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Not as "blurred" as expected? Acuity and spatial summation in the pain system

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Abstract

Spatial acuity measured by 2-point discrimination (2PD) threshold and spatial summation of pain (SSp) are useful paradigms to probe the pain system in humans. Whether the results of these paradigms are influenced by different stimulus modalities and intensities is unclear. The aim of this study was to test 2PD controlling the stimulus modality and the intensity and to investigate the effect of modality on SSp. Thirty-seven healthy volunteers were tested for 2PDs with 2 stimulus modalities (electrocutaneous and mechanical) and intensity (noxious and innocuous). For each condition, participants received stimuli to either 1 or 2 points on their lower back with different distances (2-14 cm, steps of 2 cm). It was found that 2PDs were significantly smaller for noxious stimuli for both modalities. By contrast, between-modality comparison reproduced previous reports of impaired acuity for noxious stimulation. Higher pain intensities were reported when a larger area was stimulated (SSp), independent of the modality. Furthermore, reported pain intensities were higher when the distance between 2 stimulated areas was increased from 2 to 6 cm ($P < 0.001$), 8 cm ($P < 0.01$), and 14 cm ($P < 0.01$). 2PDs determined by mechanical and electrocutaneous stimuli were significantly correlated within both stimulus intensities, ie, innocuous ($r = 0.34$, $P < 0.05$) and noxious ($r = 0.35$, $P < 0.05$). The current results show 3 novel findings: (1) the precision of the pain system might be higher than in the innocuous (tactile) system when mechanical and electrocutaneous modalities are used, (2) the pattern of distance-based and area-based SSp seems to be comparable irrespective of the modality applied (mechanical and electrocutaneous), and (3) both modalities are moderately correlated.

Keywords: Two-point discrimination, Two-alternative forced-choice task, Spatial summation, Lateral inhibition

1. Introduction

Pain perception in its pure biological role indicates potential tissue damage. Intuitively, the localization and intensity of the nociceptive input should be recognized precisely to allow for efficient threat recognition and adequate protective response.[3] However, most previous studies reported poorer acuity for nociceptive in contrast to innocuous stimulation,[20,30,32] some claimed that the accuracy is equal,[41] and the latest report suggested that this might depend on body regions.[30] This was assessed by evaluating 2-point discrimination thresholds (2PDs) which were larger in noxious and smaller in tactile paradigms.[20,30–32,49]

Explanations for this could be a less precise geometry of surrounding inhibition of Aδ and C fibers compared with the tactile stimuli-transmitting Aβ fibers.[31] As recently pointed out,[32] broad spatial tuning in the nociceptive system might reflect the lateral inhibition capabilities of wide-dynamic-range neurons with their on-center off-surround.[23] It has further been suggested that an overlap between inhibitory and facilitatory receptive fields (RFs) and higher spatial separation before a stimulus reaches the inhibitory zone, predispose the pain system to lesser precision.[32] Nevertheless, not only the discrimination of more than 1 stimulus depends on inhibition and excitation within the neuroaxis. A second phenomenon, which is related to 2PD, since it occurs simultaneously but is rarely recognized as such, is spatial summation of pain (SSp).[24,32,37,40] Previous studies have either reported 2PD as mutually exclusive[10,11,13] or to some degree coexisting[32] with SSp by showing SSp being stable over limited space but within- and between-dermatome resolution.[33] These findings were found using distance-based SSp that occurs when pain increases with the increase of the distance between 2 stimulated points.[40] Another SSp type, area-based SSp is observed when manipulating the stimulation area.[12] Compared with temporal summation of pain, SSp is poorly understood, especially in terms of the neural loci responsible for this kind of pain modulation.[36] Local integration at the single-cell level,[37] lateral inhibition,[38] and the general increase in the total number of recruited neurons are hypothesized mechanisms.[37]

Since available evidence comparing noxious and innocuous (tactile) acuity is limited, and because of between-modality

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comparisons made in previous studies,[20,30,32,49] the main aim of this study is to directly compare acuity in the tactile and nociceptive systems, using the same modality (electrocutaneous and mechanical) for both innocuous and noxious stimulation, with controlled and comparable intensities—2 factors that were not included in previous reports.[19,20,30,32] Focusing on SSp as a secondary aim, a paradigm was developed to study spatial acuity within both the tactile and the pain system with the possibility to measure distance-based and area-based SSp.[40] In line with our preregistered protocol and the previous literature,[30] it was hypothesized that acuity will be poorer for noxious stimuli. Furthermore, collecting pain ratings in both stimulus modalities allowed us to explore the magnitude and the pattern of SSp, driven by different sensory inputs. It was hypothesized that both stimulus modalities elicit an SSp effect. As the project introduces a novel method for tactile acuity assessment, we also aimed to investigate the concurrent validity of the novel electrocutaneous 2PD method.

2. Material and methods

2.1. General information

The study protocol was approved by the ethics committee at the University of Luebeck (19-226/10-07-2019) and registered in the Open Science Framework database[1] using a standardized template provided by the AsPredicted.org platform. The experiment followed the recommendations of the Declaration of Helsinki. The study was based on a repeated-measures experimental design and involved a group of healthy participants, exposed to 4 different test procedures, administered in a random order.

2.2. Participants

2.2.1. Eligibility

A group of 41 healthy, pain-free participants were recruited from the community of Luebeck using social media and word-of-mouth. Eligibility criteria were chosen in accordance with previous tactile acuity studies on healthy subjects[8,9]: Participants were included, if they were healthy (self-report) and aged between 18 and 65 years. Exclusion criteria were any condition influencing the perception of tactile and noxious stimuli such as neuropathy, pain at any body location within the previous week, a history of chronic pain or any systemic disease, electronic devices in or at the body or unremovable metal objects in the area of the lumbar spine, as well as tattoos in close proximity to the measured body area. Participants received detailed information on the study procedures before signing the informed consent. They were informed that they can drop out of the study at any timepoint without any consequences and reason for a withdrawal. Three participants were excluded because of the following reasons: communication problems (n = 1), metal objects in the body (n = 1), and epilepsy (n = 1). In total, 38 participants were assessed; however, data were not recorded from 1 subject; therefore, the statistical analysis was based on a data set from 37 participants.

