



AALBORG UNIVERSITY
DENMARK

Aalborg Universitet

Tempo-spatial discrimination is lower for noxious stimuli than for innocuous stimuli

Frahm, Ken Steffen; Mørch, Carsten Dahl; Andersen, Ole Kæseler

Published in:
Pain

DOI (link to publication from Publisher):
[10.1097/j.pain.0000000000001095](https://doi.org/10.1097/j.pain.0000000000001095)

Publication date:
2018

Document Version
Accepted author manuscript, peer reviewed version

[Link to publication from Aalborg University](#)

Citation for published version (APA):
Frahm, K. S., Mørch, C. D., & Andersen, O. K. (2018). Tempo-spatial discrimination is lower for noxious stimuli than for innocuous stimuli. *Pain*, 159(2), 393-401. <https://doi.org/10.1097/j.pain.0000000000001095>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- ? Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- ? You may not further distribute the material or use it for any profit-making activity or commercial gain
- ? You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us at vbn@aub.aau.dk providing details, and we will remove access to the work immediately and investigate your claim.

Tempo-spatial discrimination is lower for noxious stimuli than for innocuous stimuli.

Ken Steffen Frahm¹, Carsten Dahl Mørch¹, Ole Kæseler Andersen¹

¹ Integrative Neuroscience group, Center for Neuroplasticity and Pain (CNAP), SMI[®], Dept. of Health Science & Technology, Aalborg University, 9220 Aalborg, Denmark

Number of pages: 22

Number of figures: 5

Number of tables: 0

Corresponding author:

Ken Steffen Frahm

Center for Neuroplasticity and Pain (CNAP), SMI[®], Dept. of Health Science & Technology, Aalborg University

Fredrik Bajersvej 7D3 (Office A2-204)

9230 Aalborg, Denmark

ksf@hst.aau.dk

+45 99 40 98 42

www.cnap.hst.aau.dk

Abstract

The exteroceptive sensory system is responsible for sensing external stimuli in relation to time and space. The aim of this study was to investigate the tempo-spatial properties of the exteroceptive system using painful laser heat and non-painful mechanical touch stimulation.

Thirteen healthy subjects were stimulated on the volar forearm using two paradigms: a continuous stimulation along a line on the skin and a 2-point stimulation. The line stimulations were delivered in both the distal and proximal direction with lengths of 25, 50, 75, and 100mm. The 2-point stimulations were assessed by simultaneous stimuli at a point-to-point distance ranging from 10 to 100mm, in steps of 10mm. The subjects reported the intensity (0-10 NRS, 3: pain threshold) and either direction (line stimuli) or number of perceived points (2-point stimuli).

All mechanical line stimulations were reported correctly, i.e. a directional discrimination threshold of less than 25mm. For painful laser line stimulation, the directional discrimination threshold was 68.5mm and 70.2mm for distally and proximally directed stimuli, respectively. The 2-point discrimination threshold for painful laser stimulation (67.9mm) was higher than for the mechanical stimulation (34.5mm). NRS increased both with line length and distance between the two points (Linear mixed model, $p < 0.001$).

The findings indicate that the tempo-spatial acuity of the exteroceptive system is lower for noxious stimuli than for innocuous stimuli. This is possible due to the larger receptive fields of nociceptive neurons and/or less lateral inhibition.

Keywords: Laser stimulation; Tempo-spatial discrimination; Exteroceptive sensory system; Healthy subjects; 2-point discrimination

Introduction

The ability to determine and recognize stimulus characteristics in relation to time and space depends on the function of the exteroceptive sensory systems [14,18]. The skin is the largest sensory organ in the human body and forms the main external barrier that contributes to the protection of the body's integrity and homeostasis. Both the spatial and temporal exteroceptive abilities in the skin have been investigated extensively. Several studies have previously investigated the spatial acuity to both painful [12,14,17,18,31] and non-painful stimuli [14,18,22,31]. Some studies have indicated that single point localization is similar for tactile and noxious stimuli [17]. However, most studies show lower point localization accuracy for noxious stimuli compared to tactile stimuli [12,39]. Overall, this was interpreted as evidence that the somatotopical representation in the brain for noxious inputs is similar to that found for tactile stimuli [12], but with slightly lower accuracy for nociceptive information. Besides point localization, many studies have used the 2-point discrimination threshold as an indicator of the spatial acuity. In addition to spatial acuity, spatial summation of pain has also been extensively investigated [19,24,27], and it has been shown that the perceived pain intensity will increase with an increased stimulation area [19,24,27].

The temporal properties have also been investigated previously. Especially the temporal summation of pain has been studied in great depth [8,21,33]. Studies in healthy subjects have shown increased responses during repetitive stimulation [1,32], and this phenomenon has been shown to be facilitated in chronic pain patients [2,9,20] making it a potential tool to assess chronic pain patients [33].

The combined ability to recognize the tempo-spatial characteristics of a stimulus has not been studied in great detail. A previous study from our group investigated both the temporal and spatial properties of the nociceptive exteroceptive system using a graphesthesia task [18]. It was found that the exteroceptive capabilities were reduced for painful stimuli compared with non-painful stimuli [18]. Using the saltation illusion, another study found that the nociceptive system exhibited phenomena similar to the mechanoreceptive system [36]. Thus, the literature somewhat differs regarding the difference between the nociceptive and somatosensory system, and even though graphesthesia and the saltation illusion are

interesting, the outcomes may be somewhat difficult to quantify and less suitable to map changes, e.g. in chronic pain patients.

Previously, it has been found that for two identical stimuli, the most distal stimulation will be perceived less painful [25]. This 'distal inhibition' phenomenon may enable more defensive reactions to stimulations closer to the personal space than when stimuli further away [29,30]. Based on these findings, it may also be hypothesized that a continuous stimulus moving in a proximal direction, e.g. on the lower arm, will be perceived as more painful than stimuli moving distally due to the defensive purpose of the nociceptive system [25].

In this study we propose a method in which both stimulation area and duration were changed simultaneously in an easy controllable manner. The paradigm was conducted using a painful laser stimulus which was moved across the skin in a straight line of varying lengths. In addition, simultaneous 2-point discrimination tasks were included to allow for a comparison of the purely spatial mechanisms.

It was hypothesized that healthy subjects would display higher accuracy in determining the stimulus direction and lower 2-point discrimination when using innocuous mechanical stimuli as compared to noxious heat stimulation.

The primary aim of this study was to use a moveable stimulus to determine the combined tempo-spatial discriminative properties for both noxious and innocuous stimuli. Such a stimulation technique is very novel as up until now the discriminative ability of the exteroceptive system has been studied mostly based on point discrimination and point localization.

Methods

Participants

Thirteen healthy subjects participated in this study (5 females and 8 males, mean age 27 ± 4 years). During the experiment the subjects were seated in a bed with the back rest inclined. The forearm was kept horizontal and supported by a pillow if necessary. During the experiment the right volar forearm was stimulated using both non-painful mechanical and painful laser heat stimuli. All participants received both written and oral information and gave written consent prior to the experiments; thus ensuring compliance with the Declaration of Helsinki. The experiment was approved by the local ethical committee (N-20140093).

Experimental protocol

The order of the laser and mechanical stimulation was randomized between the subjects. Any excessive hair growth in the stimulation area was removed prior to stimulation.

Both the mechanical and laser stimulations consisted of two parts. First, the tempo-spatial acuity was tested by displacing a continuous stimulation along a line parallel to the forearm (Figure 1). Four different stimulation lengths were used (25, 50, 75 and 100mm) and two directions of the displacement were used; distally, i.e. towards the wrist, and proximally, i.e. towards the upper arm. The velocity of the stimulation was maintained at 35 mm/s [18] for both stimulation types. The subjects were blinded to the stimulus length and direction. Following each stimulation, the participants were asked to indicate the direction of the stimulus, either distally or proximally. This was done using a forced choice design, i.e. the subjects had to indicate the perceived direction of the stimulation. Furthermore, the subjects were asked to indicate the intensity on a numeric rating scale (NRS) anchored as 0: Perception threshold, 3: Pain threshold, 10: Maximum pain. This scale was used as the mechanical stimulations were expected to be perceived as being below and the laser stimulations were expected to be above the pain threshold. All combinations of line length and direction were administered twice in randomized order.

To test the 2-point discrimination ability, the subjects were instructed that both single and 2-point stimulations would be administered (Figure 1) [18]. Following each stimulation, the participants were asked to indicate the number of points perceived, again using a forced choice design; i.e. the participants were to rate either 1 or 2 points and indicate the perceived intensity on the NRS scale. All combinations of the point-to-point distances were administered twice in randomized order.

Laser stimulation

A Synrad Firestar ti-series 100 W CO₂ laser (Synrad, USA) was used to deliver the noxious heat stimuli to the skin. A 5X beam expander was mounted on the laser to obtain a larger beam diameter. The trajectory of the laser beam was directed through a scanner head (GSI Lumonics General Scanning XY10A) containing two mirrors mounted on galvanometers, which rapidly and accurately displaced the beam across the skin surface (Figure 1). To obtain a beam width of 5mm ($1/e^2$), the laser beam was very rapidly moved in small concentric circles along the path of the laser stimulation (dithering). The stimulation was perceived as continuous, i.e. the dithering was not perceived. The 2-point discrimination stimulation was conducted in the same manner ensuring that each spot was 5mm ($1/e^2$) in diameter.

For the line stimulations, the stimulation intensity was adjusted so that a 50mm line stimulation was perceived as 4 on the NRS scale. For the 2-point stimulations, the stimulation intensity was adjusted so that a single point stimulation was perceived as 4 on the NRS scale. The 1 and 2-point stimulations lasted for 1.5 seconds, similar to the stimulus time for the mechanical point stimulation.

