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The two-point discrimination threshold depends both on the stimulation noxiousness and modality

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Abstract

The two-point discrimination threshold (2PDT) has been used to investigate the integration of sensory information, especially in relation to spatial acuity. The 2PDT has been investigated for both innocuous mechanical stimuli and noxious thermal stimuli; however, previous studies used different stimulation modalities to compare innocuous and noxious stimuli. This study investigated the 2PDT in 19 healthy participants, using both thermal (laser) and mechanical stimulation modalities. Within each modality, both innocuous and noxious intensities were applied. Concurrent point stimuli were applied to the right volar forearm, with separation distances of 0 to 120 mm, in steps of 10 mm. 0 mm corresponds to a single point. Following each stimulus, the participants indicated the number of perceived points (1 or 2) and the perceived intensity (NRS: 0: no perception, 3: pain threshold, 10: maximum pain). The order of stimulation modality, intensity and distance was randomized. The 2PDT for innocuous and noxious mechanical stimuli was 34.7 mm and 47.1 mm, respectively. For thermal stimuli, the 2PDT was 80.5 mm for innocuous stimuli and 66.9 mm for noxious stimuli. The average NRS for thermal stimuli was 1.6 for innocuous intensities and 4.0 for noxious intensities, while, for mechanical stimuli, the average NRS was 0.9 for innocuous intensities and 3.6 for noxious intensities. This study showed that the 2PDT highly depends on both stimulation modality and intensity. Within each modality, noxious intensities modulates the 2PDT differently, i.e. noxious intensities lowers the 2PDT for thermal stimuli, but increases the 2PDT for mechanical stimuli.

Keywords: 2-point discrimination threshold, spatial acuity, laser stimulation, mechanical stimulation, pinprick stimulation, psycho-physics, sensory integration.

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Introduction

The spatial acuity of the sensory system is often investigated by determining the 2-point discrimination threshold (2PDT) (Schlereth et al. 2001; Tong et al. 2013; Mancini et al. 2014; Frahm et al. 2018). The 2PDT is the measure of the spatial distance between two cutaneous point stimuli where the perception shifts from perceiving a single point into perceiving two independent points. Often the purpose of identifying the 2PDT is to investigate possible pathological alterations in spatial acuity e.g. due to chronic pain (Catley et al. 2013) or to apply it as predictive measure of treatment effectiveness e.g. carpal tunnel surgery (Wessel et al. 2020).

The size of 2PDT depends on the size of the receptive field (RF) of the primary afferent as well as innervation density, and therefore varies across body site (Catley et al. 2013; Mancini et al. 2014). However, it also appears that the 2PDT often far exceeds the size of the individual receptive field of the primary afferents, since most 2PDT are reported to being several centimeters, except for in the hands (Catley et al. 2013; Mancini et al. 2014), while the size of the RFs are typically only a few millimeters. This discrepancy indicates that central mechanisms also play a critical role in the 2-point discrimination. Mechanisms such as lateral inhibition (LI) are likely to have an effect on the integration of the sensory information (Békésy 1962; Quevedo et al. 2017; Frahm et al. 2018). When one or more RFs are exited by a stimulus, LI will inhibit the surrounding RFs, thus creating a sharpened spatial contrast in the sensory signal. This phenomenon is believed to be part of the neural mechanism behind spatial discrimination.

A review by Catley et al (2014) suggests that the 2PDT may be used to probe the spatial acuity in patients, and that the 2PDT appears to change in pathological conditions such as complex region pain syndrome, arthritis and low-back pain, but does not appear to be affected during burning mouth syndrome (Catley et al. 2014). Another study showed that the 2PDT increased slightly during cutaneous capsaicin sensitization (Kauppila et al. 1998), which was interpreted as changes in the RFs of the wide dynamic range (WDR) neurons. Additionally, it has recently been shown that the 2PDT may be modulated both during experimental acute pain (Adamczyk et al. 2018b) and chronic low back pain (Adamczyk et al. 2018a).

The majority of studies that have investigated the 2PDT and spatial acuity have applied low-intensity mechanical stimuli, therefore, testing tactile acuity. In addition, several studies have compared innocuous and noxious stimulus intensities in relation to spatial discrimination (Schlereth et al. 2001; Martikainen and Pertovaara 2002; Ylioja et al. 2006; Mørch et al. 2010; Mancini et al. 2014; Frahm et al. 2018). Most studies conclude that the nociceptive system exhibits poorer discrimination than the mechano-receptive system. It is often hypothesized that this poorer discrimination might be due to the lower 'resolution' of the nociceptive system possibly due to larger RFs. Yet some studies have indicated more similar discrimination between nociceptive and mechano-receptive system (Schlereth et al. 2001; Mancini et al. 2014). Differences in the stimulation site, and therefore innervation density, and in LI between the nociceptive and mechano-receptive systems may also account for some of the differences in 2PDT reported. It is, however, somewhat puzzling that the vast majority of these previous studies, which compared the spatial acuity of innocuous and noxious stimuli, have applied different stimulation modalities. Most studies applied low-intensity *mechanical* stimuli to test the discrimination of innocuous stimuli – whereas high-intensity *radiant heat* (laser) were used to investigate the discrimination of noxious stimuli (Ylioja et al. 2006; Mørch et al. 2010; Mancini et al. 2014; Frahm et al. 2018). Therefore, these comparisons in fact becomes investigations across both stimulation intensity – as well as stimulus modality.

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Studies employing methods such as localization or directional discrimination indicate that spatial acuity might be dependent on both stimulus modality and intensity. Schlereth at al. investigated the spatial acuity using a localization task (Schlereth et al. 2001), and compared noxious radiant heat with both innocuous and noxious mechanical stimuli. The study found that the spatial discrimination was similar for noxious laser stimuli and innocuous mechanical stimuli, but the discrimination of noxious mechanical stimuli was more accurate (Schlereth et al. 2001). Moreover, we have recently shown how the directional discrimination of laser stimuli depends on the noxiousness of the stimulation (Frahm et al. 2019a). Taken together, this shows that both the stimulation modality and intensity may affect the discriminative abilities significantly. Thus, it may be hypothesized that a similar finding could be expected for the 2PDT.

The aim of this study was to investigate how the 2PDT depends both on stimulation modality and stimulation noxiousness. This study applied both mechanical and radiant heat (laser) stimuli, and both innocuous and noxious intensities were tested for each modality.

Methods

Participants

19 healthy participants (7 females) participated in this study (mean age 24.6 ± 4.2 years). All participants received both oral and written information prior to the experiment and gave written consent before the experiment started, ensuring compliance with the Helsinki declaration. The experiment was approved by the local ethical committee (VN-20190005).