2.2.2. Sample size

A sample size was calculated for the analysis that required the highest number of participants, which was the correlation between the 2 methods (electrocutaneous and mechanical). To detect a moderate correlation (Pearson coefficient r) between 2 different tactile acuity test procedures, a minimum of 37 subjects were required. The sample size calculation was based on reported correlations of r ranging from 0.35 to 0.48[2,26] and assuming 80% power ($\alpha = 0.05$). Thus, to detect a significant correlation of $r = 0.4$ (mean from the literature), a minimum of 37 subjects were required. The sample size calculation was performed using G*Power 3.1 software.[16]

2.3. Study design

The study was based on a within-subject repeated-measurement design. Healthy pain-free participants underwent noxious and innocuous acuity assessment using both modalities: mechanical and electrocutaneous (novel method). The order of the modality was randomized and counterbalanced (**Fig. 1**). Each modality was assessed at the lower back (at the level of L3) using innocuous (non-nociceptive) and noxious (nociceptive) stimuli; the order of the type of stimulation was also counterbalanced. The control stimulus (single stimulus application) was always applied to 1 of 2 outer spots. These control sites were in line with a previously published SSp protocol.[40]

2.4. Experimental procedure

2.4.1. Preparation

Participants were positioned in prone on a plinth with their face placed in the plinth's foramen and hands kept on the armrests. The lumbar curvature of the spine was flattened by placing a small pillow below the abdomen. The examination site, ie, lower part of the back, was shaved and cleaned using alcohol solution to reduce between-subject variability that might occur during spatial acuity assessment (**Fig. 2**). Eight numbered (1-8) areas (spots) were marked on the skin at the examination site in a horizontal line (**Fig. 3**). All the areas were distributed within the same dermatome of L3 at the middle of the back. Before test procedures, participants received example stimuli to control for abnormal sensation and to familiarize participants with the sensation of electrocutaneous and mechanical stimulation.

The distance between 2 stimulated points used in this study was set at 2.0 cm, resulting in a maximum possible separation of 14.0 cm. The mean 2PD threshold has been shown to be more than 5 cm and less than 7 cm in our previous study,[3] and it has been shown that the distance of 12 cm is wide enough to allow for a distinct perception of 2 points on the back.[3]

2.4.2. Mechanical assessment

The mechanical paradigm was performed using a mechanical sliding caliper (Powerfix, digital caliper: Z22855) with a precision of 0.01 mm. For innocuous acuity assessment, 2 identical von Frey hairs (no. 4.31, force of 2 g) were attached to the caliper's tips to ensure a constant force applied to the skin. The same principle was applied for noxious acuity assessment; however, instead of von Frey hairs, 2 (512 mN each) PinPrick needles (PinPrick; MRC Systems GmbH, Germany) were attached to the calipers' tips. This reduced the bias of variable forces of the 2 tips during the 2PD paradigm which might affect the magnitude of spatial acuity.[6,29]

Participants were exposed to 8 series of measurements for each stimulus intensity (noxious and innocuous). Each series consisted of 8 different trials, 7 of them were based on stimuli pairs with different distances between stimulated spots (2, 4, 6, 8, 10, 12, and 14 cm), and 1 trial was a control stimulus applied to 1 single spot ("catch" trial). The sequence of each stimuli type within

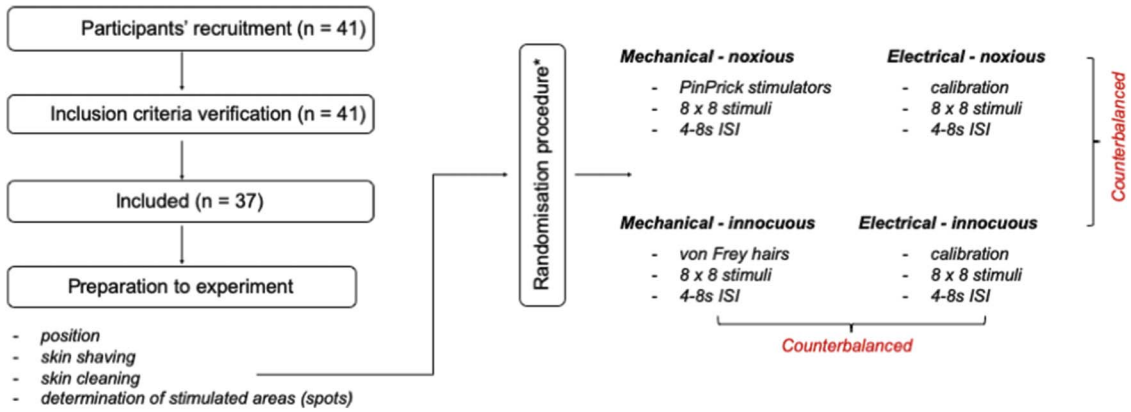

Figure 1. Study design. The order of test modalities (electrocutaneous vs mechanical), as well as the order of test stimuli (innocuous vs noxious), was counterbalanced and randomly chosen for each participant using block randomization. Participants received 8 series of 8 different stimuli types, ie, 7 different distances between electrodes were used and control stimuli activated a single electrode. ISI, interstimulus interval.

each of 8 series was generated fully randomly using a feature of the PsychoPy 2.0, open-source software.[35] However, the locations for each type of trial were fixed to replicate previous SSp reports[24,40]: For half of the participants, the control electrode was placed on the outer left spot and for the remaining participants on the outer right spot. This allowed to fully control for perceptual differences caused by laterality or site differences in innervation. A total of 128 trials (8 series × 8 trials × 2 intensities) were applied. The paradigm was based on the psychophysical method of a two-alternative forced-choice task (2AFC). After each stimuli pair, or single stimulus, participants had to decide and indicate on a keyboard if they felt the sensation in 2 or in 1 location. After the discrimination task, subjects were asked to rate the intensity of pain experienced using a numerical rating scale (NRS) ranging from "0" (no pain at all) to "10" (worst pain imaginable). Scale and anchors were displayed on a screen (**Fig. 2**). At the end of each stimuli series, participants were asked 2 control questions, ie, whether they thought stimuli were applied synchronously (when 2 were perceived) and how difficult the task was.

