abnormalities of somatosensation. Changes in perception-related arousal can be therefore assessed by comparing SSRs to stimulation applied to the affected region and to other, non-affected areas, ideally homologous zones. The SSR does not need special devices, can be performed in parallel to standard electrophysiology recordings, and allows immediate (on-line) and straightforward interpretation. Rapid SSR habituation is a drawback that should be countered by using few repetitions and low stimulus rates. The present results in healthy subjects support the clinical use of pain-autonomic responses in patients suspected of hyper or hypoalgesia.

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Conflict of interest:

The authors declare that there are no financial or other relationships that might lead to a conflict of interest.

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Figure legends

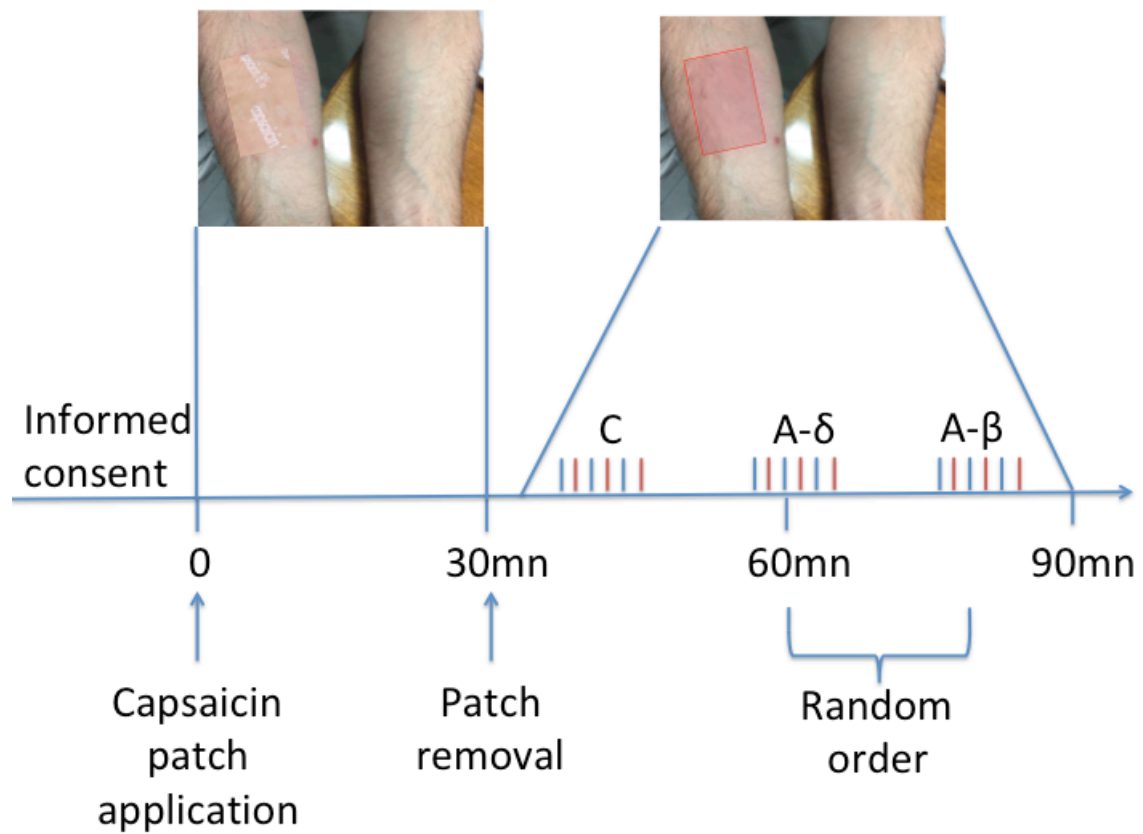


Figure 1. Stimulation protocol. C= Laser C-warmth stimulations; A δ = Pinprick A δ stimulations; A β = Brush A β stimulations