Throughout the experiment the skin temperature was monitored using an Agema 900 series infrared camera. If the skin temperature exceeded 60°C during the stimulation, the experiment was stopped. The infrared image was used to ensure that the skin temperature did not increase during the experiment, due to repeated stimulations. Both the participant and investigator wore protective goggles for the experiments. The spatial profile of the laser beam was controlled using the infrared camera ensuring a Gaussian-like profile. The inter-stimulus interval for the laser stimuli was 30-60 seconds.

Mechanical stimulation

A Somedic Senselab Brush-05 (Somedic, Sweden) was used to deliver the mechanical line stimulation (Figure 1). The width of the stimulation was approx. 5mm and the length was approx. 15mm. The mechanical line stimulation was guided along the skin using a visible HeNe (Helium-Neon) laser ensuring a speed and distance identical to the laser stimulations. The HeNe laser was inserted into the path of the CO₂ laser beam and directed through the scanner head onto the skin. During these stimuli the CO₂ laser was shut off. The mechanical stimuli were then delivered in the same manner as the laser line stimulations, i.e. four lengths (25, 50, 75 and 100mm) and two directions (distally and proximally).

The mechanical 2-point discrimination threshold was tested using a Vernier caliber with two blunt plastic filaments both with a diameter of 5mm (Figure 1). During the stimulations care was taken to ensure that that the two filaments contacted the skin simultaneously. Again the subjects were instructed that either one or two points could be administered and asked to indicate the intensity and number of perceived points.

Data analysis

The data analysis was performed using Matlab (Natick, MA, USA).

Line stimulations

To analyze the responses from the line stimulations, the responses were fitted to a sigmodal curve [18,31] (Eq. 1) in which 0.5 corresponds to merely guessing the direction. b corresponds to the point where $y = 0.75$, and a is the slope of the curve at this point. 0.75 corresponds to the point where the subjects answer 75 % correct, i.e. 50% better than merely guessing.

$$y = \frac{0.5}{1 + e^{(a(b-x))}} + 0.5 \quad \text{Eq. 1}$$

2-point stimulations

The responses to the 2-point discrimination were analyzed similarly to [18,31] by fitting a sigmodal curve (Eq. 2). Prior to the fit, the responses to the 2-point stimulations (either 1 or 2) were subtracted 1, meaning that 1s were converted to 0, and 2s were converted to 1 [18]. b corresponds to the point where $y = 0.5$, i.e. when the subjects were capable of distinguishing between the two points. a corresponds to the slope of the curve at point b .

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

Eq. 2

Statistics

Normality was confirmed before statistical tests were applied.

Differences in NRS for the line stimulations were analyzed using a linear mixed model (LMM) with line direction as factor set and line length as a continuous covariate. This model does not give the option of comparing the NRS response between each line length; however, it does allow an estimation of how much the NRS change for a certain change in line length. Differences in NRS for the 2-point stimulations were analyzed using a linear mixed model with distance between points as covariate. Independent analyses were made for each stimulation modality.

The difference between NRS in relation to the correctness of the response (direction or number of points) was analyzed for both stimulation modalities (and for both directions for the line stimulations) using a Student t-test.

Student's t-tests were used to calculate the difference between NRS in relation to correctness of the indicated direction.

For the laser line stimulations, a Chi-squared test was used to investigate the relationship between the correctness of the answer in relation to whether the stimulation was perceived as painful or not. In addition, the odds ratio for answering correctly if the stimulation was perceived as painful was calculated.

A p-value of 0.05 was considered significant. The statistical tests were performed using SPSS 23 (IBM, Armonk, NY, USA)

Results

The skin temperature of the participants never exceeded 60°C during the experiments. No subjects reported skin damages following the laser stimulations.

Line stimulations

The laser line stimulations revealed very similar directional discrimination thresholds for both directions. For distally directed stimuli the threshold was 68.5mm ($r^2=0.59$; 95% CI: -15.3-152.4mm) and for proximally directed stimuli the threshold was 70.2mm ($r^2=0.90$; 95% CI: 42.8-97.7mm). When combining the directions, the threshold was 69.5 mm ($r^2=0.84$; 95% CI: 26.9-112.2mm) (Figure 2). For the mechanical stimuli the line stimulations revealed a response accuracy of 100% (208 correct responses out of 208 stimulations) for both directions and all four stimulation lengths (not depicted), i.e. the directional discrimination threshold for mechanical stimuli appears to be less than 25mm.

Significant differences were found in the NRS responses for the laser stimulation depending on the length of the line (LMM, $p<0.001$), but not for the direction of the stimulation (LMM, $p=0.843$; Figure 3). The LMM showed that NRS increased by 0.23 for each step of 25mm, indicating an average NRS difference of 0.69 between the shortest (25mm) and longest (100mm) line stimulation.

For the mechanical line stimulations no differences were found in the NRS responses for neither stimulation length nor direction (LMM, $p=0.214$; Figure 3).

A significant difference was demonstrated in the perceived intensity (NRS) for the distally directed stimuli depending on the correctness of the response (Student's t-test, $p<0.01$ – correct responses had higher intensities). However, this was not the case for the proximal direction (Student's t-test, $p=0.10$, Figure 3). A Chi-squared test revealed that the answers more often were correct if the stimulation was perceived as

painful (Chi square, $p < 0.01$, Figure 3). The odds ratio for answering correctly was 2.4 times higher if the stimulus was perceived as painful as compared with non-painful (Figure 3).

2-point discrimination

The 2-point discrimination thresholds for the laser stimulation were 67.9mm (95% CI: 63.5-72.3mm) and 34.5mm (95% CI 32.3-36.7mm) for the mechanical stimulation (Figure 4).

For the laser stimulation significant differences were reported in NRS in relation to the distance between the point (LMM, $p < 0.001$; Figure 4). The LMM showed that NRS increased by 0.10 for each step of 10mm, meaning that larger distances between the points resulted in higher reported NRS and that the LMM indicated a NRS difference of 0.9 between the shortest (10mm) and longest (100mm) distance between the points (Figure 4). For the mechanical stimulation no significant differences were found in NRS in relation to the distance between the points (LMM, $p = 0.19$).

No difference was detected in the perceived intensity (NRS) depending on number of perceived points, neither for laser stimulation (Student's t-test, $p = 0.31$) nor mechanical stimulation (Student's t-test, $p = 0.19$; Figure 4).

Discussion

The current study investigated the exteroceptive abilities of the sensory system using a continuous stimulus that moved at a constant speed in a straight line across the skin in both proximal and distal directions. The ability to discriminate between the directions of a stimulus was shown to be far greater for innocuous mechanical stimulation than for noxious laser stimulations. For noxious laser stimulation, the perceived intensity increased with stimulation length and thereby stimulus duration. Furthermore, it was found that the 2-point discrimination threshold for the noxious laser stimuli was almost twice that of the innocuous mechanical stimuli. However, the results indicated that neither the perceived intensity nor the discriminative abilities depend on the stimulus direction.

The line stimulation method used in this study is quite simple but offers insight into the exteroceptive sensory system and the differences between the tactile mechanoreceptive and nociceptive system.

Perceived intensities

The stimulation setup used in this study was identical to previous studies from our group [7,18]. The stimulation intensity for the laser line stimulation was set to correspond to a NRS value of 4 for a 50mm stimulation. However, most NRS scores were reported as lower than 4, indicating that the stimulations were perceived more intense during threshold determination compared with the following stimulations (Figure 3). This may be due to receptor fatigue or habituation [6,35,37] despite an inter-stimulus interval of 30-60s which should be sufficient to minimize habituation [35]. However, some studies suggest as much as 'minutes' should pass between stimuli [23]. This may also reflect other mechanisms affecting the perceived intensity such as lateral inhibition [3] which may also affect the discriminative abilities. In addition, so-called in-field inhibition may affect the perceived intensity when stimulating within the same receptive field [13].

Directional discrimination threshold

It was not possible to determine the mechanical directional discrimination threshold, but the findings suggest that this threshold is less than 25mm. However, mechanical stimulation lengths of less than 25mm would be a considerable challenge to conduct accurately and repeatedly since the stimulation brush is approx. 5mm wide and 15mm long and applied manually. In addition, the scope of this study was merely to investigate differences between noxious and innocuous stimuli.

Lateral inhibition is known to affect and aid the spatial acuity of the skin [3,16] through a centrally facilitated area surrounded by an inhibitory area (Figure 5). If lateral inhibition is reduced, so is the contrast of the overall activation, and then the combined neural activation will linger over a prolonged period possibly affecting the discrimination of stimuli (Figure 5). When moving the stimulus across the skin, the stimulus will then be applied in areas which are inhibited by the preceding stimulus and may thus make the

discrimination more difficult. This effect would increase with larger receptive fields and will also depend on the speed of the laser beam (Figure 5). In fact, the average receptive field size for heat-sensitive nociceptive neurons is approx. 4-5mm in both the human and primate forearm [4,34], whereas for human mechanoreceptive non-nociceptive neurons the average receptive field has been reported to be 2-3mm [4] down to approx. 1-2mm in the human forearm [38]. Since the receptive fields of nociceptive afferents are larger than non-nociceptive mechanoreceptive afferents, this may possibly explain the higher directional discrimination thresholds for noxious stimuli. However, it is worth noting that the directional threshold is far larger than the sizes of the individual receptive fields of the afferent neurons. This most likely indicates that the directional discrimination threshold also depends on the integration of afferent input in higher order neurons. Previously, animal studies using single-unit recordings from the primary sensory cortex have shown a subset of sensory neurons primarily responsive to the direction of a tactile stimulus [5,10]. Some neurons responded to several stimulus directions, whereas others showed reduced [5] or no [10] response in the opposite direction. The receptive field for these complex cortical neurons appears larger than simpler neurons, e.g. mediating information regarding stimulus intensity [5]. The number of neurons responding to distally-proximally or proximally-distally directed stimuli in the volar forearm of monkeys appears similar for both directions [5]. These direction-sensitive neurons show a very poor response to single point stimuli [5,10]. This indicates that the direction-discrimination task used in this study is indeed very novel since it reflects a very different mechanism than the 2-point discrimination task. The response of these neurons does not appear to depend on the texture of the moving stimulus, i.e. metal, cotton, finger all elicited similar responses [10], but no noxious stimuli were tested. The findings in this study may indicate that similar mechanisms exist for nociceptive direction-sensitive neurons; however, with larger receptive fields. This has also been hypothesized by other studies [12]. The use of single-unit recording is not feasible in healthy humans and thus the investigation must rely on psychophysical methods as used in this study. The results showed that longer laser stimulation lengths were perceived as more intense (Figure 3). This finding is likely due to a higher degree of spatial summation [19] due to larger area being stimulated. This agrees