2.4.3. Electrocutaneous paradigm

Electrocutaneous stimuli have been used frequently in projects assessing the integrity of the sensory and nociceptive systems[17,18,22,25] and can be used with electrodes small enough to mimic the stimuli delivered in the mechanical paradigm. The 8-mm-diameter, planar concentric electrodes (WASP electrodes; Brainbox Ltd., Cardiff, United Kingdom) used in the paradigm had 2 gold-plated solder pads, platinum cathode in the centre and a concentric anode.[15,42] Electrodes were placed on the same spots used for the mechanical paradigm, in a horizontal line across the lumbar spine. Electrocutaneous (square) pulses (100 μs) were used as previously described.[43] A DS8R model (Digitimer; Welwyn, Garden City, England) served as the stimuli generator, and a D188 (Digitimer; Welwyn) remotely selected and activated the required electrode or pair of electrodes according to the fully random sequence generated by the software. External control of the DS8R and D188 was ensured through the Labjack U3-LV control device (LabJack Corporation, Lakewood, CO). The procedure was fully automatic and operated by the PsychoPy 2.0 software.[35] Electrode placement was performed using double-sided stickers, commonly used in electroencephalography (EEG) studies. After electrode placement, a single stimulus of low intensity (6.0 mA) was applied to each electrode manually with an interstimulus interval of 5 seconds to test electrode function and attachment.

Before the innocuous and noxious acuity assessment, a calibration procedure was conducted to determine the low and high intensity of the electrocutaneous stimuli. This phase was mandatory because (1) the stimulation intensity had to be strong enough to perceive the stimulus, and not too intense to be perceived as noxious for the innocuous acuity assessment, and (2) the stimulation should be above pain threshold to induce pain in the noxious acuity assessment, but not too high to still be comparable to pain elicited by the mechanical stimulation. Therefore, the calibration phase involved the determination of the sensory detection threshold (t) and the pain threshold (T) according to the methods of limits: Two ascending series of electrocutaneous stimuli were delivered in steps of 1.0 mA (intertrial interval = 5 seconds), starting from 2 mA. The intensity of the electrocutaneous stimuli was gradually increased until the participant detected the first sensation (t). The intensity was subsequently further increased until the participant reported that the sensation became painful (T). To adjust for possible differences in sensation occurring in different skin sites at the L3 spinal level, and to make the calibration as valid as possible for this experiment, the first stimuli run was applied to electrodes placed on the outer side of the stimulation area (eg, electrodes 1 and 2), while the second run activated the 2 outmost electrodes (1 and 8). Finally, the average values of t and T were calculated, and subsequently, the intensity of stimulation $a_{innocuous}$ was calculated using the following formula I:

$$\text{I. } a_{innocuous} = ((T - t)/4)) + t$$

$$\text{II. } a_{noxious} = ((T - t)/2)) + T.$$

This formula (I) ensured that the stimuli were clearly below pain threshold and unlikely to be perceived as a painful. For the noxious stimulation, the formula II was applied to ensure stimuli were above pain threshold and likely to be perceived as painful. After the calibration, participants were exposed to 8 series of 8 different trials for each stimulus intensity (noxious and innocuous) similarly to the mechanical paradigm (**Fig. 2**). After the application of each stimuli pair or stimulus, subjects first discriminated locations and then rated pain intensity (**Fig. 2**). The same control questions were asked as for the mechanical paradigm (see above). The data were saved automatically in the data set generated by the PsychoPy software.

2.5. Outcome measures

Main outcomes in this study were SSp and 2PD thresholds (innocuous and noxious) measured using protocols based on 2 modalities: mechanical and electrocutaneous. In addition to that, the following data were collected to characterize the sample: age, sex, lumbar spine awareness using the German version of the Fremantle Back Awareness Questionnaire,[14] the Pain and Vigilance Awareness Questionnaire using the German version,[27] body mass, height, and fear of pain measured on a 0 (no at all) to 10 (very much) scale. Additional data were collected to characterize testing procedures: Perceived synchronicity of applied stimuli measured on 0 (2 stimuli applied asynchronously) to 10 (synchronously) scale and the level of difficulty of the discrimination task measured on a scale ranging from 0 (very easy task) to 10 (very hard task).

2.6. Data processing and statistical analysis

Raw data from the discrimination task and pain ratings were first extracted from the files generated by the PsychoPy software using MATLAB R2017b (MathWorks, Inc, Natick, MA). 2PD thresholds were extracted from psychometric functions determined for each participant, individually. Psychometric sigmoid functions were determined in MATLAB using the proportion of trials in which the sensation of 2 points were reported on the y-axis and the physical parameter "separation distance" on the x-axis. The 50% point from the curves indicating the threshold at which participants reported 2 points at the probability of 0.5 was used for statistical analysis. As every type of stimuli ("distance") was repeated 8 times within each paradigm type, pain ratings representing the same stimuli type were averaged for the purpose of statistical analysis.