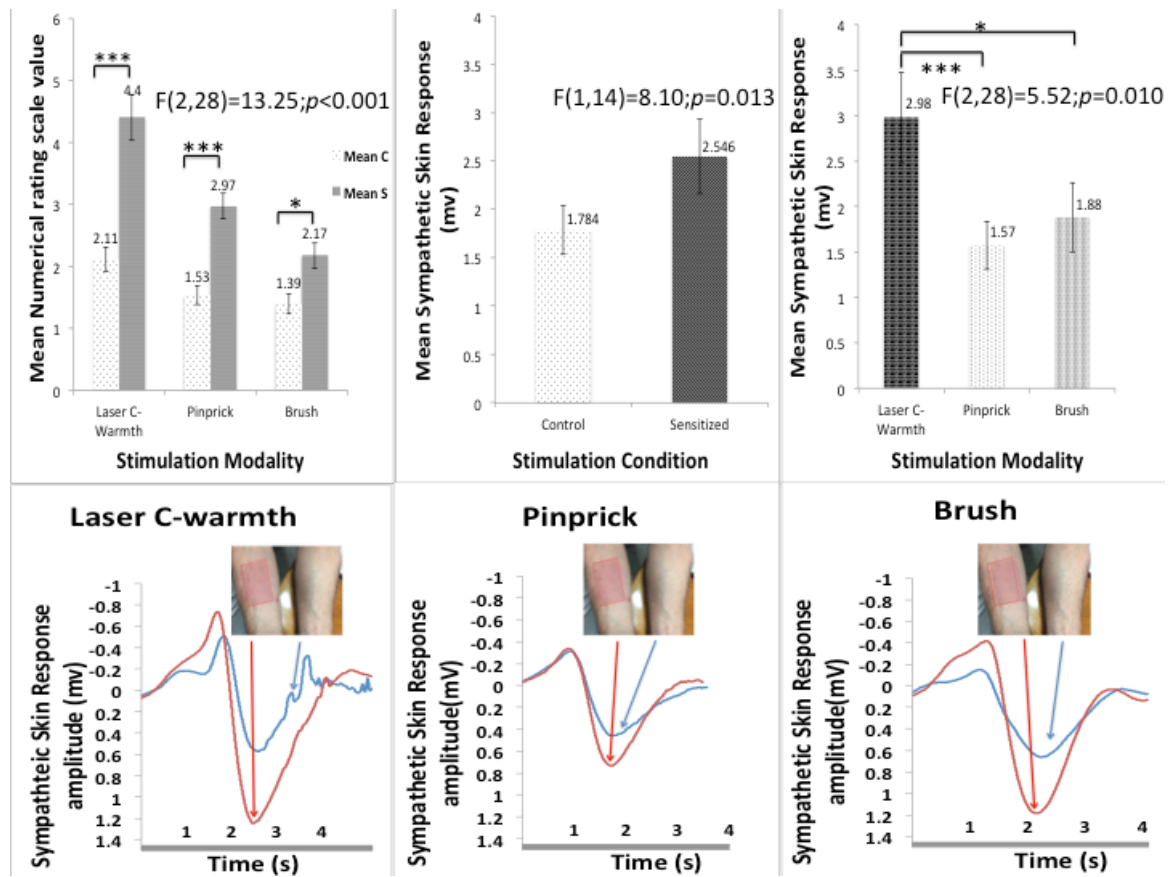


Figure 2. **Top:** Bar graphs showing Numerical Rating Scale (**left**) and Sympathetic Skin Response (**middle and right**) mean values to Laser C-warmth, Pinprick and Brush stimulations on control (C) and sensitized (S) sides. Error bars= Standard Error of Mean (SEM). **Bottom:** Grand-averages of Sympathetic Skin Responses on control and sensitized sides to all three stimulation modalities.