Experimental protocol

During the experiment, the participants were lying in a bed with their forearm supinated. Stimulations of different modalities and intensities were delivered to the participants' right volar forearm.

The 2PDT was determined for both laser and mechanical stimulation modalities. For each modality, both innocuous and noxious stimulation intensities were tested. The order of the modalities and intensities was randomized.

To test the 2PDT, stimuli were delivered with point-to-point distances from 0 to 120 mm, in steps of 10 mm. The distance of 0 mm corresponded to a single point stimulus. The stimuli were delivered along the proximal-distal axis of the forearm. Each point-to-point stimulation distance was delivered twice for each distance and the order of the distances was randomized. The 0 mm (single point) distance was repeated four times, as a control. The stimulation location was always changed slightly to avoid habituation and skin irritation/damage. Single point stimuli were delivered in the same area as the two point stimuli.

Following each stimulus, the participants had to indicate the number of perceived points (1 or 2 points, forced-choice) and the perceived intensity. Prior to the experiment, the subjects were instructed that they should first focus on the number of perceived points and then on perceived intensity. The subjects were informed that both 1 and 2 points would be delivered. The perceived intensity was reported using a Numerical Rating Scale (NRS) anchored as 0: no perception, 3: pain threshold, 10: maximum pain. This NRS scale was used as both painful and not painful responses were expected. The use of this scale also allows for comparison to previous studies (Frahm et al. 2018, 2019b).

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For each combination of modality and intensity, a total of 28 stimuli were delivered, i.e. a total of 112 stimuli. Stimuli were delivered with an interval at least 30 seconds between consecutive stimuli. The entire session lasted approx. 90 minutes.

Ten of the 19 participants took part in a follow-up session. The 2PDT was reassessed in the follow-up session following the same procedure as in the first session. The investigation time, instructions and investigator were identical between the two sessions. The investigation time, instructions and investigator were identical between the two sessions. The first and the follow-up session were separated by at least 48 hours.

Thermal stimulation

The thermal laser stimuli was delivered using a Synrad Firestar ti-series 100 W CO_2 laser (Synrad, USA). The beam diameter was 5 mm. The laser beam was directed through a scanner head (GSI Lumonics General Scanning XY10A) onto the participants' skin. The scanner head enabled very rapid displacement of the laser beam across the skin. To obtain an almost simultaneous stimulation of the two points, the laser alternated between stimulating each point in fast succession. I.e. the laser stimulated one point, then rapidly displaced to the other location and stimulated the second point. During movement, the laser was turned off. This sequence was repeated 20 times per second (Frahm et al. 2018, 2019b). This procedure caused a continuous and concurrent perception at each point, ensuring equal intensity at each point. The total stimulus duration was 1 second. The laser and scanner were controlled using a computer with custom-made Labview software.

During stimulation, the skin temperature was continuously measured using an Agema 900 infrared (IR) camera (FLiR, Sweden), with a framerate of 30 Hz. The intensity of the thermal stimuli was adjusted to reach skin temperatures of 42±1 °C and 49+1 °C to deliver innocuous and noxious intensities, respectively. These intensities were based on pilot experiments with the aim of identifying standardized stimulation intensities that would ensure either a clear non-painful warm sensation or a clear painful-hot sensation. The pilot experiments were made in same skin area, using the same setup as the final experiments. Regarding the lower intensity, the pilot experiments showed that lower intensities (below 42±1 °C) often led to non-perception of the stimulation, while increasing the intensity lead to some stimuli being perceived as painful. Regarding the higher intensity, the pilot experiments showed that this intensity led to a perception of pain, whilst avoiding skin erythema. The experiment was stopped if the skin temperature exceeded 55 ^{o}C.

Mechanical stimulation

To test the 2PDT for mechanical stimuli, two custom-made Vernier calibers were used, each caliber had two custom-made probes attached to the jaws of the caliber (Figure 1). The probes used to test innocuous stimuli were made of two blunt plastic filaments (5 mm diameter) (Figure 1A). It was ensured that, during the stimulation, both filaments impacted the skin simultaneously. See (Mørch et al. 2010; Frahm et al. 2018, 2019b) for reference.

The probes used to test noxious stimuli were blunted acupuncture needles (200 µm diameter) each suspended with a 60 grams weight i.e. pin-prick stimulators (Figure 1B). Also in this case, it was ensured that, during the stimulation, both needles impacted the skin simultaneously.

For both mechanical stimulators the stimulation duration was approx. 1 second. To test single point stimuli, only one probe was used for either intensity.

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[Insert Fig 1]

Data analysis & statistics

The maximum skin temperature, following the laser stimuli, was extracted from the IR data for each stimulus and averaged for each intensity (innocuous vs. noxious). A Students t-test was used to test for significant differences in stimulation temperature.

The 2PDT was calculated for each condition separately. Prior to the analysis, the point responses (1 or 2) was subtracted 1 (becoming 0 or 1). Then, the threshold was estimated using a logistic regression to fit the data to a sigmoidal curve (Eq. 1).

$$y = \frac{1}{1+e^{(a(b-x))}} \qquad \text{Eq. 1}$$

where *x* is the distance between the points (mm), *b* corresponds to the point where *y* = 0.5, and *a* is the slope of the curve at y = 0.5. Thus, y = 0.5 corresponds to the point separation distance where the participants perceived 50 % of the stimuli as two points (Mørch et al. 2010; Frahm et al. 2018). During the logistic regression, the parameters *x* and *y* originated from the experimental data and the fitting estimated the parameters *a* and *b*, for reference see (Mørch et al. 2010; Frahm et al. 2018). To investigate any possible order effect on the discrimination responses a Pearson's correlation analysis was performed between stimulation number and response correctness, this was made for each session and for both modalities and intensities. Finally, the agreement of the 2PDT responses between the two sessions was compared using a Bland-Altman (BA) plot. In the BA plot, the averaged response (1 or 2 point) was compared for each point separation distance (0 to 120 mm). A BA plot was constructed for each modality and each intensity.

To analyze differences in the perceived intensities a 4-way Analysis of Variance (ANOVA) was used. Factors were point separation distance (0 to 120 mm), stimulation modality (thermal or mechanical), stimulus intensity (innocuous or noxious) and session (first or follow-up). A Tukeys post-hoc was applied in case of significant main effect. The Bonferroni correction was used to adjust for multiple comparison.

To investigate whether the perceived number of points depended on the stimuli being perceived as painful a X^2 (Chi squared) test was used, the dependent variable was the correctness (correct response of either 1 or 2 points), and the painfulness of the stimuli, i.e. NRS below or above 3, was the independent variable. A separate χ^2 was conducted for both mechanical and thermal stimuli.