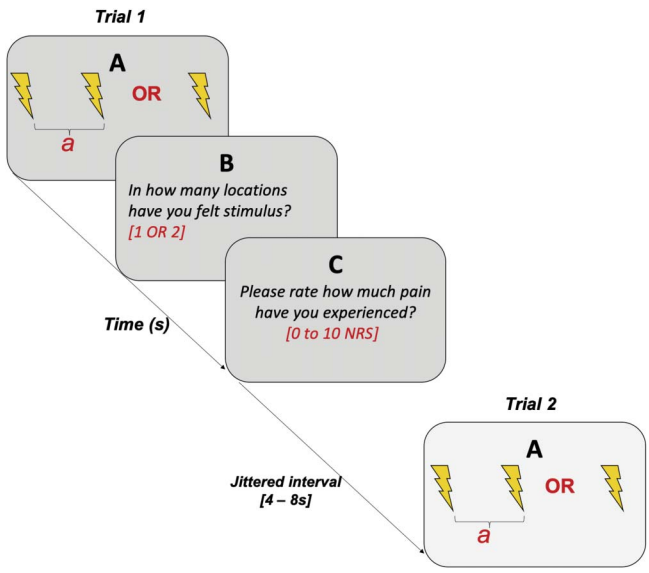

Figure 2. Single trial design. The figure presents single trial design with electrocutaneous stimuli as an example. A, Stimulus presentation: Participants received single or 2 stimuli with different spatial relations. B, Discrimination task: They had to discriminate between 1 or 2 locations being stimulated in the two-alternative forced-choice task (2AFC). In the next step (C), they had to decide how much pain they experienced in the last trial using a 0 to 10 numerical rating scale (NRS). Note: for the mechanical paradigm, electrocutaneous stimuli were replaced by mechanical tactile (von Frey hairs) and noxious (PinPrick needles) stimuli; "a" refers to the distance (2, 4, 6, 8, 10, 12, or 14 cm) between 2 electrodes used in the given trial.

Two-point discrimination thresholds, synchronicity and difficulty data, were analysed using general linear model (GLM) analysis with stimulus modality ("electrocutaneous" vs "mechanical") and stimulus intensity ("noxious" vs "innocuous") as within-subject factors. In case of statistically significant interaction effects, F tests were followed by planned paired *t* tests with Bonferroni correction for multiple comparisons to explore within-modality differences. Pain ratings were entered into GLM analysis with modality ("electrocutaneous" vs "mechanical") and distance (8 possibilities) as within-subject factors. Area-based SSp was assessed by comparing pain ratings from single stimulus (smaller area) application to pain from different stimuli pair (larger area). Distance-based SSp was assessed by comparing pain ratings from the smallest separation distance (2 cm) to larger distances (4-12 cm).

Pearson *r* correlation coefficients were applied to test concurrent validity: 2PD thresholds determined by the reference standard (mechanical assessment) were correlated with 2PD thresholds estimated by electrocutaneous assessment. Furthermore, SSp and 2PD dependencies were assessed by correlating (Spearman rank coefficients) the 2PD results with the distance at which SSp was considered the maximal (highest pain reported). Analyses were conducted using the STATISTICA data analysis software, version 13 (StatSoft, Inc., Tulsa, OK). The level of significance was set at $P < 0.05$, and Bonferroni correction was applied to control for familywise error. When *P* value did not exceed α levels after correction, it was then marked as not significant (ns).

3. Results

The mean (SD) age of the included sample (n = 37, 46% females) was 28.24 (SD 8.75) years (**Table 1**). In general, the stimuli calibration was successful, and 84% and 91% of noxious stimuli type were considered painful (≥0.5 on the NRS) in the electrocutaneous and mechanical modalities, respectively. In the

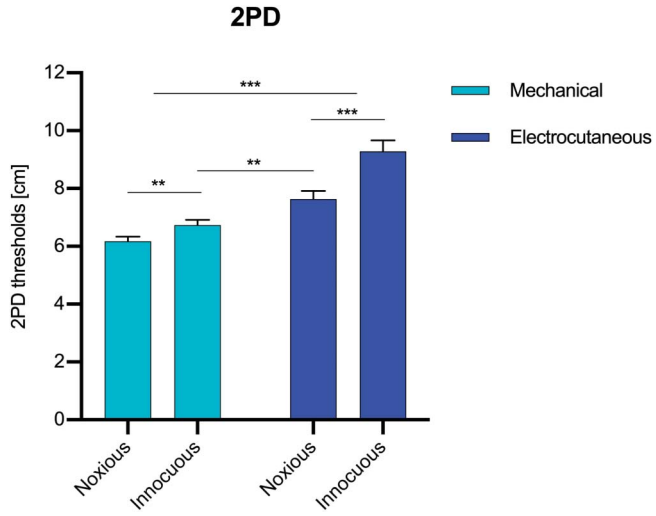

Figure 3. Lumbar innocuous and noxious acuity. Two-point discrimination (2PD) thresholds were lower for noxious stimuli compared with innocuous stimuli within both studied modalities. Interestingly, a significant difference in acuity between innocuous mechanical and noxious electrocutaneous might erroneously imply that the innocuous system has a higher precision. In general, the electrocutaneous modality produced larger thresholds (top comparison). Note: data presented as mean and SEM. ***$P < 0.001$, **$P < 0.01$.

Table 1

Descriptive statistics.

Variable	Mean ± SD
Fear of pain [0 to 10 scale]	2.14 ± 2.30
Age (y)	28.24 ± 8.75
Body mass (kg)	70.74 ± 13.52
Height (cm)	175.57 ± 10.45
FreBAQ (score)	1.22 ± 2.41
PVAQ (score)	35.32 ± 11.79
t (mA)	2.77 ± 0.77
T (mA)	11.93 ± 7.73

Data presented as mean and SD.
FreBAQ, Fremantle Back Awareness Questionnaire; PVAQ, Pain and Vigilance Awareness Questionnaire; t, tactile threshold; T, pain threshold.

innocuous domain, 88% and 93% of stimuli were considered nonpainful (<0.5 on the NRS).