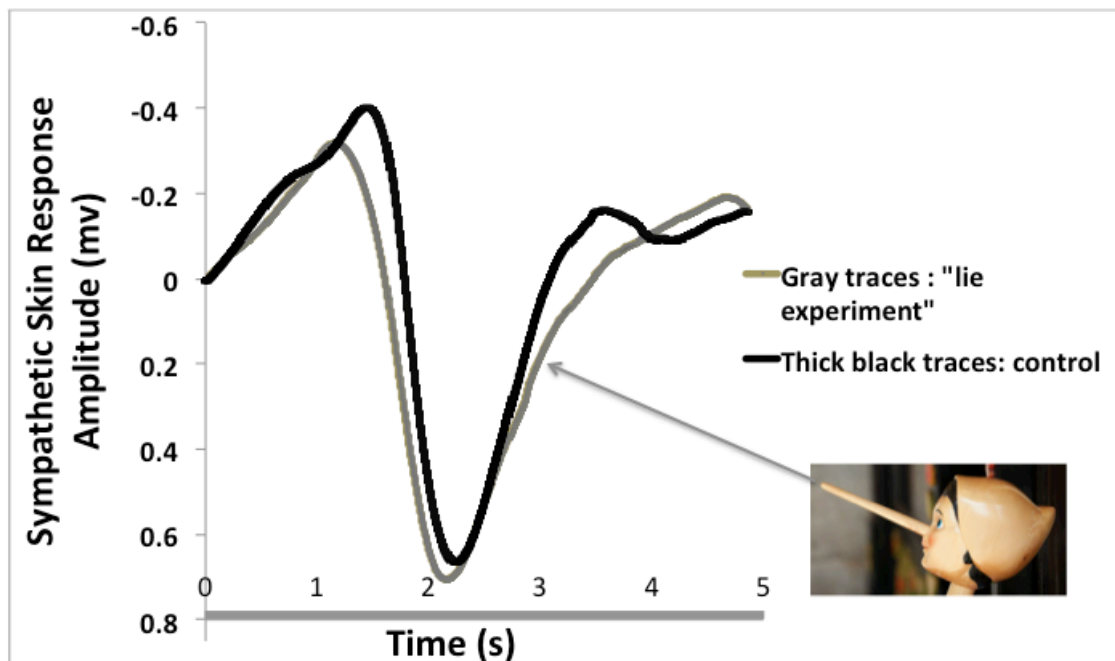


Figure 3. Grand-average of Sympathetic Skin Response values to Laser C-warmth and Pinprick A- δ stimulations during the “lie experiment”. (i) Responses to stimulation of the test region (pseudo-sensitized) superimposed to (ii) responses to stimulation of the contralateral control zone.

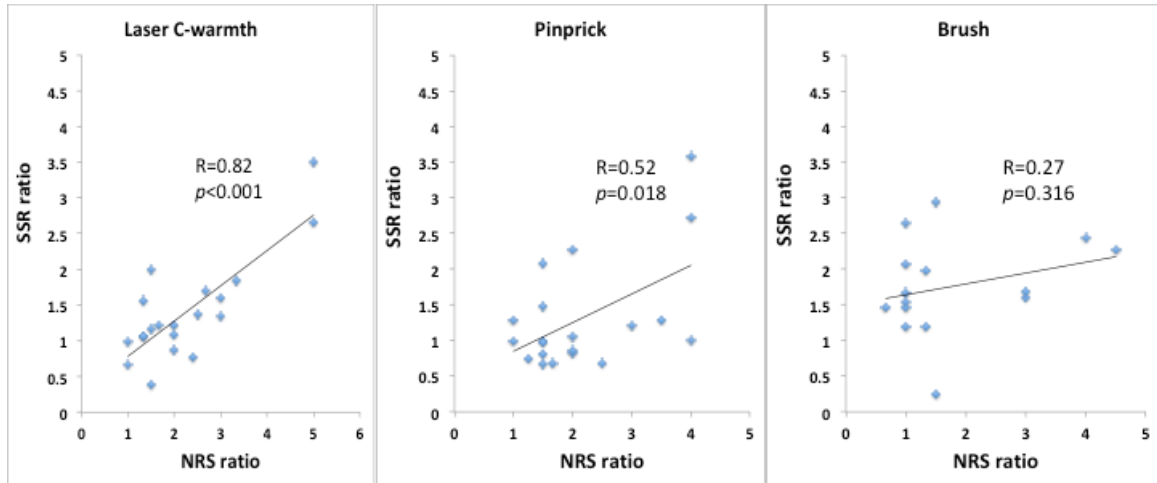


Figure 4. Correlation of the ratio of maximal Sympathetic Skin Response amplitude on sensitized and control side versus the ratio of their corresponding Numerical Rating Scale.