with the literature as a previous study showed that this effect would be largest when the stimulated areas are separated by approx. 50-100mm [27]. In this case it would mean that longer stimulations, i.e. 75 and 100mm, would result in higher perceived intensity. Overall, the perceived intensity will be the net sum of the spatial summation and the lateral inhibition. Therefore, the tempo-spatial element becomes important as the stimulation time is longer due to the relatively slow stimulation speed across the skin, i.e. the duration of the total nociceptive afferent volley is up to four times longer for the longest stimulus. This may also lead to temporal summation of the stimuli [8] resulting in higher perceived intensity despite the fact that the stimulus is moving across the skin because the nociceptive afferent input will be integrated over time further up the neuroaxis. Thus, the increased NRS may in fact be a result of a tempo-spatial summation. The results also show that line stimulations which are perceived as painful are more often perceived correctly (Figure 3). This could indicate that the exteroceptive system is more accurate for nociceptive input compared with innocuous thermal stimuli. However, it could also reflect that longer stimulations are easier to perceive correctly (Figure 2) and because of the larger stimulation area, longer stimulations will be perceived as more intense due to a tempo-spatial summation as the results also indicate.

The spatial acuity in the radial-ulnar direction appears better than in the proximal-distal direction [16,31]. Therefore, it would be interesting to investigate if this was also the case for the line stimulations conducted in this study. However, it is not feasible to test the radial-ulnar direction on the volar forearm due to the curvature of the arm rendering variation in the stimulus direction impossible with the current setup. However, future studies could investigate this, e.g. on the abdomen or back.

Finally, there appeared to be little difference in the response accuracy or perceived intensity depending on the direction of the stimulus. This may indicate that the concept of distal inhibition [25] plays only a minor role when the stimulus is moved in the proximal-distal direction across the skin. This is somewhat surprising since proximally directed stimuli could be conceived as more 'dangerous', similar to studies showing that,

stimuli delivered closer to the personal space create stronger defensive reactions than stimuli delivered further away [29,30].

2-point discrimination threshold

This study found a larger 2-point discrimination threshold for noxious stimuli compared with innocuous tactile stimuli. There is a large variation in the reported 2-point discrimination threshold in the literature [11,14,16,18,22,31]. Some studies reported a similar 2-point discrimination threshold between noxious and innocuous stimuli [14,31], while most report higher thresholds for noxious stimuli [16,18]. However, the reason for these different observations is most likely due to experimental differences. Studies using simultaneous stimuli, including the present study, generally find larger differences between noxious and innocuous thresholds [16,18] compared with studies applying sequential stimuli [15,31]. Thus, the findings in the present study correspond well with values in the literature. Part of the reason for the larger 2-point discrimination threshold for nociceptive neurons may be the larger receptive field of the nociceptive neurons. However, other factors, such as integration of the afferent input, will also affect this threshold. This is evident since the 2-point discrimination threshold is far larger for both mechanical (34.5mm) and noxious heat (67.9mm) than the size of the respective receptive fields of the non-nociceptive neurons (~2mm [4,38]) and nociceptive neurons (~5mm [4,34]). Furthermore, it is worth noting that the directional discrimination and 2-point discrimination thresholds for the noxious laser stimulation are very similar (69.5 vs. 67.9mm, respectively). In comparison, the mechanical directional threshold is lower than the mechanical 2-point discrimination threshold (<25mm vs. 34.5mm, respectively). These findings could indicate that the tempo-spatial processing of the mechanoreceptive and nociceptive exteroceptive systems differs in more aspects than just merely spatial lower resolution in the nociceptive system; thus resulting in lower tempo-spatial acuity for noxious stimuli.

The reported NRS was increased for points separated by larger distances compared with smaller separation distances. This is most likely a result of the lateral inhibition mechanism as stimuli located further apart will be less affected by lateral inhibition, since the two stimuli are located outside the inhibitory area of the concurrent stimuli, and will thus be perceived as more intense. In fact, points which were located closer together resulted in lower reported NRS supporting a role of lateral inhibition [3,16] This effect increases with decreasing distance between the points, i.e. when the overlapping of the inhibitory areas is substantial. In addition it is worth noting that for several 2-point stimulations with small separation the reported NRS was lower than the threshold intensity rated at NRS=4 (Figure 4). This was determined using a single stimulus point, demonstrating how lateral inhibition from each of the two closely located concurrent point stimuli lowers the perceived intensity. Besides lateral inhibition, the so-called neuronal population code could also affect the perceived intensity of the two points [26]. According to this theory, two stimuli located side-by-side or close together will activate the same population of neurons. However, when the distance between the points increases, the populations of the activated neurons will differ leading to a larger number of recruited neurons. In turn, this leads to more spatial summation of pain [26]. A recent study showed that noxious 2-point stimulation with a certain distance between the points is perceived as more painful than a stamped line stimulation of the same distance [28] most likely reflecting the mechanism of lateral inhibition. This despite the fact that the line stimulations should activate more neurons, due to a larger stimulation area, which should result in increased pain perception due to spatial summation. However, this was not observed [28], thus demonstrating a strong effect of lateral inhibition. These findings fit well with the NRS responses seen in the current study where noxious 2-point stimulations were perceived as more intense when the distance between the points was increased.

Conclusion

The main finding of this study is lower directional discrimination in the nociceptive system compared with the mechanoreceptive system. Part of the reason for this may be the larger receptive fields of nociceptive neurons. However, this could also reflect larger receptive fields of direction-sensitive neurons in the primary sensory cortex. Furthermore, it was found that longer line stimulations resulted in higher reported NRS likely reflecting the net sum of spatial and temporal summation and lateral inhibition.

Acknowledgement

This study was funded by the Danish National Research Foundation (DNRF121).

Conflict of interest

There are no conflicts of interest.

References

- [1] Andersen OK, Spaich EG, Madeleine P, Arendt-Nielsen L. Gradual enlargement of human withdrawal reflex receptive fields following repetitive painful stimulation. *Brain Res.* 2005;1042:194–204.
- [2] Arendt-Nielsen L, Drewes AM, Hansen JB, Tage-Jensen U. Gut pain reactions in man: An experimental investigation using short and long duration transmucosal electrical stimulation. *Pain* 1997;69:255–262.
- [3] Békésy GV. Lateral inhibition of heat sensations on the skin. *J. Appl. Physiol.* 1962;17:1003–1008.
- [4] Bromm B, Jahnke MT, Treede RD. Responses of human cutaneous afferents to CO₂ laser stimuli causing pain. *Exp. Brain Res.* 1984;55:158–166.
- [5] Costanzo RM, Gardner EP. A Quantitative Analysis of Responses of Direction-Sensitive Neurons in Somatosensory Cortex of Awake Monkeys. *J. Neurophysiol.* 1980;43:1319–41.
- [6] Ferraro D, Vollono C, Miliucci R, Viridis D, De Armas L, Pazzaglia C, Le Pera D, Tarantino S, Balestri M,

Di Trapani G, Valeriani M. Habituation to Pain in “Medication Overuse Headache”: A CO2 Laser-Evoked Potential Study. *Headache* 2012;52:792–807.

- [7] Frahm KS, Andersen OK, Arendt-Nielsen L, Mørch CD. Spatial temperature distribution in human hairy and glabrous skin after infrared CO2 laser radiation. *Biomed. Eng. Online* 2010;9:69.
- [8] Granot M, Granovsky Y, Sprecher E, Nir RR, Yarnitsky D. Contact heat-evoked temporal summation: Tonic versus repetitive-phasic stimulation. *Pain* 2006;122:295–305.
- [9] Graven-Nielsen T, Aspegren Kendall S, Henriksson KG, Bengtsson M, Sörensen J, Johnson A, Gerdle B, Arendt-Nielsen L. Ketamine reduces muscle pain, temporal summation, and referred pain in fibromyalgia patients. *Pain* 2000;85:483–491.
- [10] Hyvarinen J, Poranen A. Movement-sensitive and direction and orientation-selective cutaneous receptive fields in the hand area of the post-central gyrus in monkeys. *J. Physiol* 1978;283:523–537.
- [11] Johnson KO, Phillips JR. Tactile Spatial Resolution . I . Two-Point Discrimination, Gap Detection, Grating Resolution, and Letter Recognition. *J. Neurophysiol.* 1981;46:1177–1191.
- [12] Koltzenburg M, Handwerker HO, Torebjork HE. The ability of humans to localise noxious stimuli. *Neurosci.Lett.* 1993;150:219–222.
- [13] Laskin SE, Spencer WA. Cutaneous Masking. II. Geometry of Excitatory and Inhibitory Receptive Fields of Single Units in Somatosensory Cortex of the Cat. *J. Neurophysiol.* 1979;42:1061–1082.
- [14] Mancini F, Bauleo A, Cole J, Lui F, Porro CA, Haggard P, Iannetti GD. Whole-body mapping of spatial acuity for pain and touch. *Ann. Neurol.* 2014;75:917–924.
- [15] Mancini F, Sambo CF, Ramirez JD, Bennett DLH, Haggard P, Iannetti GD. A Fovea for Pain at the Fingertips. *Curr. Biol.* 2013;23:496–500.