3.1. Innocuous and noxious acuity

General linear model analysis revealed statistically significant main effects for the factors "modality" ($F_{(1,36)} = 54.23$, $P < 0.001$, $\eta_p^2 = 0.60$) and "intensity" ($F_{(1,36)} = 43.92$, $P < 0.001$, $\eta_p^2 = 0.55$), indicating that in general, 2PD thresholds were lower for noxious stimuli and higher if these were provided by electrocutaneous stimulation (**Fig. 3**). Interestingly, a significant interaction was found between both factors ($F_{(1,36)} = 12.88$, $P < 0.001$, $\eta_p^2 = 0.26$) and post hoc planned comparisons revealed that 2PD thresholds were lower for noxious stimuli within both stimulus modalities, ie, electrocutaneous ($t_{(36)} = -5.92$, $P < 0.001$, $d = 0.97$) and mechanical ($t_{(36)} = -3.57$, $P < 0.01$, $d = 0.59$), respectively. To verify whether the pain system has a poorer acuity if comparisons are made across modalities, electrocutaneous noxious (M = 7.64 cm, SD = 1.66) and mechanical innocuous thresholds (M = 6.73 cm SD = 1.10) were contrasted. It was found that 2PD determined by noxious electrocutaneous stimuli had significantly larger values compared with 2PD determined by innocuous (tactile) mechanical stimuli ($t_{(36)} = -3.57$, $P < 0.01$, $d = 0.59$).

No differences between the level of synchronicity were found (**Table 2**) as indicated by not significant effects of factors "modality" ($F_{(1,36)} = 0.004$, $P = 0.95$, $\eta_p^2 = 0.0001$), "intensity" ($F_{(1,36)} = 1.58$, $P = 0.22$, $\eta_p^2 = 0.04$), or their interaction ($F_{(1,36)} = 1.05$, $P = 0.31$, $\eta_p^2 = 0.03$). The GLM on difficulty of ratings revealed a significant main effect of "intensity" ($F_{(1,36)} = 9.96$, $P < 0.01$, $\eta_p^2 = 0.22$) and "modality" ($F_{(1,36)} = 34.52$, $P < 0.001$, $\eta_p^2 = 0.49$), indicating that, in general, electrocutaneous and innocuous paradigms were more difficult to rate the number of stimuli (**Table 2**). No interaction between modality and intensity was found ($F_{(1,36)} = 2.25$, $P = 0.14$, $\eta_p^2 = 0.06$) in terms of difficulty level.

3.2. Spatial summation of pain

A clear relationship between the number of stimulated spots, separation distances, and pain was found (**Fig. 4**, Supplementary file 1, available at http://links.lww.com/PAIN/B172). General linear model analysis showed a significant effect for the factor "distance" ($F_{(7,252)} = 40.82$, $P < 0.001$, $\eta_p^2 = 0.53$), indicating that higher pain ratings were reported when the stimulated area was larger and when the distance between 2 stimulated spots was increased (**Fig. 4** and **Table 3**). Exact comparisons revealed that stimulation of 1 spot (the control electrode) was always less painful compared with 2 stimuli with separation of 2 cm ($t_{(36)} = -7.80$, $P < 0.001$, $d = 0.91$), 4 cm ($t_{(36)} = -8.97$, $P < 0.001$, $d = 1.04$), 6 cm ($t_{(36)} = -9.37$, $P < 0.001$, $d = 1.09$), 8 cm ($t_{(36)} = -9.25$, $P < 0.001$, $d = 1.08$), 10 cm ($t_{(36)} = -9.59$, $P < 0.001$, $d = 1.12$), 12 cm ($t_{(36)} = -9.23$, $P < 0.001$, $d = 1.07$), and 14 cm ($t_{(36)} = -10.75$, $P < 0.001$, $d = 1.25$, **Fig. 4**). Comparing pain induced by 2 stimuli with a separation of 2 cm to that of 4 cm did not show a significant pain increase ($t_{(36)} = -0.72$, $P = 0.48$, $d = 0.08$). Nevertheless, pain increased when the separation was 6 cm ($t_{(36)} = -3.68$, $P < 0.001$, $d = 0.43$), 8 cm ($t_{(36)} = -3.23$, $P < 0.01$, $d = 0.37$), 10 cm ($t_{(36)} = -2.31$, $P < 0.05$/ns., $d = 0.27$), 12 cm ($t_{(36)} = -2.46$, $P < 0.05$/ns., $d = 0.29$), and 14 cm ($t_{(36)} = -3.13$, $P < 0.01$, $d = 0.36$), indicating that distance-based SSp was induced successfully (**Fig. 4**, Supplementary file 1, available at http://links.lww.com/PAIN/B172). However, the factor "modality" was not significant ($F_{(1,36)} = 3.98$, $P = 0.054$, $\eta_p^2 = 0.10$) nor was the "distance" × "modality" interaction ($F_{(7,252)} = 1.41$, $P = 0.20$, $\eta_p^2 = 0.04$), suggesting that SSp was modality independent and both modalities produced approximately similar pain levels (**Table 3**).

3.3. Correlations between test paradigms

Two-point discrimination thresholds determined by the novel electrocutaneous method were positively correlated with the standard innocuous acuity assessment using mechanical sliding caliper (**Fig. 5**). The correlation was found to be significant for innocuous ($r = 0.34$, $P = 0.04$) and for noxious stimuli ($r = 0.35$, $P = 0.03$). A significant correlation was found between the absolute magnitude of the SSp effect induced through electrocutaneous stimuli and mechanical stimuli ($r = 0.43$, $P < 0.01$, **Fig. 5**). The distance at which maximal SSp was reported was not correlated with the 2PD threshold in both electrocutaneous ($r_s = 0.16$, $P > 0.05$) and mechanical stimuli ($r_s = -0.09$, $P > 0.05$).

4. Discussion

The main aim of this study was to assess the precision of the perception of noxious and innocuous (tactile) stimuli when

Table 2

Noxious and innocuous acuity: mean and SD.