SSR= Sympathetic Skin Response; NRS= Numerical Rating Scale

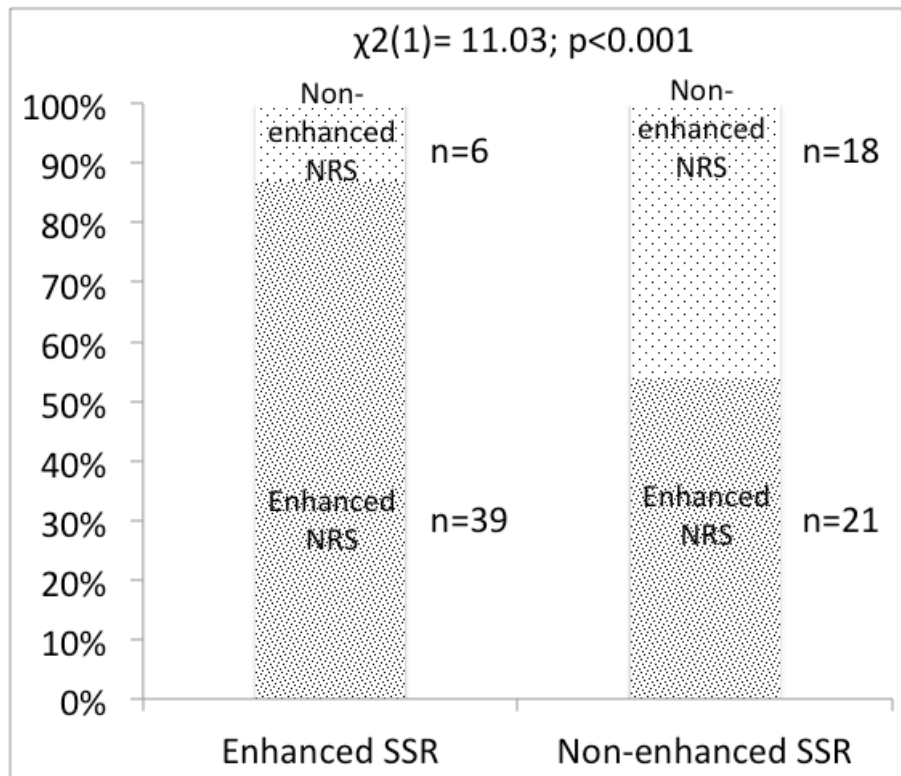


Figure 5. Distribution of pooled Sympathetic Skin Response and pooled Numerical Rating Scale values to all stimulation modalities.

Enhanced NRS: NRSS – NRSC \geq 1 point. Enhanced SSR: $SSR_{Sensitized} / SSR_{Control} \geq 130\%$ and $SSR_{Sensitized} \geq 3$ times the baseline. SSR= Sympathetic Skin Response; NRS= Numerical Rating Scale. S= sensitized side. C= control side