P-values smaller than 0.05 were considered significant. The data analysis was conducted in Matlab (R2019a, Mathworks, Natick, MA, USA) and the statistical tests were performed using SPSS 25 (IBM, Armonk, NY, USA).

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Results

Stimulation intensity

The average stimulation intensities used for the thermal stimuli is depicted in Figure 1. For innocuous thermal stimuli the average skin temperature was 41.7 ± 1.5 °C and for the noxious thermal stimuli the skin temperature was 48.6 ± 1.8 °C. There was a significant difference between the stimulation intensities (Students t-test, $t(986) = 57.4$, $p < 0.001$). At no time did the skin temperature exceed 55 °C and no skin damages were reported.

[Insert Fig 2]

For the innocuous thermal stimulation, 13.4 % and 7.7 % of the innocuous stimuli were perceived as painful (NRS ≥ 3) in session 1 and session 2, respectively. For noxious thermal stimulation, 79.6 % and 81.5 % were perceived as painful for session 1 and 2, respectively. For innocuous mechanical stimulation, 0.0 % of the stimuli were perceived as painful in either sessions. For noxious mechanical, 74.4 % and 82.3 % of the stimuli were perceived as painful in session 1 and 2, respectively. Across both sessions, 11.4 % of the innocuous and 80.3 % noxious thermal stimuli were perceived as painful. 0.0 % of the innocuous mechanical stimuli were perceived as painful and 77.1 % of the noxious mechanical stimuli were perceived as painful.

Two-point discrimination

The average perceived number of points and the fitted thresholds are depicted in Figure 3.

[Insert Fig 3]

The fitted 2-point discrimination thresholds for both sessions, modalities and noxiousness are depicted in Table 1. For thermal stimuli the discrimination is better for noxious intensities (indicated by a lower 2PDT), whereas for mechanical stimuli the discrimination is better for innocuous stimuli (Table 1).

[Insert Tab 1]

The stimulation order had no significant effect on the correctness of the discrimination in neither sessions, and for none of combinations of stimulus modality intensity (Pearson's correlation, $p > 0.05$).

[Insert Fig 4]

The BA plot (Figure 4) showed that the differences in the 2PDT measures between sessions are equally distributed in relation to the zero error reference line and are consistent across the different point distances. Furthermore, the BA plots showed that the majority of the measures were within the 95 % CI.

Perceived intensities

The average perceived intensities are depicted in Figure 5. There was a significant interaction between stimulation modality and noxiousness (ANOVA, $F(1,18) = 5.93$, $p < 0.05$), see (Table 2). The analysis also showed that the perceived intensities were significantly higher for noxious stimuli (ANOVA, $F(1,18) = 2493.86$, $p < 0.001$) and thermal stimuli (ANOVA, $F(1,18) = 51.24$, $p < 0.001$). The perceived intensity did also depend significantly on the distance between the points (ANOVA, $F(12,18) = 3.4$, $p < 0.05$). The post-hoc test showed that the distance of 0 mm (i.e. 1 point) was perceived significantly less intense compared to all other distances (Tukey post-hoc, $p < 0.05$), except for the smallest separation distance (10 mm, Tukey post-hoc $p > 0.1$) and the two largest separation distances, 110 and 120 mm (Tukey post-hoc Tukey post-hoc $p > 0.1$). There was

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no other statistical difference in the perceived intensities between the point separation distances. There were no differences in the perceived intensities between the two sessions (ANOVA, F(1,18) = 1.71, p = 0.19).

[Insert Fig 5]

[Insert Tab 2]

The Chi squared test showed that mechanical stimuli were more often correctly discriminated as two points, when the stimuli were not perceived as painful (χ^2, p < 0.001). The odds ratio was 0.62. For thermal stimuli, this dependency was not significant (χ^2, p = 0.078).

Discussion

In this study, we show how the 2-point discrimination threshold depends both on stimulation modality and on stimulus noxiousness. Interestingly the noxiousness affects the discriminative abilities differently for different modalities (mechanical vs. thermal). For thermal stimulation, noxious stimuli are discriminated better than innocuous stimuli, whereas for mechanical stimulation, innocuous stimuli are discriminated better than noxious stimuli.

Two-point discrimination

Generally, the 2PDTs for the mechanical stimuli were lower than the 2PDT for the thermal stimuli. This indicates a higher spatial acuity for mechanical stimuli. In fact, the highest spatial acuity was found for innocuous mechanical stimuli (lowest 2PDT), whereas the lowest spatial acuity was found for innocuous thermal stimuli (highest 2PDT) (Figure 3 & Table 1). It is worth noting that the discrimination threshold does not show a clear relation with the size of the individual RFs of the primary afferents, the RFs of the primary afferents are several orders of magnitude smaller than the discrimination thresholds. In this study both different stimulation modalities and intensities were applied, thus it can be expected that different subsets of cutaneous afferents will be activated. Noxious stimuli may activate both nociceptive as well as non-nociceptive afferents - non-nociceptive afferents will particularly be activated at the edge of the stimulation site, where the stimulation intensity is reduced. During innocuous mechanical stimulation it is expected that the stimulus will activate primary afferents belonging to the Aβ fiber type, shown to have RF diameters of approx. 1-6 mm (Bromm et al. 1984; Vallbo et al. 1995). Noxious mechanical stimulation will additionally activate nociceptive Aδ fibers with RFs diameters of approx. 5-8 mm (Bromm et al. 1984) as well as mechanosensitive C fiber nociceptors with RFs diameters of approx. 3-5 mm (Lamotte and Campbell 1978; Treede et al. 1990). The innocuous thermal stimuli will mostly activate C-fiber warm receptors which have very small RFs (~1 mm) sometimes even reported as ‘punctate’ or ‘spot-like’ (Hensel and Iggo 1971; Lamotte and Campbell 1978; Darian-Smith et al. 1979). The noxious thermal stimuli will additionally activate both C and Aδ fiber nociceptors. Heat-sensitive C fiber nociceptors have RFs of approx. 4-5 mm, but have been reported to have diameters of up to 17 mm (Schmidt et al. 2002). Heat-sensitive Aδ fibers have RF diameter of 5-8 mm (Bromm et al. 1984). Overall, the sizes of the different receptors’ RFs differs substantially from the found 2PDTs (~35 – 80 mm, Table 1). It is also very interesting to note that the smallest RFs are reported for C warmth receptors, but the current findings show that the spatial acuity is lowest for innocuous thermal stimuli. This further demonstrates the apparent lack of an association between the RF size and spatial acuity. Additionally it is worth noting that previous studies have showed differences in the 2PDT across different body regions (Mørch et al. 2010; Catley et al. 2013; Mancini et al. 2014). The 2PDT is often reported to be highest in the back and lower limbs (Mancini et al. 2014), indicating lower spatial acuity in these areas. Furthermore, the effect of different body regions should also be taking into account when using the 2PDT to assess the sensory function

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in different pathologies, as the findings presented in this study, may not be directly applicable across all body regions.