Modality	Innocuous			Noxious		
	2PD	Difficulty*	Synchronicity†	2PD	Difficulty*	Synchronicity†
Mechanical (n = 37)	6.73 ± 1.10	2.25 ± 1.72	8.17 ± 1.30	6.18 ± 0.98	1.89 ± 1.74	8.19 ± 1.37
Electrocutaneous (n = 37)	9.29 ± 2.27	3.96 ± 2.47	7.97 ± 2.53	7.64 ± 1.66	3.08 ± 2.01	8.32 ± 2.16

2PD, Two-point discrimination threshold [cm].
* Difficulty of the task measured on a 0 to 10 scale: General linear model (GLM) analysis showed that electrocutaneous stimuli were more difficult to discriminate ($P < 0.001$) and innocuous were more difficult than noxious ($P < 0.01$).
† Perceived synchronicity of 2 different stimuli on a 0 to 10 scale: GLM analysis showed no differences between paradigms ($P > 0.05$).

Table 3

Pain ratings: mean and SD.

Intensity	Modality	0 cm*	2 cm	4 cm	6 cm	8 cm	10 cm	12 cm	14 cm
Noxious	Mechanical	1.51 ± 1.13	2.11 ± 1.45	2.19 ± 1.36	2.27 ± 1.45	2.35 ± 1.42	2.33 ± 1.40	2.33 ± 1.43	2.50 ± 1.48
	Electrocutaneous	1.09 ± 1.07	1.84 ± 1.32	1.84 ± 1.40	2.06 ± 1.56	2.1 ± 1.52	1.92 ± 1.46	1.98 ± 1.37	1.98 ± 1.38
Innocuous	Mechanical	0.05 ± 0.14	0.11 ± 0.25	0.18 ± 0.36	0.17 ± 0.41	0.16 ± 0.34	0.17 ± 0.32	0.14 ± 0.22	0.15 ± 0.30
	Electrocutaneous	0.05 ± 0.15	0.24 ± 0.50	0.14 ± 0.38	0.26 ± 0.51	0.25 ± 0.54	0.26 ± 0.57	0.22 ± 0.42	0.27 ± 0.50

* 0 cm refers to the control trial in which always only 1 point was stimulated.

controlling for stimulus modality and intensity. A secondary aim was to investigate the effect of stimulus modality on SSp. In contrast to our a priori hypothesis which was based on previous reports, the current results indicate a higher precision of the pain system compared with the innocuous system. This finding was consistent and showed the same pattern in both modalities, electrocutaneous and mechanical. Thus, one may argue that the pain system itself is not as "blurred" as it seemed to be and its precision could be—to some degree—underestimated. Regarding SSp, both types, ie, area-based and distance-based, followed the same pattern regardless of stimulus modality. Finally, the study showed that the 2 modalities used for stimulus discrimination and summation are closely related as indicated by significant positive correlation coefficients. To the best of our knowledge, this is the first experimental study that investigated and compared spatial characteristics of the pain and tactile systems using different modalities and intensities in the same group of subjects. The results of this experiment improve our understanding of the pain system, mechanisms of the SSp effect, and provide novel tools that can be used to study 2PD and SSp in both healthy subjects and patients with chronic pain.

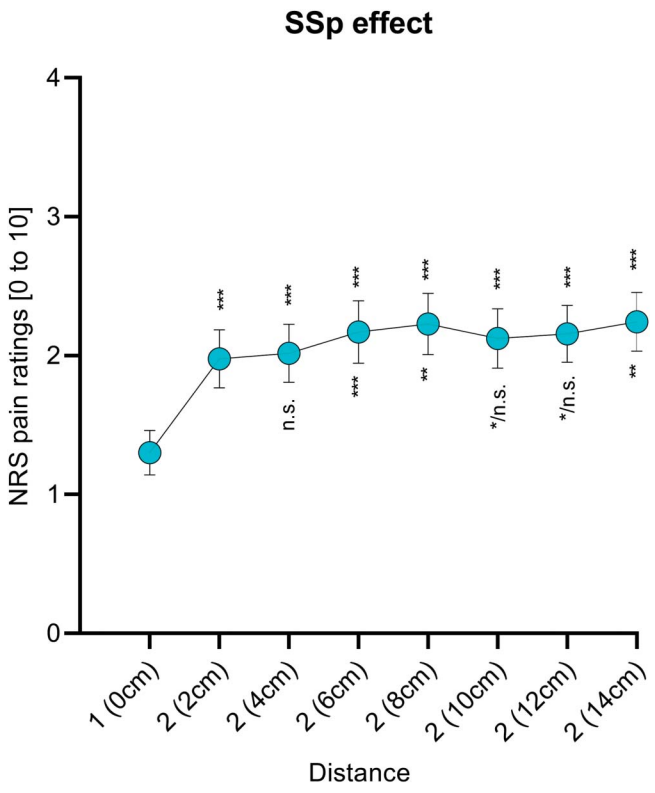

Figure 4. Spatial summation of pain (SSp) based on pooled noxious data from 2 modalities. More pain was felt when 2 electrodes were activated (upper comparisons), indicating significant area-based SSp. All comparisons showed significantly less pain when only 1 spot was stimulated. More pain was felt when wider distance was applied (lower comparisons), indicating a significant distance-based SSp effect: Compared with the shortest distance of 2 cm, pain increased during the 6-cm separation and remained high for all other separations. Note: data presented as mean and SEM. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, n.s., not significant after Bonferroni correction. SSp, spatial summation of pain.