- [16] Martikainen IK, Pertovaara A. Spatial discrimination of one versus two test stimuli in the human skin: dissociation of mechanisms depending on the task and the modality of stimulation. *Neurosci. Lett.* 2002;328:322–324.
- [17] Moore CEG, Schady W. Cutaneous localisation of laser induced pain in humans. *Neurosci. Lett.* 1995;193:208–210.
- [18] Mørch CD, Andersen OK, Quevedo AS, Arendt-Nielsen L, Coghill RC. Exteroceptive aspects of nociception: Insights from graphesthesia and two-point discrimination. *Pain* 2010;151:45–52.
- [19] Nielsen J, Arendt-Nielsen L. Spatial summation of heat induced pain within and between dermatomes. *Somatosens. Mot. Res.* 1997;14:119–125.
- [20] Nikolajsen L, Hansen CL, Nielsen J, Keller J, Arendt-Nielsen L, Jensen TS. The effect of ketamine on phantom pain: A central neuropathic disorder maintained by peripheral input. *Pain* 1996;67:69–77.
- [21] Pedersen JL, Andersen OK, Arendt-Nielsen L, Kehlet H. Hyperalgesia and temporal summation of pain after heat injury in man. *Pain* 1998;74:189–97.
- [22] Peters ML, Schmidt AJM. A comparison of two-point discrimination threshold of tactual, non-painful stimuli between chronic low back pain patients and controls. *Pain* 1991;44:57–60.
- [23] Plaghki L, Mouraux A. How do we selectively activate skin nociceptors with a high power infrared laser? Physiology and biophysics of laser stimulation. Comment activer selectivement des nocicepteurs cutanés avec un laser infrarouge de forte puissance? *Physiologie et biophysiq.* 2003;33:269–277.
- [24] Price DD, McHaffie JG, Larson MA. Spatial Summation of Heat-Induced Pain: Influence of Stimulus Area and Spatial Separation of Stimuli on Perceived Pain Sensation Intensity and Unpleasantness. *J. Neurophysiol.* 1989;62:1270–1279.

- [25] Quevedo AS, Coghill RC. An Illusion of Proximal Radiation of Pain Due to Distally Directed Inhibition. *J. Pain* 2007;8:280–286.
- [26] Quevedo AS, Coghill RC. Attentional Modulation of Spatial Integration of Pain: Evidence for Dynamic Spatial Tuning. *J. Neurosci.* 2007;43:11635–11640.
- [27] Quevedo AS, Coghill RC. Filling-In, Spatial Summation, and Radiation of Pain: Evidence for a Neural Population Code in the Nociceptive System. *J. Neurophysiol.* 2009;102:3544–3553.
- [28] Quevedo AS, Mørch CD, Andersen OK, Coghill RC. Lateral Inhibition during Nociceptive Processing. *Pain* 2017;158:1046–1052.
- [29] Sambo CF, Forster B, Williams SC, Iannetti GD. Behavioral/Systems/Cognitive To Blink or Not to Blink: Fine Cognitive Tuning of the Defensive Peripersonal Space. *J. Neurosci.* 2012;32:12921–12927.
- [30] Sambo CF, Liang M, Cruccu G, Iannetti GD. Defensive peripersonal space: the blink reflex evoked by hand stimulation is increased when the hand is near the face. *J Neurophysiol.* 2012;107:880–889.
- [31] Schlereth T, Magerl W, Treede R. Spatial discrimination thresholds for pain and touch in human hairy skin. *Pain* 2001;92:187–194.
- [32] Spaich EG, Arendt-Nielsen L, Andersen OK. Repetitive painful stimulation produces an expansion of withdrawal reflex receptive fields in humans. *Artif.Organs.* 2005;29:224–228.
- [33] Staud R, Price DD, Fillingim RB. Advanced Continuous-Contact Heat Pulse Design for Efficient Temporal Summation of Second Pain (Windup). *J. Pain* 2006;7:575–582.
- [34] Treede R-D, Meyer RA, Campbell JN. Comparison of Heat and Mechanical Receptive Fields of Cutaneous C-Fiber Nociceptors in Monkey. *J Neurophysiol.* 1990;64:1502–1513.
- [35] Treede RD, Lorenz J, Baumgärtner U. Clinical usefulness of laser-evoked potentials.

Neurophysiol.Clin. 2003;33:303–314.

- [36] Trojan J, Stolle AM, Kleinbo D, Mørch CD, Arendt-Nielsen L, Hözl R. The saltation illusion demonstrates integrative processing of spatiotemporal information in thermoceptive and nociceptive networks. *Exp. Brain Res.* 2006;170:88–96.
- [37] Valeriani M, De Tommaso M, Restuccia D, Le Pera D, Guido M, Iannetti GD, Libro G, Truini A, Di Trapani G, Puca F, Tonali P, Cruccu G. Reduced habituation to experimental pain in migraine patients: A CO₂ laser evoked potential study. *Pain* 2003;105:57–64.
- [38] Vallbo AB, Olausson H, Wessberg J, Kakuda N. Receptive field characteristics of tactile units with myelinated afferents in hairy skin of human subjects. *J Physiol.* 1995;483:783–795.
- [39] Ylioja S, Ve Carlson S, Raij TT, Pertovaara A. Localization of touch versus heat pain in the human hand: A dissociative effect of temporal parameters on discriminative capacity and decision strategy. *Pain* 2006;121:6–13.

Figure legends

Figure 1. Experimental setup. Both painful (laser) and non-painful (mechanical) stimuli were delivered to the volar forearm. Left: setup for testing the directional discrimination thresholds. Right: setup for testing 2-point discrimination.

Figure 2. Directional discrimination threshold for the laser stimulations. The data is pooled for both the proximal and distal directions. Right: single subject data for the directional discrimination. Generally it appears as if the directional discrimination ability increases with stimulation length as only one subject (light green color coding) clearly stands out from this trend, however, notice that each point is only the pooled data of four stimuli (two stimulus directions; each direction was repeated twice). Left: average

responses across subjects and a sigmoidal fit to determine the threshold. The black line indicates a fitted sigmoidal curve and the dashed lines indicate the 95% CI of the fit. The combined discrimination threshold was 69.5mm (purple circle).

Figure 3. Reported intensities for laser (left) and mechanical (right) line stimulations. Colors indicate stimulation direction. Top: Stimulation length vs. perceived intensity (NRS). The lines indicate a linear regression and dashed lines indicate 95% CI for the fit. For the laser stimulations a significant difference was found in NRS depending on the length of stimulation (LMM, $p < 0.001$), but not stimulus direction (LMM, $p = 0.843$). For the mechanical stimuli no differences were found in NRS (LMM, n.s.). Bottom: Correctness of the response vs. perceived intensity (NRS). The answers are more often correct if the stimulation was perceived as painful (Chi-squares, $p < 0.01$). The odds ratio for answering correctly was 2.4 times higher if the stimulation was perceived as painful (NRS > 3). All subjects detected the stimulation direction correctly for the mechanical stimulations. Note: y-axes have been truncated for better visualization. The horizontal line in NRS=3 indicates pain threshold.

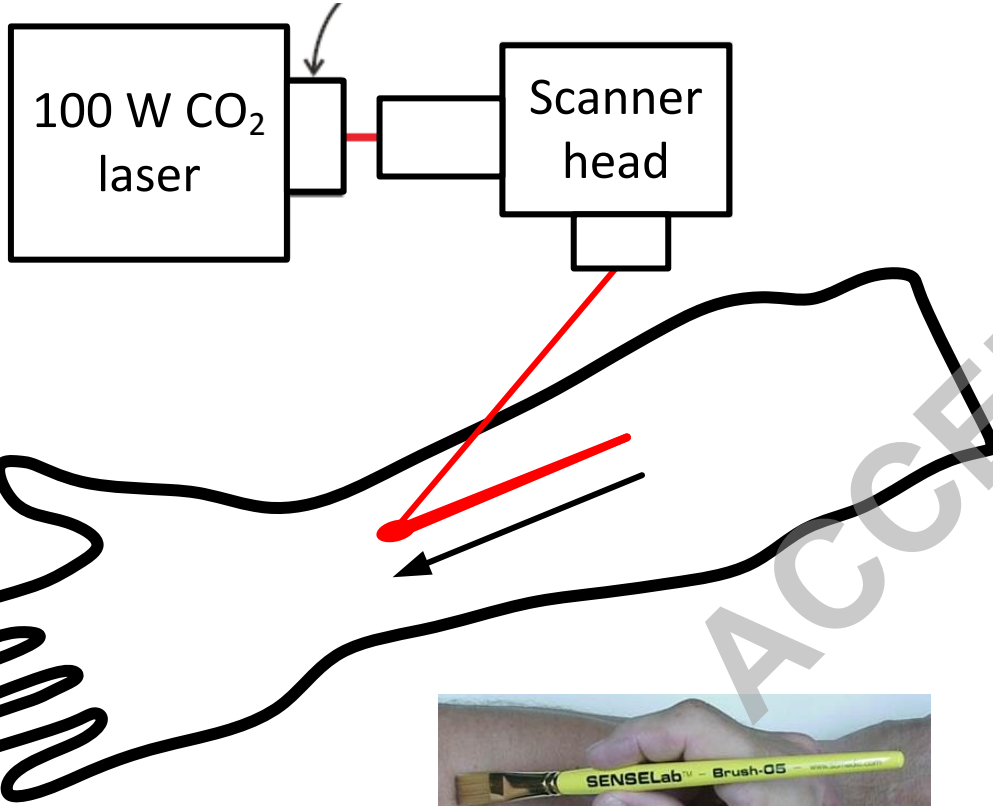
Figure 4. 2-point discrimination thresholds and NRS responses for laser (left) and mechanical (right) stimulation. Top: mean values of reported stimulation points. The line indicates a sigmoidal fitted curve and the dashed lines are the 95 % CI for the fit. Thresholds are indicated with a purple circle and were 67.9 mm for the laser stimulation and 34.5 mm for the mechanical stimulation. Middle: distance between the two points vs. the reported intensity (NRS). Bottom: perceived number of points vs. the reported intensity. Note: y-axes (NRS) have been truncated for better visualization.