It is worth noting that within each modality, the noxiousness affects the discrimination oppositely. For example, noxious thermal stimuli are better discriminated than innocuous thermal stimuli; but for mechanical stimuli the opposite is true. This notion is particularly of interest as many studies which investigated the discriminative abilities between the nociceptive and non-nociceptive systems, applied different modalities for the innocuous and noxious stimuli (Martikainen and Pertovaara 2002; Mørch et al. 2010; Mancini et al. 2014; Frahm et al. 2018).

The co-activation of both nociceptive and non-nociceptive afferents may lead to a synergy between the two sensory subsystem improving the discriminative abilities (Schlereth et al. 2001). However, the current findings show that noxious mechanical stimuli are discriminated worse than innocuous mechanical stimuli. This finding is somehow in contrast to what was shown by Schlereth et al. (2001) that indicated a more accurate discrimination of noxious mechanical stimulation than of innocuous mechanical stimulation. The study by Schlereth et al. (2001), however, investigated spatial acuity using a localization task, which is based on a single point stimulation, while in the 2 points stimulation paradigm, neural mechanisms such as spatial summation and lateral inhibition may affect the spatial acuity differently than during a localization task due to multiple stimuli and larger stimulation area.

The different dependence of noxiousness in relation to stimulus modality might also be, at least in part, due to afferents transmission properties. The different fiber types that most likely are activated during this study (Aβ, Aδ and C fibers) have different conduction velocities, thus, the delay, at which the sensory information reaches the cortex, differs substantially. For mechanical stimuli, the innocuous information is most likely conveyed by Aβ fibers, whereas noxious information is most likely conveyed by Aδ fibers (with possible co-activation of non-nociceptive Aβ). This means that the innocuous stimuli will purely be transmitted via faster fibers. For thermal stimuli, the tactile information, from the innocuous stimuli, is only transmitted via slow conducting C fibers, whereas nociceptive information, from the noxious stimuli, is transmitted via a combination of faster Aδ and slower C fibers. These differences in conduction velocity may explain the differences in the 2PDT. The highest spatial acuity was found for innocuous mechanical stimuli (mediated by fast Aβ fibers), whereas, the lowest spatial acuity was found for innocuous thermal stimuli (mediated by C fibers). Therefore, this may suggest that sensory information conveyed by faster afferents are easier to discriminate compared to sensory information conveyed by slower afferents.

Finally, the estimation of the 2PDT was repeated in two separate sessions. Even though the sample size in the follow-up session is roughly half that of session 1, it is encouraging to see that the BA plots showed good agreement between the two sessions (Figure 5). This indicates a good degree of reproducibility of the 2PDT methods used in this study.

Perceived intensities

As mentioned above, the different stimulation intensities and modalities applied in this study are likely to activate different subsets of afferent fibers. For the thermal stimuli, the temperature at the skin surface during the lower (innocuous) intensity was on average 41.7 °C. This intensity was based on pilot experiments to ensure a robust sensation of non-painful warmth. According to the literature, the selected temperature is well above the thermal threshold of C warm fiber, which has been reported to be as little as 1 °C above normal skin temperature (Lamotte and Campbell 1978; Darian-Smith et al. 1979), with maximum activity around 40-41 °C (Hensel and Iggo 1971; Hallin et al. 1981). The selected temperature is, however, also similar

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to the thermal threshold of C fiber nociceptors (40-41 °C (Churyukanov et al., 2012; Schmidt et al., 2002; Treede et al., 1995)). The reported intensities showed that approx. 11 % of these stimuli were perceived as painful, indicating that thermoreceptive C fiber nociceptors may have been activated in some cases. The higher (noxious) stimulation temperature was on average 48.6 °C, which is above the thermal threshold of both Aδ fiber nociceptors (thermal threshold 46-47 °C, (Treede et al. 1995; Churyukanov et al. 2012)) as well as C fiber nociceptors, as mentioned above. This agrees with the reported intensities where approximately 80 % of the stimuli were perceived as painful.

For the mechanical stimulation, the probes were designed to elicit either a clear tactile non-painful sensation or a clear painful pin-prick sensation. The innocuous stimulator has previously been used in studies investigating the tactile 2PDT (Mørch et al. 2010; Frahm et al. 2018, 2019b), and the results showed that this probe did not cause any stimuli to be perceived as painful (0.0 %). The design of the weighted 60 grams pin-prick stimulator was chosen to ensure an intensity above pain threshold. The average pain threshold for pin-prick stimuli has been reported to be approx. 100 mN (~10.2 grams) in the hand (Rolke et al. 2006). Rolke et al (2006) also reported the higher limit of the 95 % CI for the pin-prick pain threshold to be approx. 450 mN (~45.8 grams), thus, the 60 grams probe used in the current study should be above pain threshold. Hence, it is slightly surprising that more than 20 % of the pin-prick stimuli were not perceived as painful. However, since 77.1 % of the stimuli were perceived as painful, and none of the innocuous stimuli were perceived as painful it is believed that the design of the two probe types are still adequate to investigate the discriminative abilities of both innocuous and noxious mechanical stimuli.

The significant interaction between stimulus modality and noxiousness indicates that the increase on the perceived intensity innocuous and noxious stimuli is not the same for thermal and mechanical stimuli (Table 2). As seen in table 2, the largest difference between innocuous and noxious stimuli are found for the mechanical stimuli.

Finally, the perceived intensities showed that one point stimulations were perceived significantly less intense than two single points (except when comparing to the smallest and the two largest separation distances). This finding, fits well with literature, and it is most likely an effect of spatial summation (Price et al. 1989; Defrin and Gideon Urca 1996; Nielsen and Arendt-Nielsen 1997), due to the increased stimulation area when two points are stimulated, compared to only a single point. The lack of a significant difference between 0 mm (1 point) and the smallest point separation distance (10 mm) is likely due to an increase effect of lateral inhibition when the two points are located in close proximity (Békésy 1962; Quevedo et al. 2017). The lack of significant differences between 0 mm (1 point) and 110/120 mm is most likely because spatial summation is less pronounced for larger separation distances (Quevedo and Coghill 2009). In the intermediate distances, the populations of neurons activated by the stimuli may partially overlap, provoking a facilitatory process that might influence the intensity of the perceived pain (Quevedo et al. 2017).