4.1. Acuity of the pain system

The current study showed better acuity for the perception of noxious compared with innocuous stimuli. Previous studies, generally reporting the contrary, assessed innocuous stimuli applied with standardized von Frey hairs and contrasted these with noxious laser-induced heat stimuli.[20,30–32] The reasoning for this approach is that Aβ fibers and nociceptive fibers (polymodal C-nociceptors and Aδ) are activated selectively using these 2 modalities, respectively. The disadvantage is the effect of stimulus modality. Indeed, these current results show, that controlling for stimulus modality, reversed the pattern of acuity, ie, noxious stimuli provoked smaller 2PD thresholds. When assessing intermodality comparisons, comparing electrical noxious stimuli with mechanical innocuous (tactile) stimuli (**Fig. 3**), these replicated previous findings,[20,30,32] confirming the hypothesis that previous findings might report a modality effect and not an effect of innocuous vs noxious stimulation. One may notice, however, that within-modality comparisons induce the problem of coactivation, eg, mechanical PinPrick stimulators might activate both Aβ and Aδ fibers.[7]

A better precision in the pain system is not simply explained by fibre-type activation and the number of recruited axons, although more input is applied to the sensory system. With the increased stimulus intensity, more fibers are recruited as indicated by elevated compound action potentials (CAPs) and intensity-dependent increases in the size of the RFs.[34,46] Following the elevated CAPs, the relative spatial gap between 2 adjacent RFs become "shortened" contributing to poorer discrimination judgements and, as a result, higher 2PD thresholds.[31] However, this mechanism does not sufficiently explain the current findings. Considering only the mechanical paradigm, PinPrick stimulation induced an enhanced discrimination compared with tactile (innocuous) von Frey hair stimulation, which is consistent with findings by Schlereth et al.[41] Both types of stimuli had similar shapes and probe sizes of approximately <1 mm. One potential explanation for the better precision of noxious stimuli is that needles could have activated both tactile and nociceptive fibres and synergistic central processing of those 2 resulted in smaller 2PD thresholds. However, this might be more evident for electrocutaneous stimuli considering its nonspecific effect. On the other hand, there was no pure activation of tactile receptors in the electrocutaneous paradigm and still discrimination thresholds were smaller for noxious stimuli. It might be suggested that the peripheral noise induced by burst-like stimuli—stimuli of high

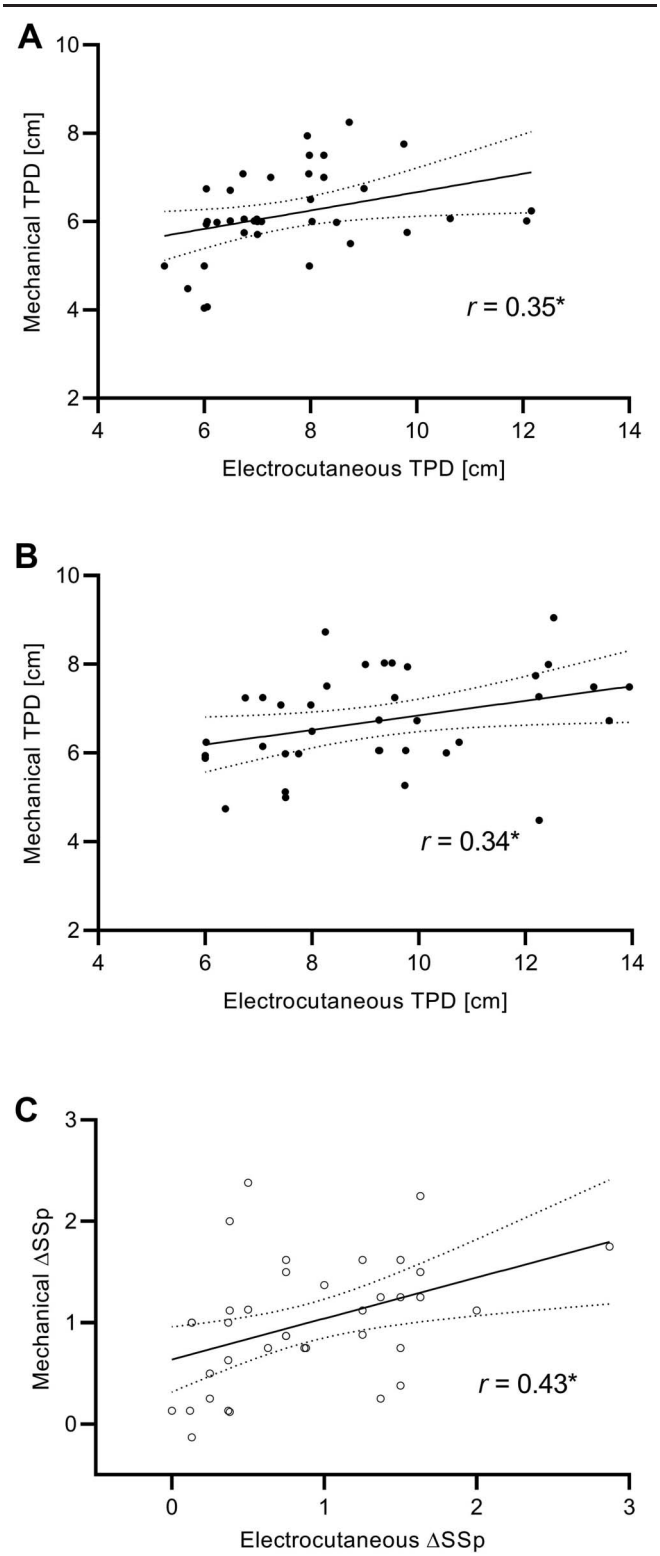

Figure 5. Concurrent validity. Correlations between paradigms, (A) noxious stimuli, (B) innocuous stimuli, and (C) absolute spatial summation observed, ie, difference between the widest separation and single electrode activation.

energy concentration—is compensated by centrally driven tuning when it comes to noxious processing. Such explanation has biological value, as in the presence of bodily threat/pain, the improved acuity might contribute to more precise response to painful event.

However, this might be of importance in regions which are not in the visual field, such as lumbar spine. Indeed, it has been shown in the work by Mancini et al.[30] that the clear pattern of lower tactile compared with noxious 2PD values can be observed in upper extremities but not at the lower back. Furthermore, our data are in line with a general somatotopic trend, showing that tactile acuity is better at the finger tips and over the hand region compared with the lumbar spine.[30,47] Current noxious 2PD with the mean above 7 cm is slightly higher than noxious thresholds found in the forearm[20,32] and the hand[30] in previous reports, further confirming the somatotopic organization of the pain system.