Figure 5. Conceptual effect of lateral inhibition during the moving laser line stimulation. In this example, the stimulus starts in the receptive field (RF) of neuron 2 then discretely moves across the skin into the RF of neurons 3 and 4. Left: Conceptual activation with lateral inhibition. Right: Conceptual activation without lateral inhibition. The traces indicate how the stimulus creates an excitatory area (black) in the middle of the receptive field and an inhibitory area (cyan) surrounding that (left side only). The red line indicates the

overall excitation projected to the third order relay neurons. The thin horizontal black lines indicate zero excitation/inhibition. This conceptual figure has been produced by adding Gaussian curves for both the excitatory and inhibitory areas. The width of the Gaussian curves for inhibition and excitation was determined by sigma (the standard deviation). Sigma for the inhibitory curves was five times higher than for the excitatory curves. In addition the excitatory curve had twice the amplitude of the inhibitory. When the stimulus moves into a new receptive field, the influence of the previous neuron is estimated to be reduced by 50 %. However, this percentage will greatly depend on the speed of the laser beam. The dotted line indicates the responses of the previous neuron and the dashed line indicates the responses of the neuron previous to that. Notice how the excitation (red) becomes more blurry after having moved through several receptive fields. This blur will increase with increasing receptive field sizes. Comparing left to right it is evident how lateral inhibition (left) will increase the contrast in the overall activation, while without lateral inhibition (right) the overall activation becomes less contrasted and lingers over a prolonged period of time.

Line stimulation

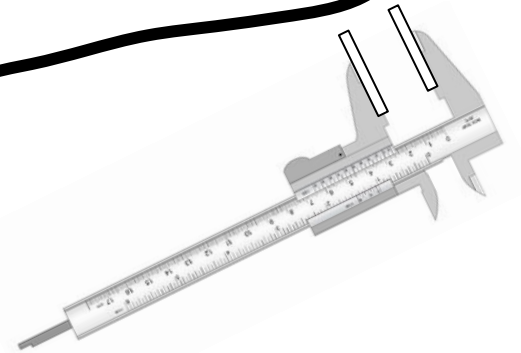
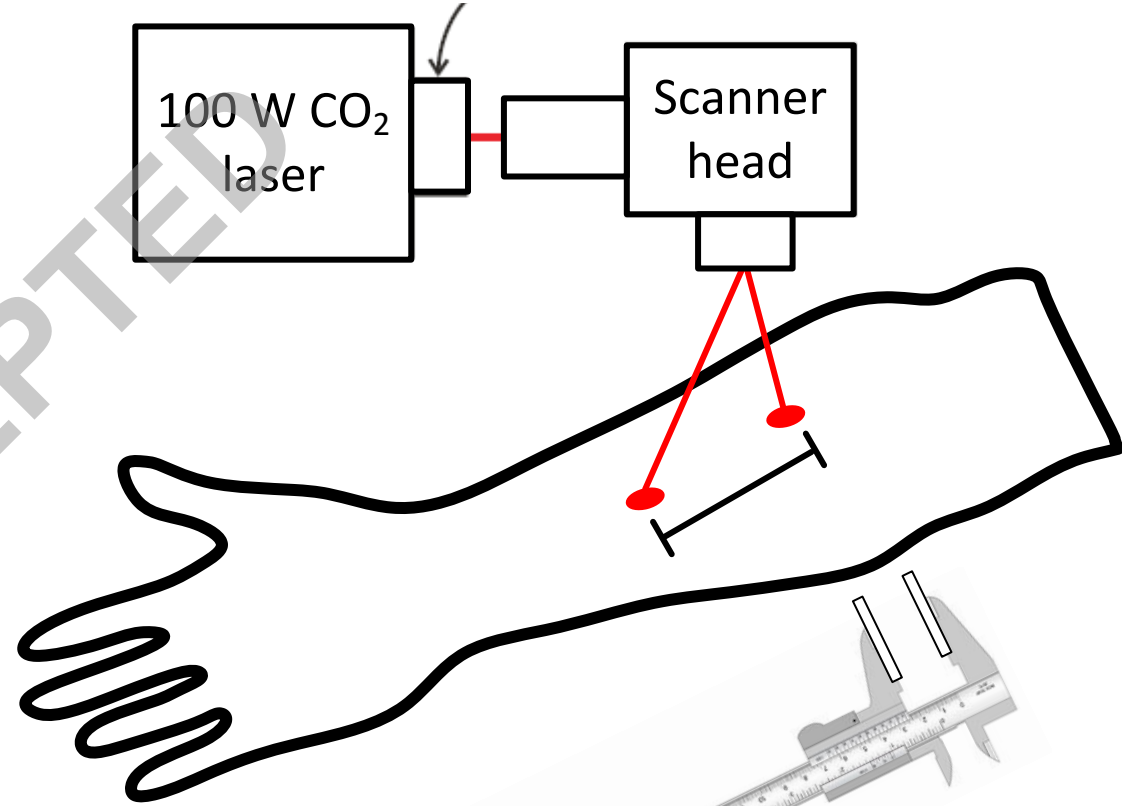
Beam expander