Impact

The 2PDT is very simple measure to conduct both in research but also clinically (Kauppila et al. 1998; Catley et al. 2014; Adamczyk et al. 2018b). This technique is indeed being used to investigate spatial acuity in patient populations. However, it is still unclear how noxiousness affects spatial acuity assessed by 2PDT. Most of the studies that investigated the discriminative abilities between the nociceptive and non-nociceptive systems, applied different stimulation modalities for the innocuous and noxious stimuli. The current study shows that the noxiousness does affect the acuity, but in opposites directions depending on stimulation modality. This indicates that different intensities cannot be compared without considering the modality, as previously done, and therefore has important implications for the design of future studies.

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Limitations

The current study may be influenced by some experimental limitations. First, the sample size, particularly in session 2 could be considered rather small; nevertheless, it is encouraging that there seems to be good agreement between the two sessions, as shown in the BA plots (Figure 4). Additionally, in future studies it may be relevant to increase the number of control stimuli (0 mm), as previous studies have used a higher percentage of control stimuli (25-50 % of the stimuli) than in the current study (Mørch et al. 2010; Mancini et al. 2014). The overall number of trials, in a forced-choice design as used in the current study, is likely to affect the robustness of the parameter estimation. A larger number of trials will likely lead to a more robust estimation of the 2PDT. However, a larger number of trials will increase the session length, requiring the subjects to concentrate for a longer amount of time, which could lead to less precise responses, thus also limiting the 2PDT estimation.

Intensity scales are routinely used to measure the perceived intensity of a stimulus. Most scales are anchored from 'No pain' to 'Maximum imaginable pain', however, such anchors are often unsuited when administering stimuli which will be perceived both as painful and non-painful (Kemp et al. 2012), leading to incorrect responses (floor effects). Therefore, the current study adopted a scale, which contained both the non-painful and painful domain, since both innocuous and noxious intensities were applied. Similar scales containing both painful and non-painful domains, have previously been used in studies anticipating both painful and non-painful perceptions e.g. (Broeke and Mouraux 2015). Similarly, Madden et al. showed the feasibility of using a scale containing both painful and non- painful domain (Madden et al. 2016).

The perceived intensities showed that not all noxious intensities were perceived as painful, and a small percentage of the innocuous thermal stimuli were perceived as painful. These findings could indicate that the used intensities may not ideal; however, as discussed above, the used intensities do appear to be well suited to activate the relevant receptors. The lack of reported pain for all noxious stimuli, may simply reflect that nociception does not necessarily lead to the perception of pain, but other phenomena, such as habituation, may also have affected the perceived intensity.

Finally, the size of the stimulation area differs between the noxious mechanical probe and the other stimuli. Whereas each point stimulation for both thermal stimuli and the mechanical innocuous probe are of identical size (5 mm in diameter), the point stimulation of the pinprick stimulator used to deliver the noxious mechanical stimuli is only 200 µm in diameter. That means that it is likely that the probe used to deliver the noxious mechanical stimulus will activate fewer receptors, due to the smaller stimulated area. This discrepancy could potentially affect the findings in this study, in particular for the noxious mechanical stimulation, and should be taking into consideration when interpreting the results.

Conclusion

Improved knowledge of the 2PDT may enable better understanding of the mechanism behind this measure and optimize its use as a neurophysiological test. The current findings provides ins ~~the~~ discrimi~~in~~ative abilities of the nociceptive, thermo-receptive and mechano-receptive subsystems within the sensory system, and aids the understanding of key differences between these subsystems. The 2PDT appear to depend on both the stimulation modality and noxiousness. However, based on the current findings, it cannot be generally concluded that the 2PDT is enhanced or reduced for noxious stimuli, because the spatial acuity of noxious stimuli compared to innocuous stimuli might depend on the stimulation modality. Thus,

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further research is needed to elucidate how noxiousness affects the discrimination.

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Conflicts of interest:

None to declare.

Ethics approval:

Compliance with ethical standards. The experiment was approved by the local ethical committee (VN-20190005).

Data availability:

All data generated or analysed during this study are included in this published article

Authors' contributions:

KSF and SG designed the study. KSF collected the data. KSF and SG analyzed and interpreted the data. KSF drafted the manuscript. All authors read and approved the final manuscript.

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Figure captions

Fig 1 Mechanical stimulators. The mechanical stimulators were two Vernier calibers to which two probes were attached. To test innocuous stimuli the two probes were blunt plastic filaments (A). To test noxious stimuli the two probes were weight-loaded pin-prick stimulators (B). It was ensured that both probes impacted the skin simultaneously. For single point stimuli, only one probe was applied to the skin

Fig 2 Stimulation intensity during thermal stimuli. The figure depicts average of the maximum recorded skin temperature in each condition (mean ± SD). There was a significant difference in the stimulus temperature (* Students t-test, $t(986) = 57.4$, $p < 0.001$)

Fig 3 Perceived number of points and fitted 2-point discrimination thresholds for thermal and mechanical stimulation modality for the two sessions. Black: innocuous stimuli. Red: noxious stimuli

Fig 4 Bland-Altman plot to compare the discrimination responses (1 or 2 points) during session 1 and 2. Each point represents the average response (1 or 2 points) for a given point separation distance (0 to 120 mm). Full line: average difference between the sessions, dashed line: 95 % CI of the difference.

Fig 5 Perceived intensities (NRS) across stimulation modality, intensity and session. Black: innocuous stimuli. Red: noxious stimuli. There was a significant interaction between stimulation modality and intensity (ANOVA, $F(1,18) = 5.93$, $p < 0.05$). The perceived intensities were significantly lower for 0 mm (a single point stimulus), compared to all other distances, except 110 and 120 mm (Tukey post-hoc, $p < 0.05$). There were no significant differences between the sessions (ANOVA, $F(1,18) = 1.71$, $p = 0.19$). The horizontal line in NRS = 3 indicates pain threshold.

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Table 1 – 2-point discrimination thresholds for both sessions. Including the 95 % confidence interval (CI) and r^2 of the sigmodal fits.

		Session 1 (N=19)			Session 2 (N=10)		
Modality	Noxiousness	Threshold (mm)	95% CI (mm)	r^2	Threshold (mm)	95% CI (mm)	r^2
Thermal	Innocuous	80.5	65.0 - 96.0	0.76	80.6	68.1 - 93.1	0.83
	Noxious	66.9	61.4 - 72.4	0.96	59.8	52.4 - 67.1	0.93
Mechanical	Innocuous	34.7	33.4 - 36.1	0.99	37.6	36.1 - 39.2	0.99
	Noxious	47.1	45.0 - 49.1	0.99	46.3	44.4 - 48.2	0.99

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Table 2 – Average perceived intensities across each modality and noxiousness. There was a significant interaction between stimulation modality and noxiousness (ANOVA, $F(1,18) = 5.93$, $p < 0.05$). The perceived intensities were significantly higher for noxious stimuli (ANOVA, $F(1,18) = 2493.86$, $p < 0.001$) and for thermal stimuli (ANOVA, $F(1,18) = 51.24$, $p < 0.001$). There were no significant differences in perceived intensities between the two sessions (ANOVA, $F(1,18) = 1.71$, $p = 0.19$).