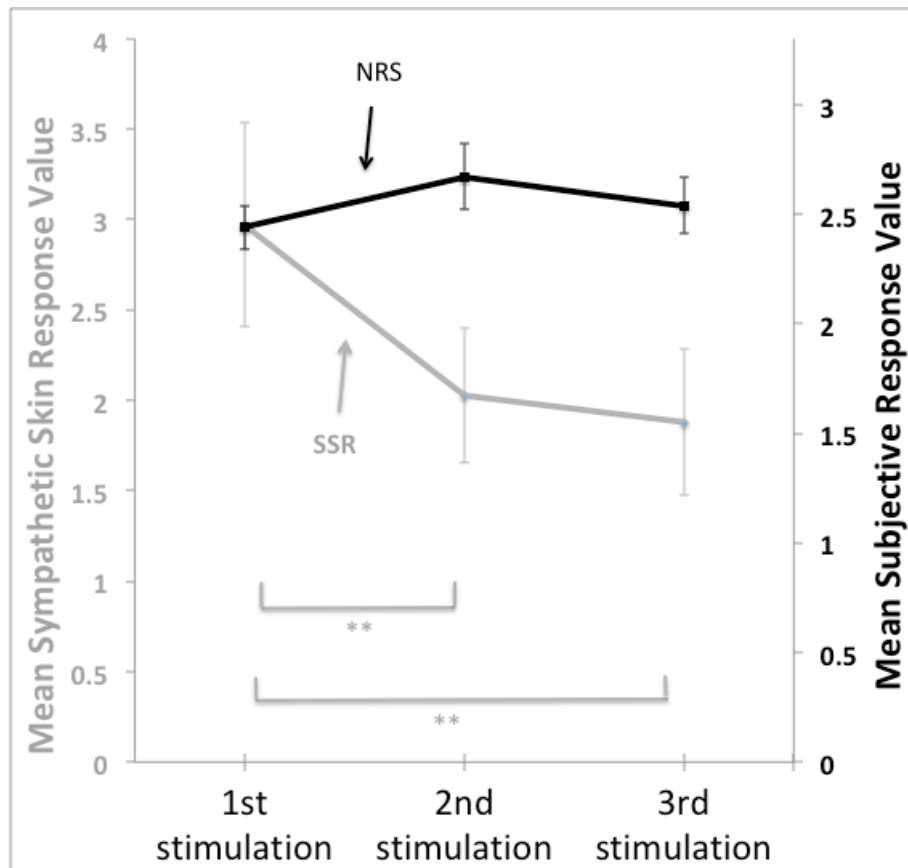


Figure 6. Sympathetic Skin Response habituation phenomena. While pain subjective ratings remained stable to the 1st, 2nd and 3rd stimulations, the Sympathetic Skin Response significantly decreased form the 2nd stimulus.

SSR= Sympathetic Skin Response; NRS= Numerical Rating Scale

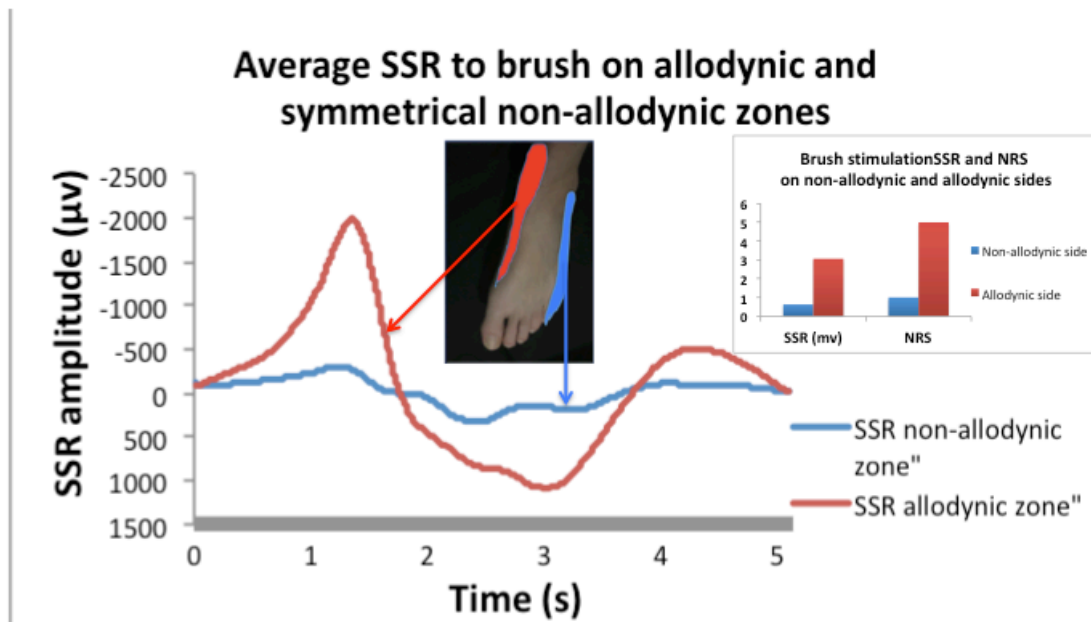


Figure 7. Clinical use of SSR in a 76 year-old patient with foot allodynia following iatrogenic saphenous nerve lesion after saphenous graft for coronary bypass. On physical examination, brush stimulation to the medio-dorsal aspect of the foot triggered dynamic mechanical allodynia, which was not reported when stimulating the lateral aspect of the same foot. Sympathetic skin responses (SSRs) to brush stimulation were more than 3-fold greater when the allodynic territory was stimulated, compared with the control area, and were consistent with subjective pain responses. Left panel: SSRs to brush stimulation of the allodynic (red) and non-allodynic (blue). Right panel: Amplitude histograms of SSR and NRS values on allodynic (red) and non-allodynic (blue) sides.