Mechanical stimulator

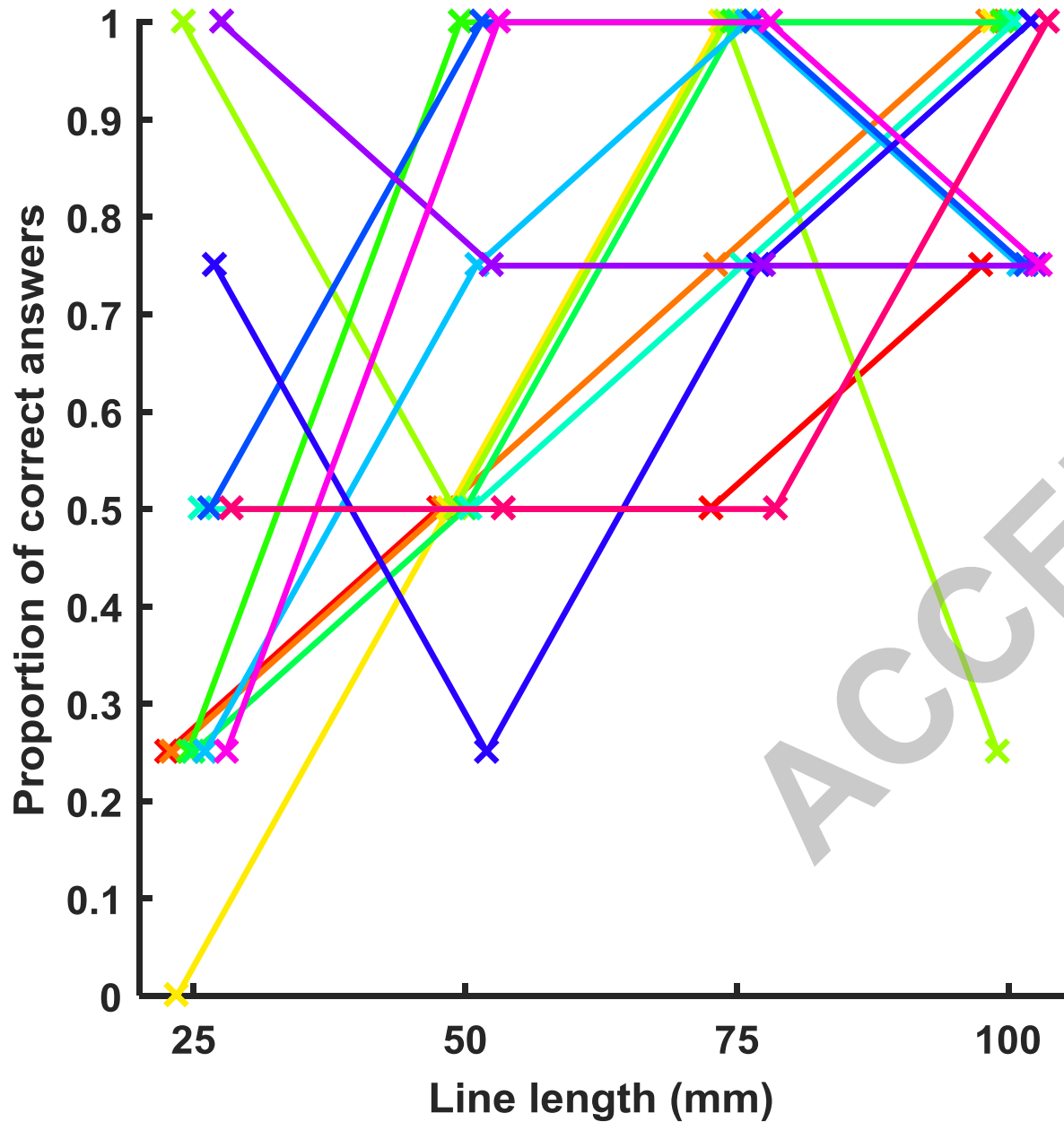
2-point stimulation

Beam expander

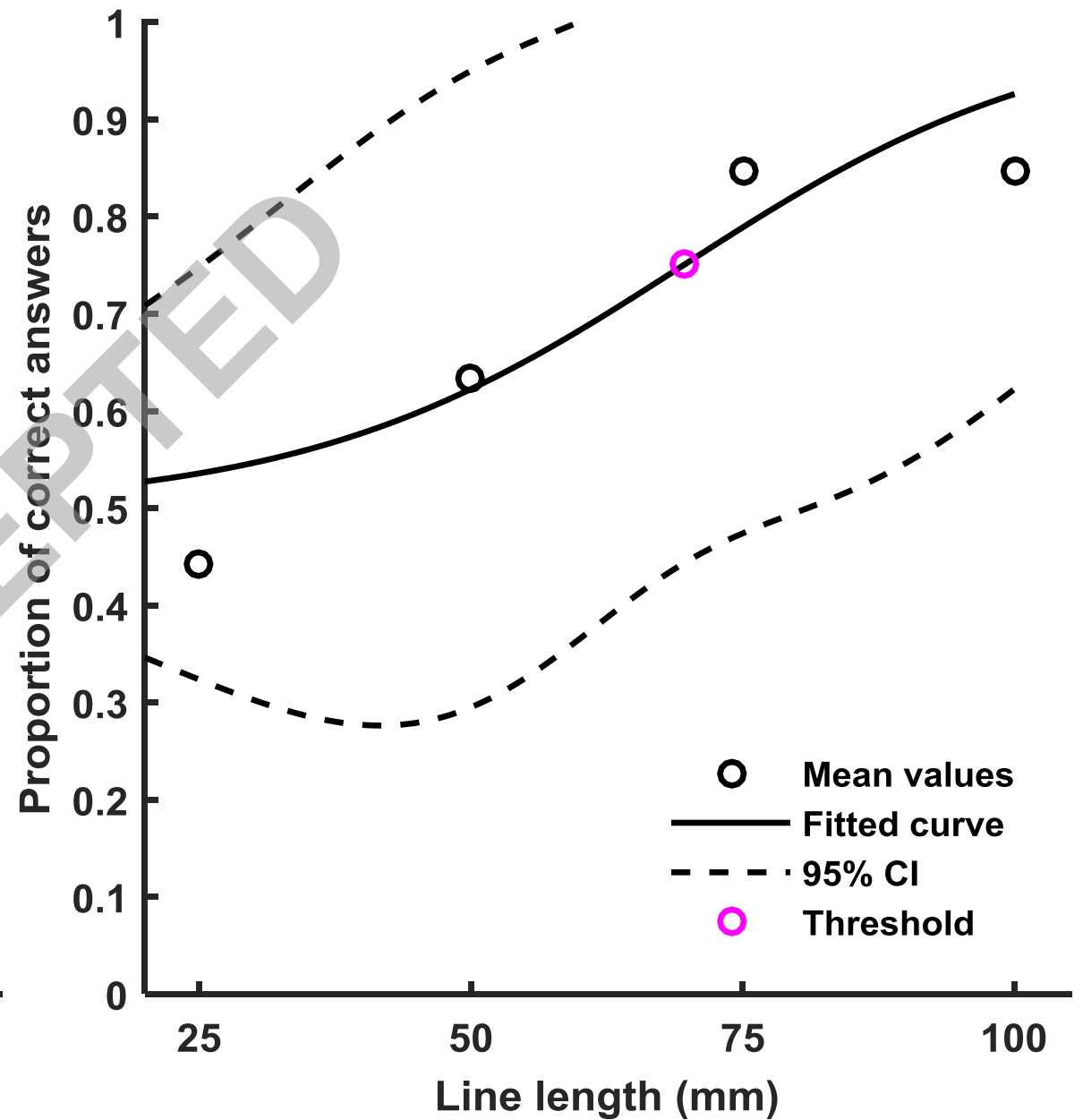


Mechanical stimulator

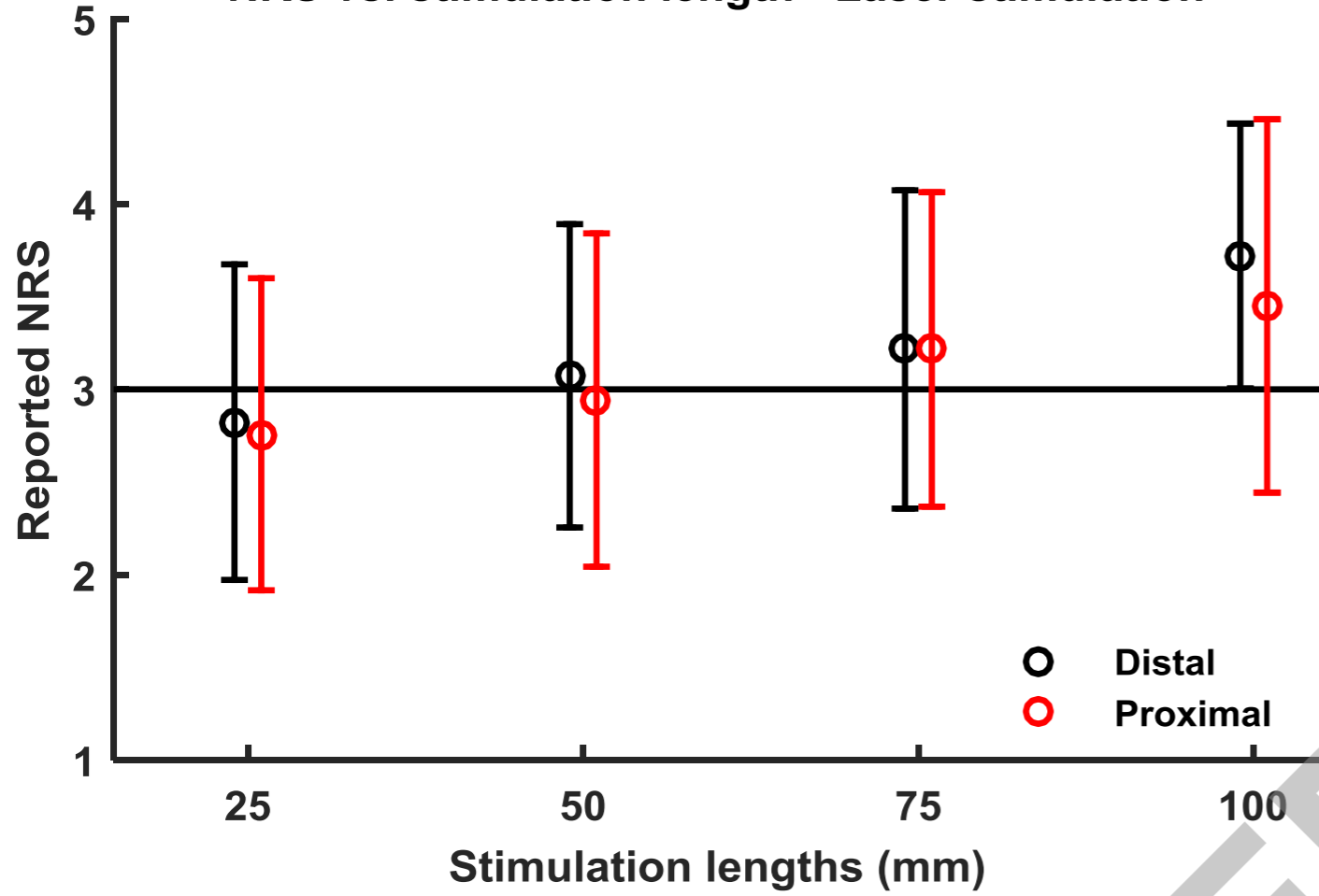
Directional discrimination per subject - Laser stimulation



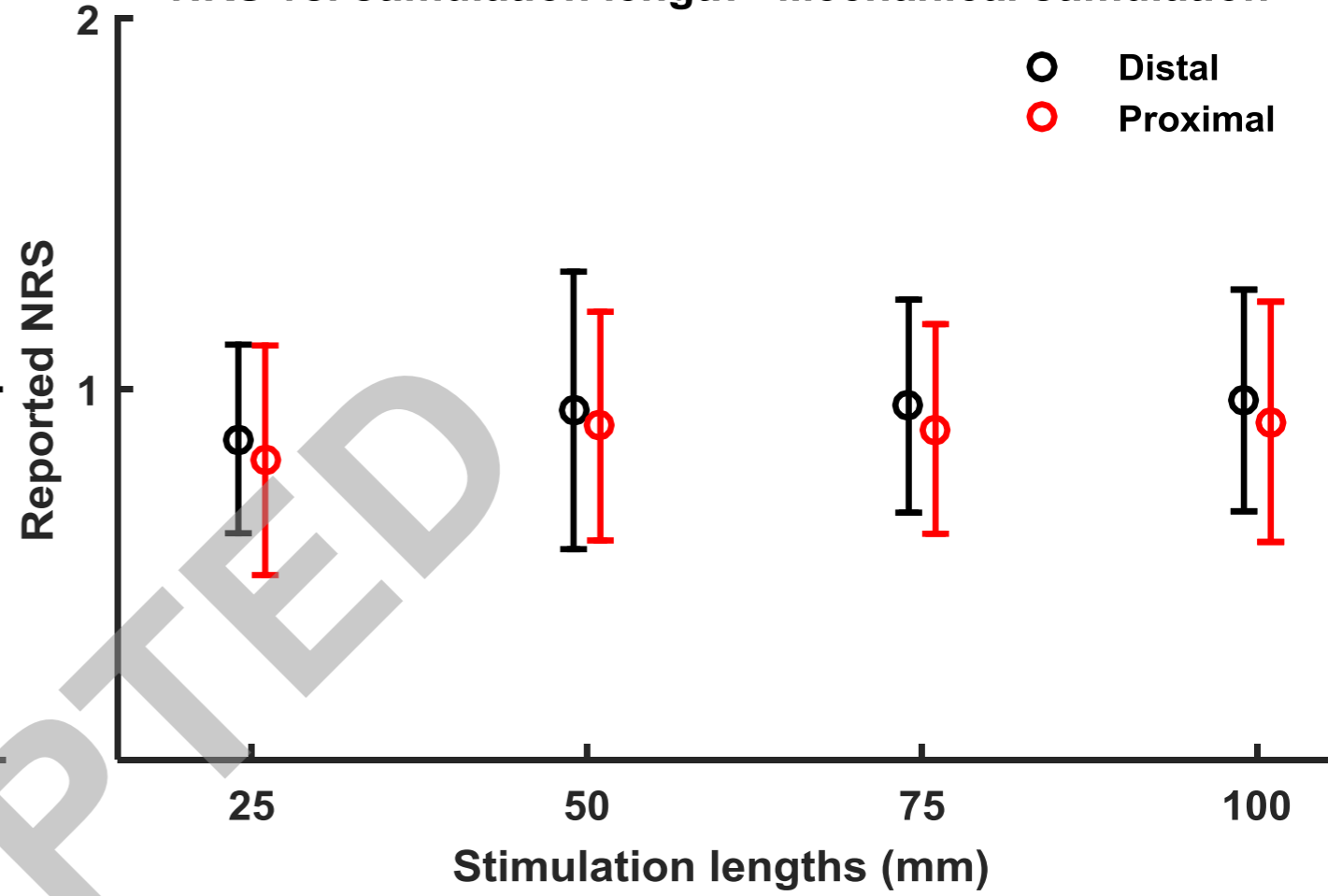
Directional discrimination - Laser stimulation