		Session 1 (N=19)	Session 2 (N=10)
Modality	Noxiousness	NRS (mean ± SD)	NRS (mean ± SD)
Thermal	Innocuous	1.6 ± 1.1	1.4 ± 0.9
	Noxious	4.0 ± 2.0	3.8 ± 1.3
Mechanical	Innocuous	0.9 ± 0.3	1.1 ± 0.4
	Noxious	3.6 ± 1.6	3.7 ± 1.3

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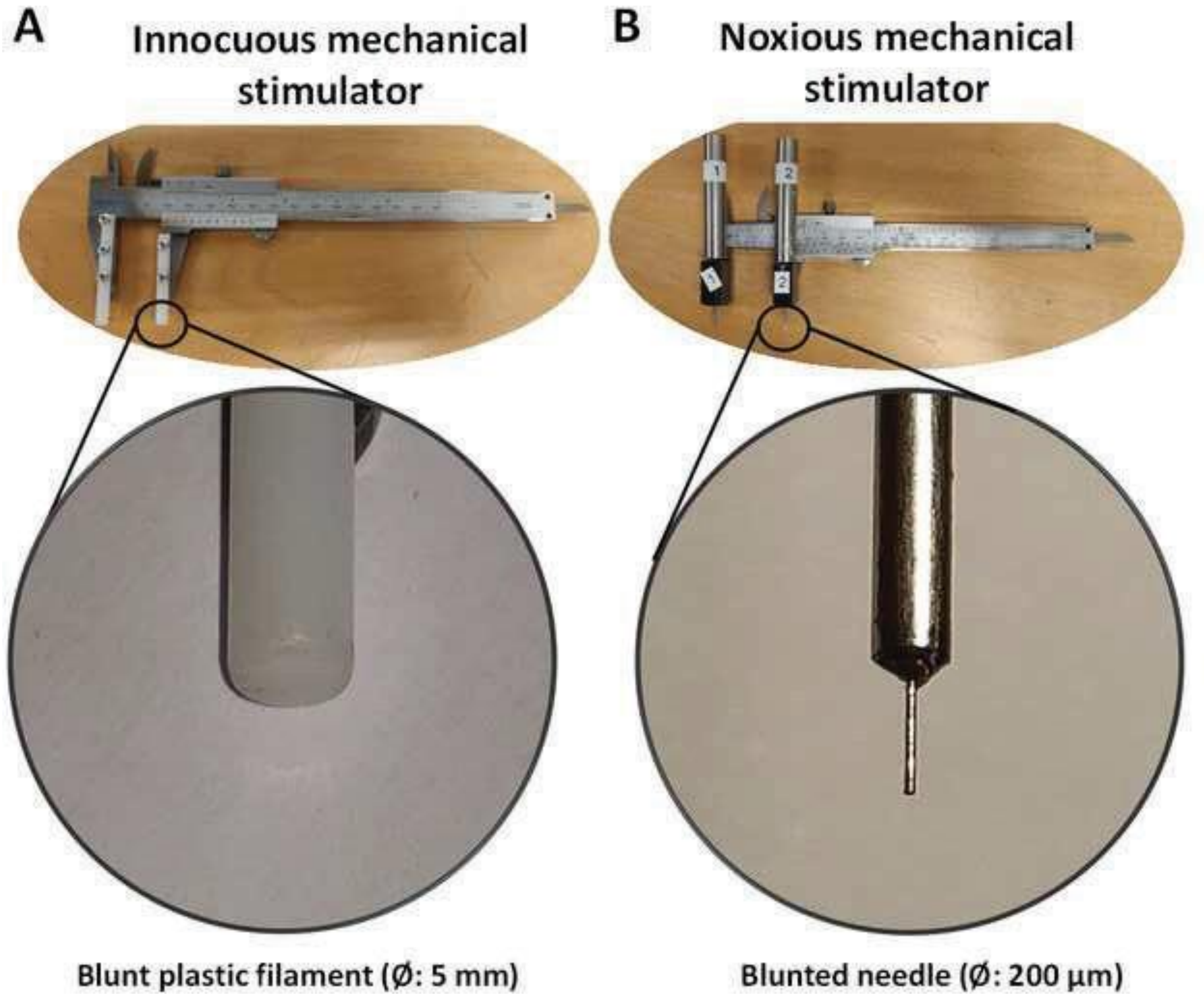

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Figure 2

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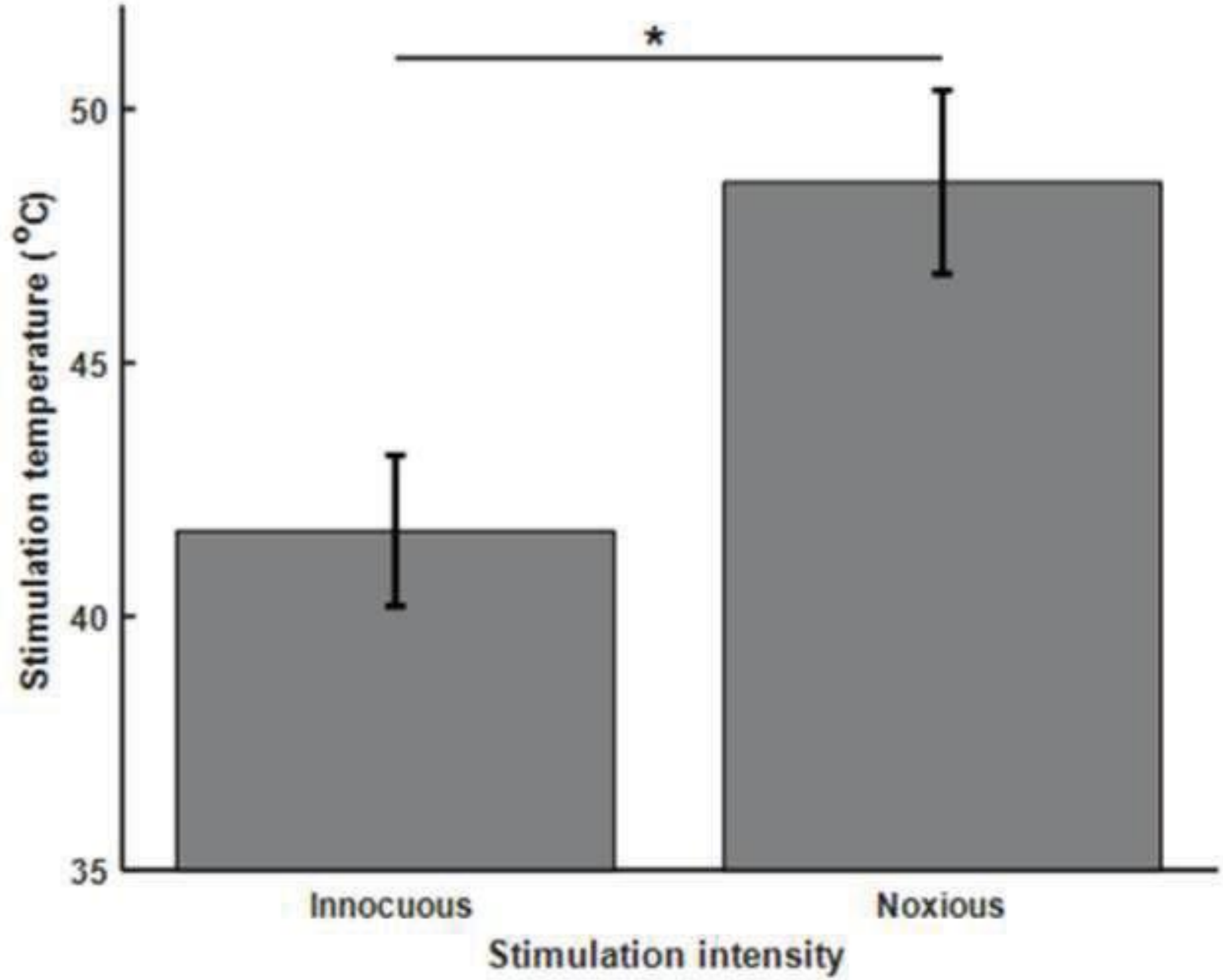