4.2. Spatial summation of pain as a modality-independent effect

Spatial summation of pain has been studied using a variety of stimulus modalities; however, only 2 studies applied electrocutaneous stimulation.[24,40] Despite the fact that SSp has been induced by, eg, pressure stimuli,[12] laser,[32] electrocutaneous,[40] or noxious heat,[37] the effect of stimulus modality on SSp has not been addressed, yet. Regardless of the application type, distance-based and area-based SSps were induced effectively in this current data set. Increasing the size of the stimulated area led to an increase in pain perception. At the same time, pain increased gradually with the increased separation between 2 stimulated areas. Assuming that the PinPrick might activate (mostly) Aδ fibers[21] and the electrocutaneous both Aβ and Aδ fibers,[28] it can be suggested that different peripheral inputs similarly shape the SSp effect. Interestingly, Aβ fibers—activated through squared electrical pulses—have different projections to the central nervous system compared with, eg, Aδ fibers.[20] Aβ fibres reach secondary neurons in dorsal column nuclei instead of the spinal laminae, where connections with wide-dynamic-range neurons form the spinal location for descending pain modulation.[44] Thus, a putative mechanism explaining the subadditivity which is characteristic for SSp can be attributed to wide-dynamic-range neurons and their projections to spinal nociceptors. On the other hand, Aβ projections (1) do not seem to interfere with SSp and (2) do not contribute to the SSp effect. Therefore, the mechanism of SSp might—at least partially—be explained by the descending pain inhibitory loop that has been shown to be involved in diffuse-noxious inhibitory control of pain.[45] However, considering peripheral mechanisms, it has been shown by comparing SSp effects in hairy and glabrous skin that the former leads to higher SSp,[39] but not in every modality.[13] Further studies are mandatory to explore spinal and supraspinal mechanisms of SSp.

In line with previous work that used different modalities, our study produced the strongest SSp effect between 6 and 8 cm.[37,38,40] This further supports that SSp is modality independent and has similar characteristics regardless of the peripheral input, ie, Aβ, Aδ, or C fibers. An interesting finding is the difference between area-based and the distance-based SSp with the larger effects in the former. These 2 types of SSp, however, can only be contrasted at very small body areas because of the interarea distance of 2 cm used in this study. Previous studies focused either on distance- or area-based SSp, exclusively. Further studies should aim to compare different SSp types using higher numbers of experimental conditions (areas and separations). Such a line of research is fundamental to better understand the spatial properties of the pain system and disentangling spatial summation from, eg, lateral inhibition.[38] It might be important to point out that the current variability of pain responses might have

been influenced by lateral inhibition. Especially the responses to stimuli separated by 10 and 12 cm are characterized by a slight reduction in pain intensity, which correspond to an early observation by von Békésy,[5] who showed similar pattern (**Fig. 4**., pp. 1010), although the stimuli used in that previous experiment were not noxious. Interestingly, greater pain at the 14 cm compared with, eg, 10 cm might also indicate lateral inhibition as at higher separations, this effect might be less prominent.[20]

4.3. Validation of the paradigm

Correlational analyses showed that the 2 innocuous (mostly tactile) acuity and the 2 noxious acuity tests were significantly correlated to each other. This is the first study that validated an electrocutaneous 2PD paradigm and contrasted its results against the standard mechanical assessment. Correlations were significant yet moderate presumably because of the significantly higher 2PD thresholds observed in electrocutaneous modality. Nevertheless, 2AFC task, which was based on series of stimuli applied in random sequences, reduced subjects' predictability compared with, eg, the staircase method commonly used in previous works. Furthermore, the test paradigm used in the current study was developed to reduce as many sources of random and systematic errors as possible. This might be considered an advantage; however, caution is required before using the novel paradigm in clinical populations because the sample size needed for case–control studies might be considerable, since the correlation is only moderate. Noxious stimulus intensities used in the current study were relatively low (elicited pain $<$ 2.5/10) compared with pain levels used in previous studies.[11,13] The highest pain induced by the PinPrick stimulators was possible with the 512-mN probe because this was the maximum force available in the PinPrick set. To make the intensity comparable, a formula for electrocutaneous stimuli was used and piloted to induce pain of the same intensity as for the mechanical stimuli. What could be considered a limitation of this study is the nonautomatic nature of the mechanical testing, the exact location of the target spots was difficult to match and required examiner training. As the nature of the study was limited to behavioural data, no physiological recording took place; thus, the inference regarding the activation of different afferent fibers is only indirect. Notwithstanding, previous literature showed a clear pattern of fibre recruitment when using mechanical or electrocutaneous stimuli.[21,28] It is also important to note that current results may have been influenced by habituation that might occur during ongoing exposure to noxious stimulation of relatively low intensity.[48] To overcome this problem, noxious stimuli of higher intensity are required for studies assessing, eg, 2PDs.

4.4. Conclusions

The 2 spatial properties of the pain system, acuity, and summation are conceptually distinct but provide deeper insight into the efficiency of the pain system in humans. 2PDs are smaller for noxious stimuli compared with innocuous stimuli when using the same stimulus modality (mechanical or electrocutaneous). When different modalities are used for noxious and innocuous stimuli, the pattern of the results is reversed and one may conclude that the pain system is less precise. Furthermore, SSp produces more robust results when applied as an area-based compared with a distance-based paradigm. Electrocutaneous and mechanical stimuli produce a similar SSp effect regarding magnitude and spatial characteristics; however, both SSp types were found to be subadditive, meaning that pain increased disproportionally with increased area or distance. Such a finding warrants further research aiming to investigate the central components of SSp. Finally, 2PDs determined by mechanical and electrocutaneous stimuli were significantly but moderately correlated, providing preliminary evidence for concurrent validity of the new paradigm.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Appendix A. Supplemental digital content

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