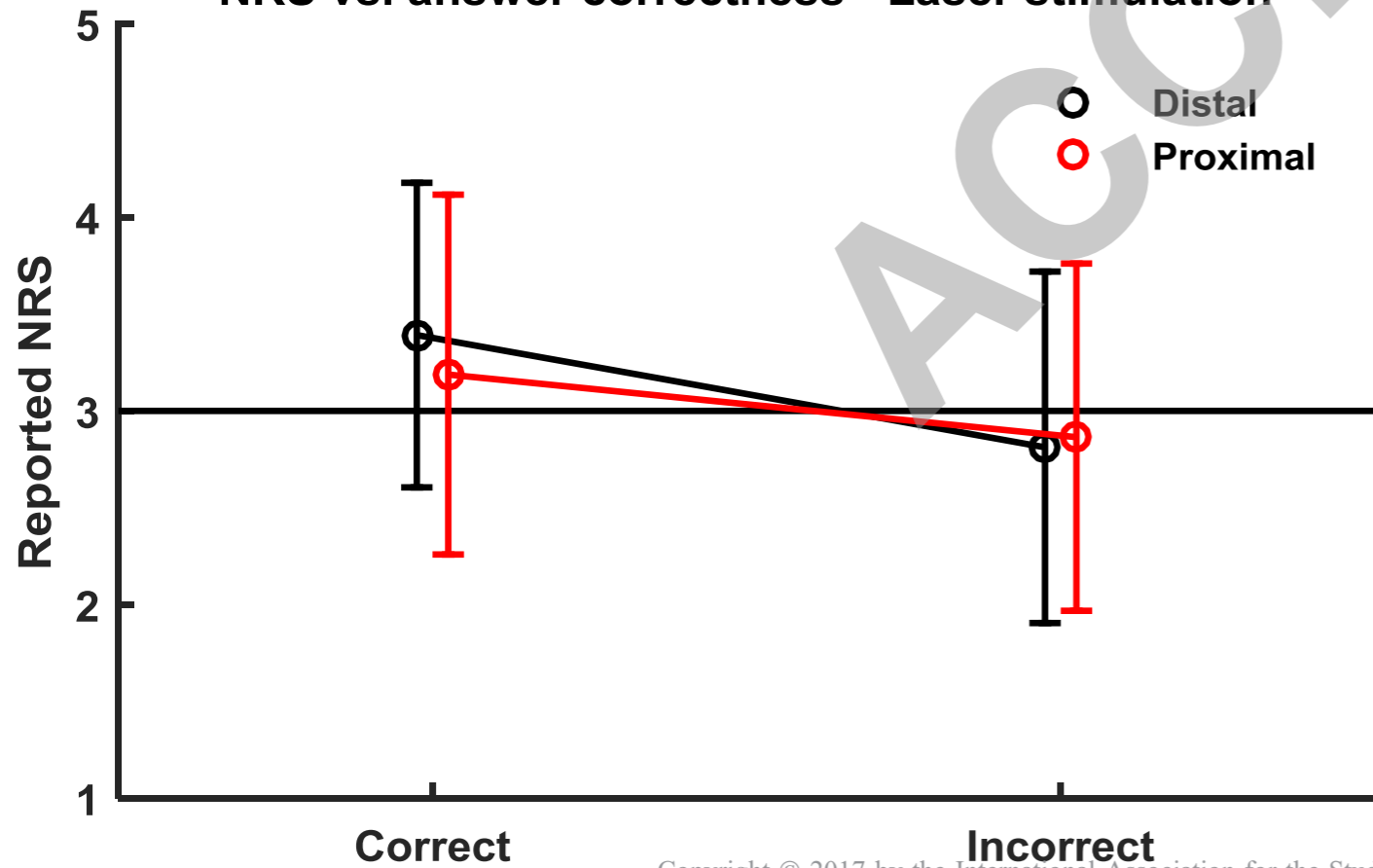
NRS vs. stimulation length - Laser stimulation



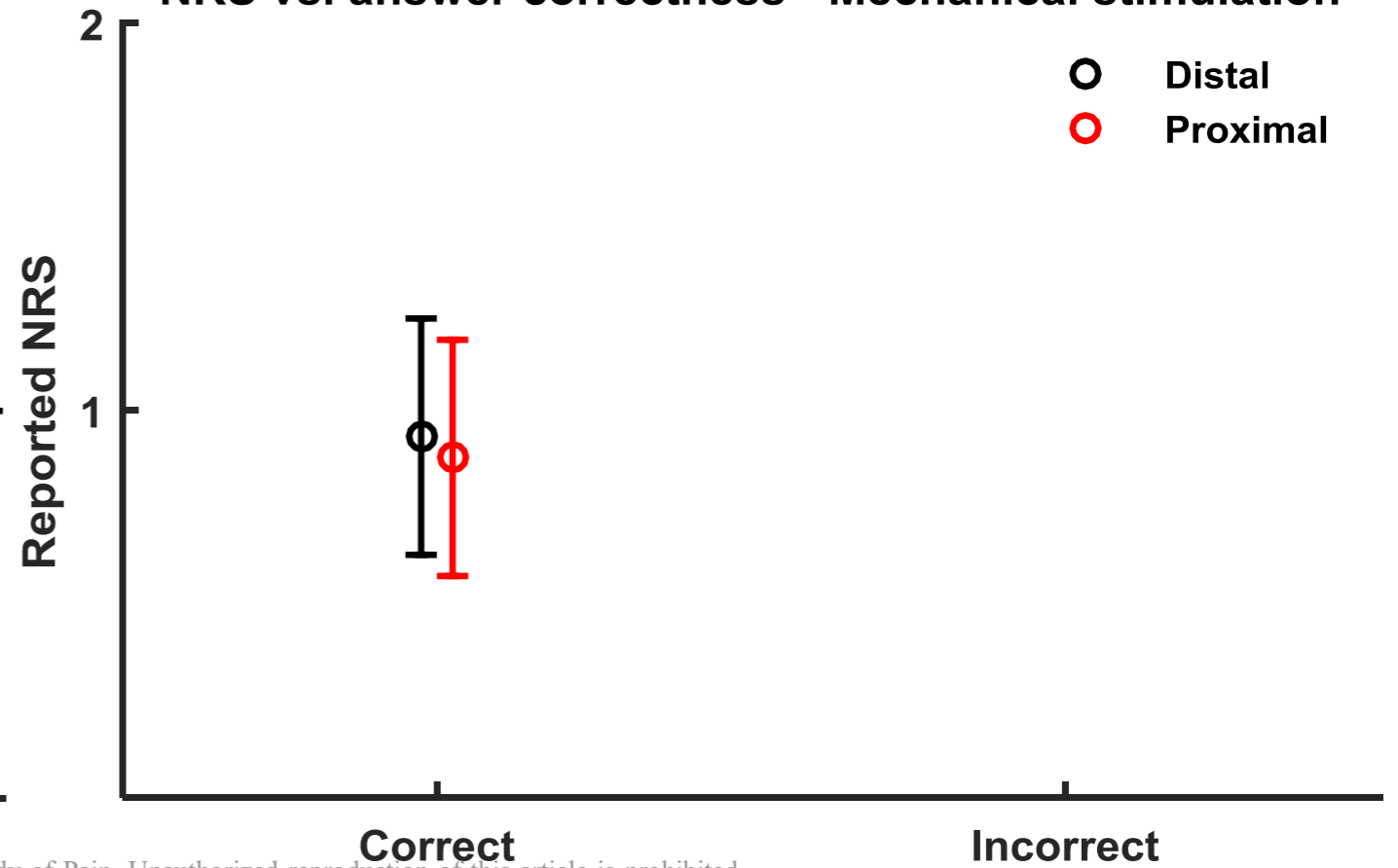
NRS vs. stimulation length - Mechanical stimulation