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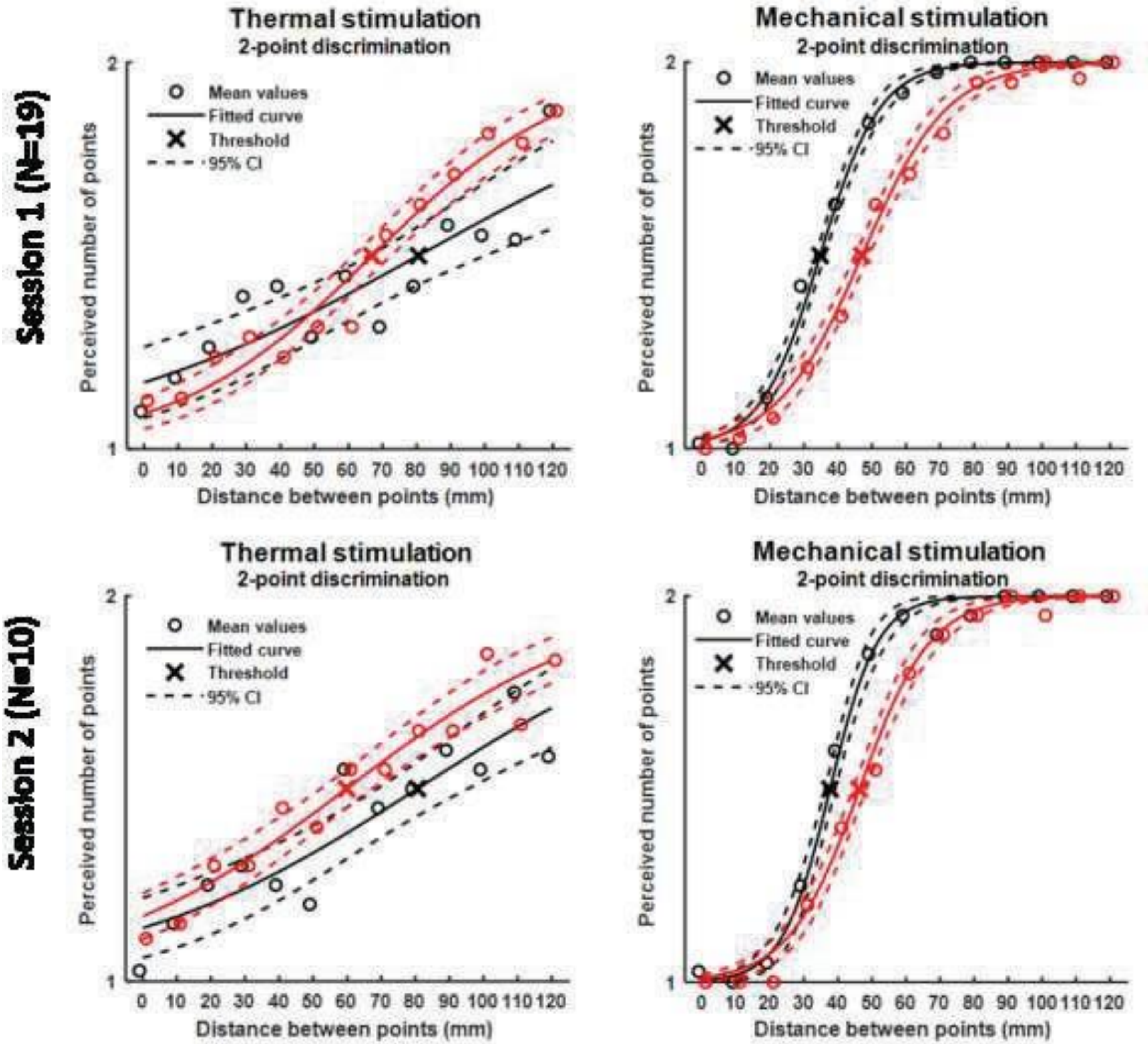

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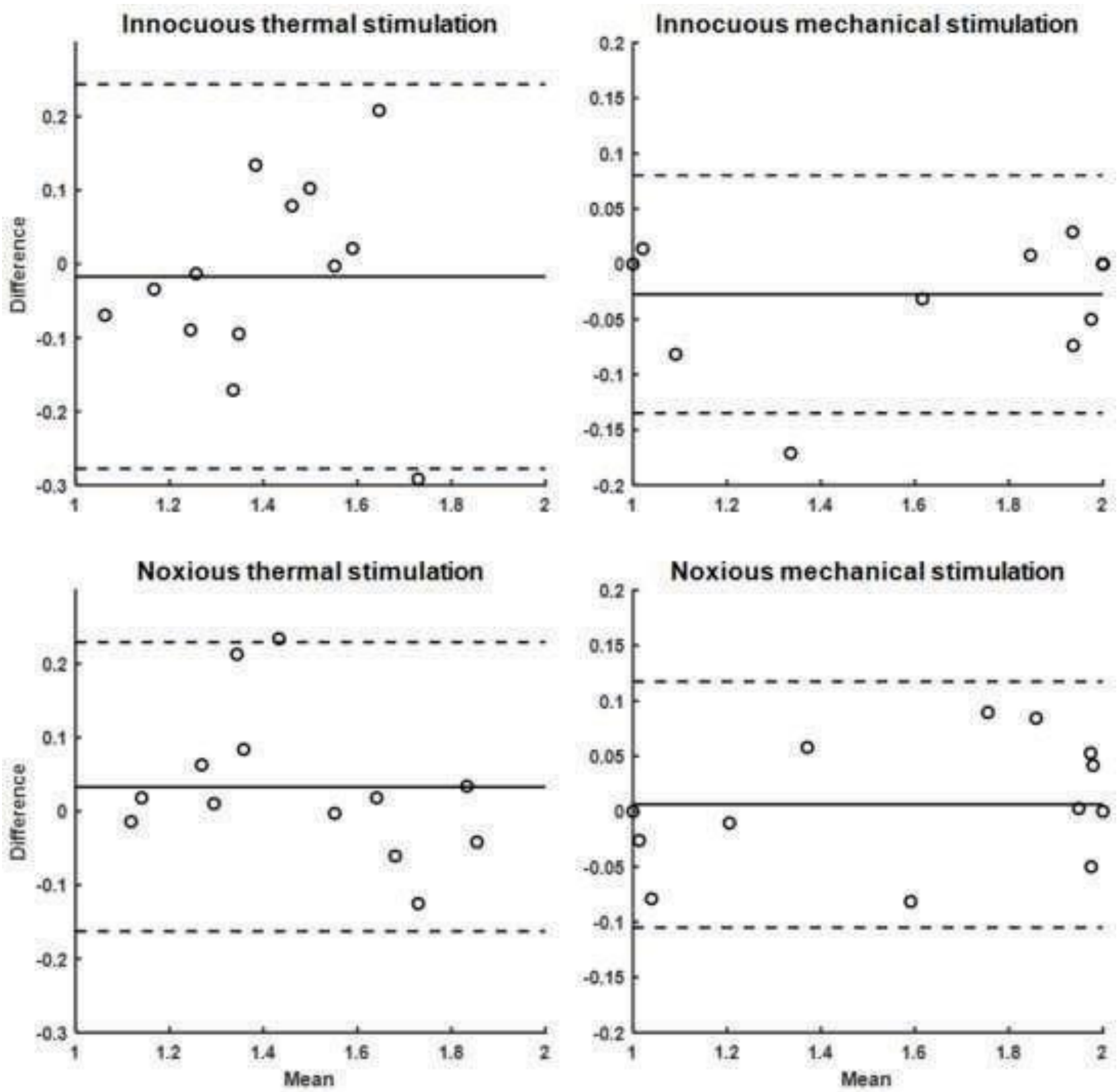

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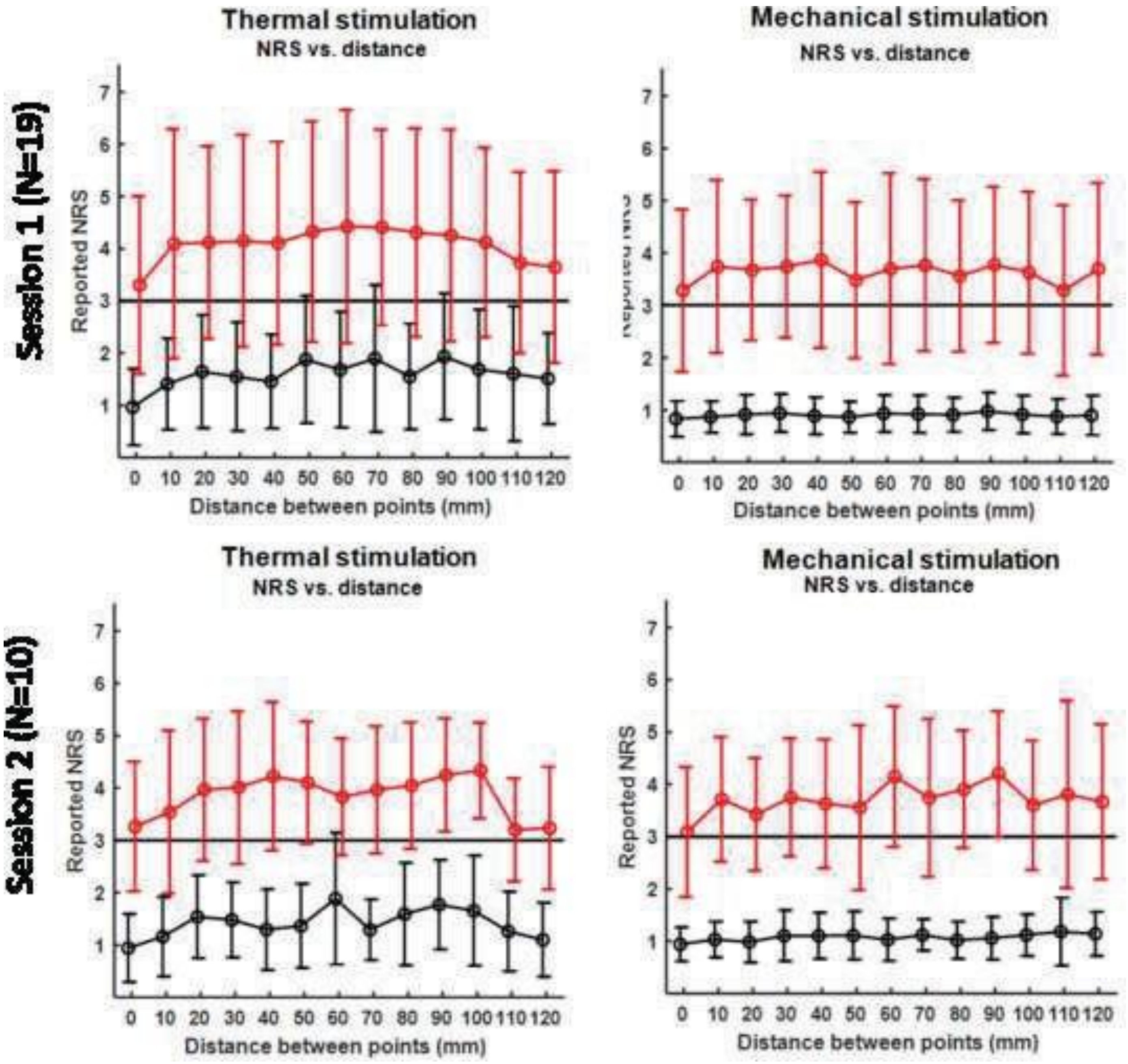

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