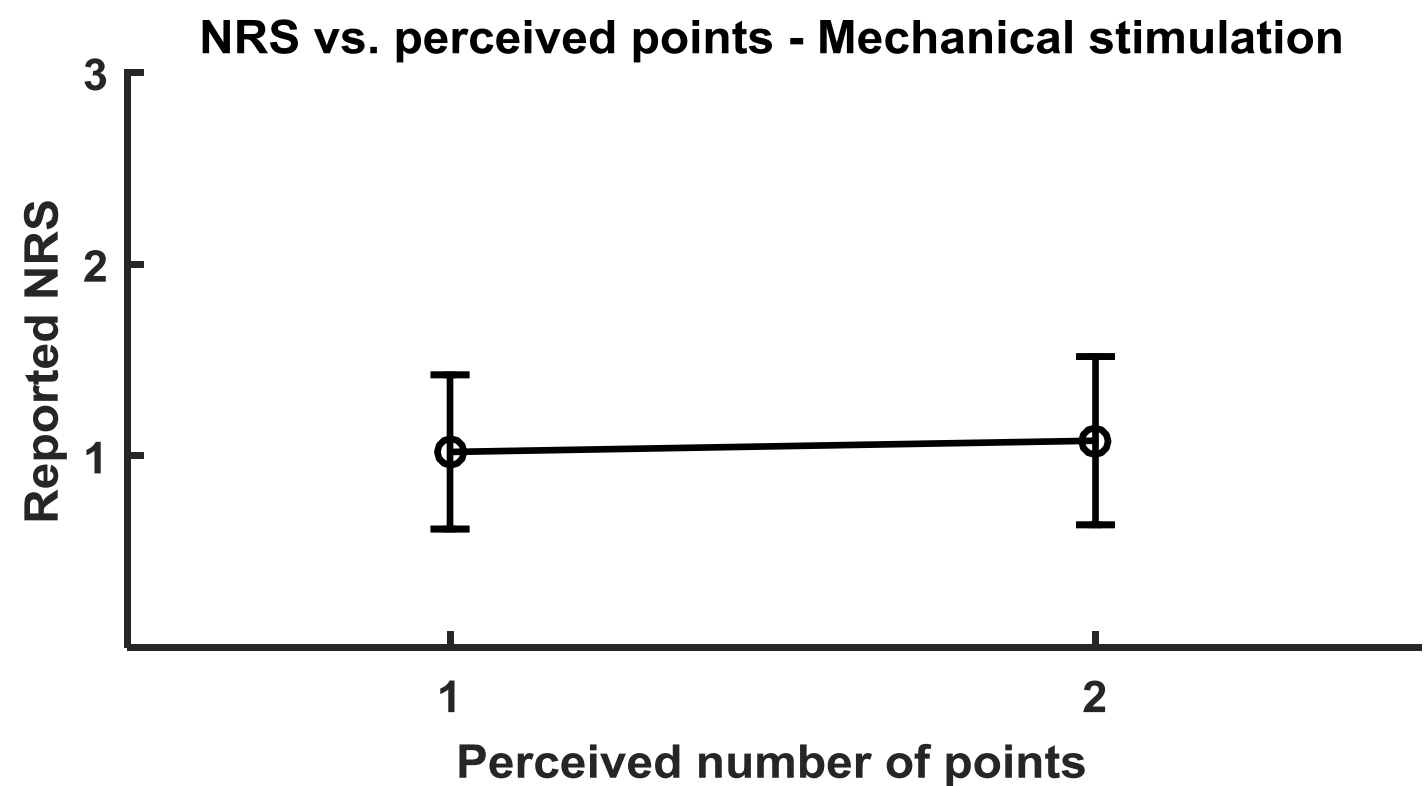
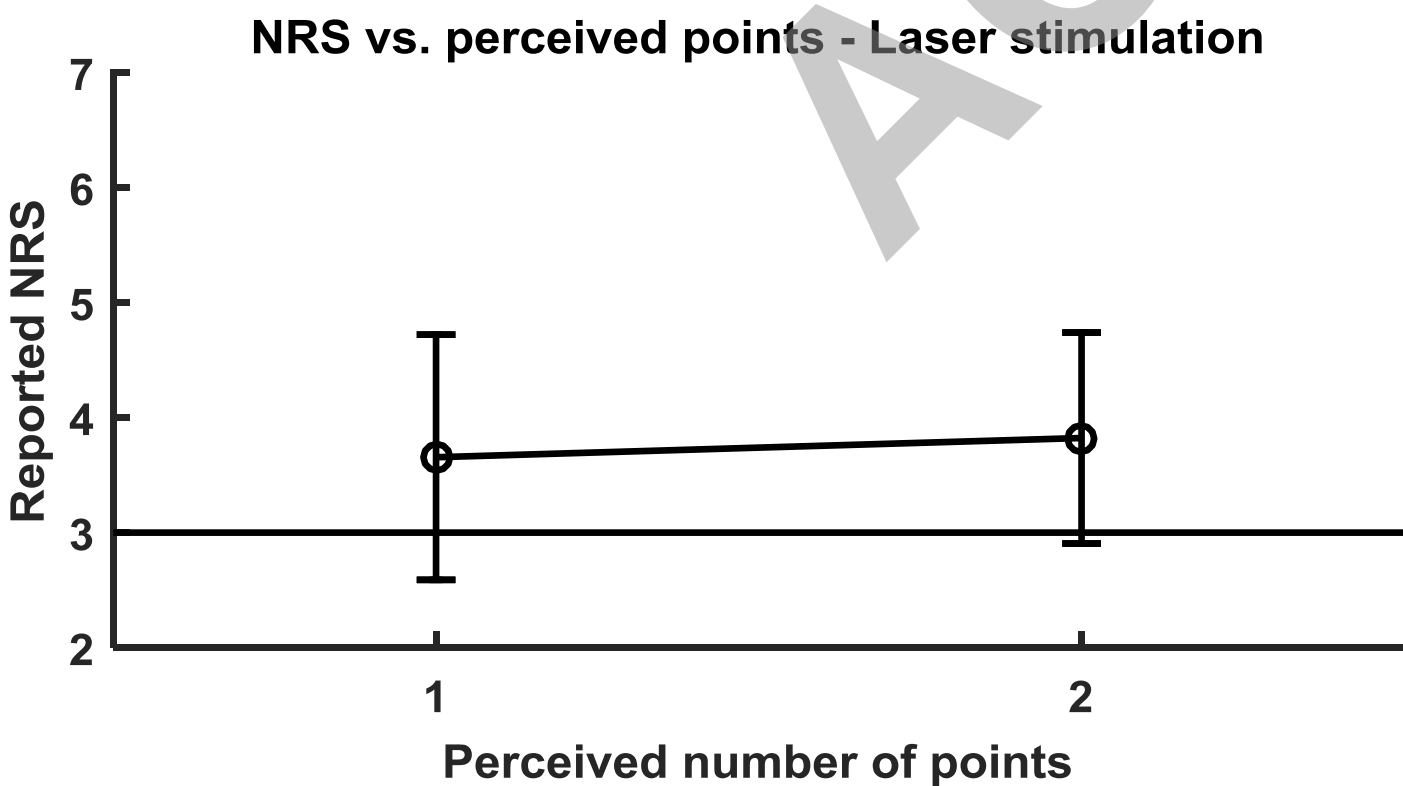
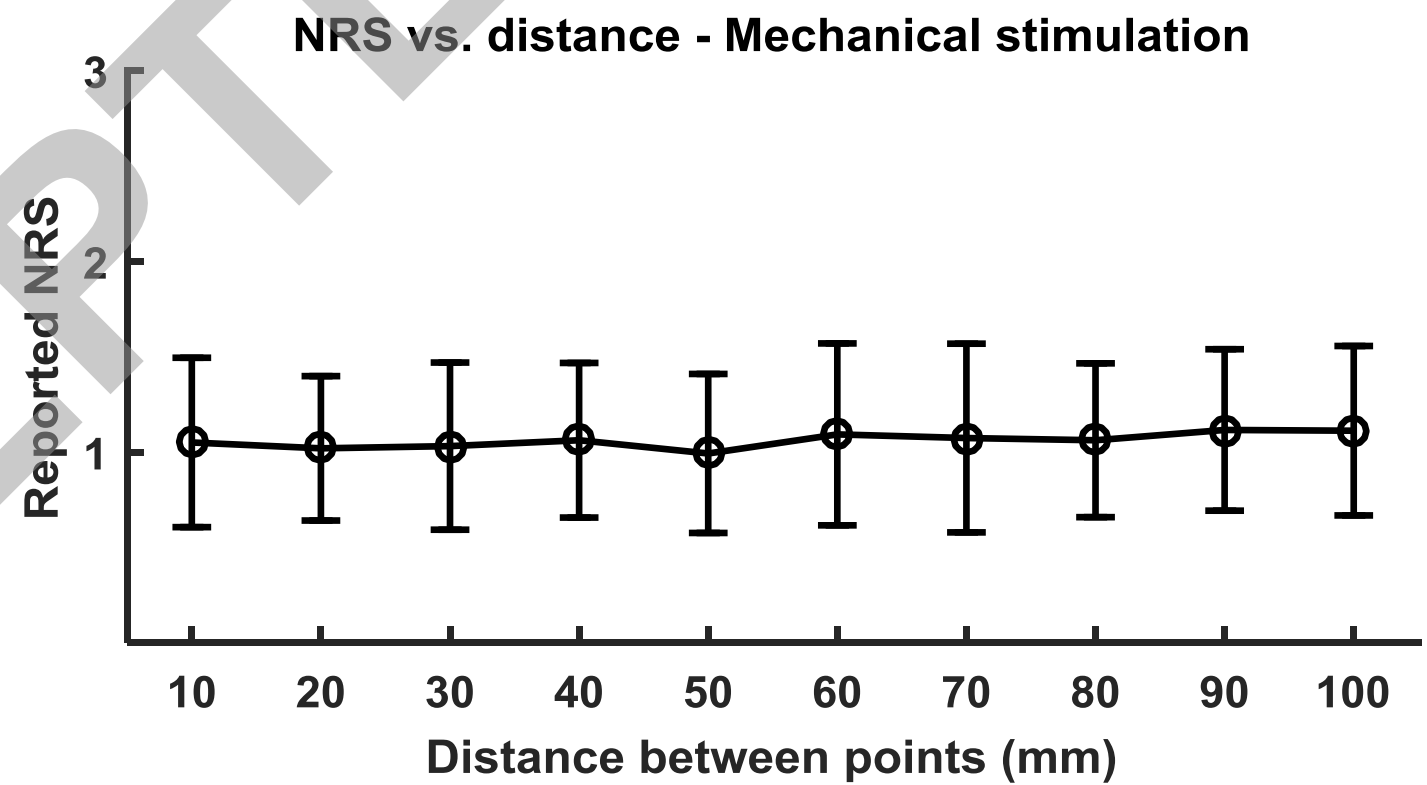
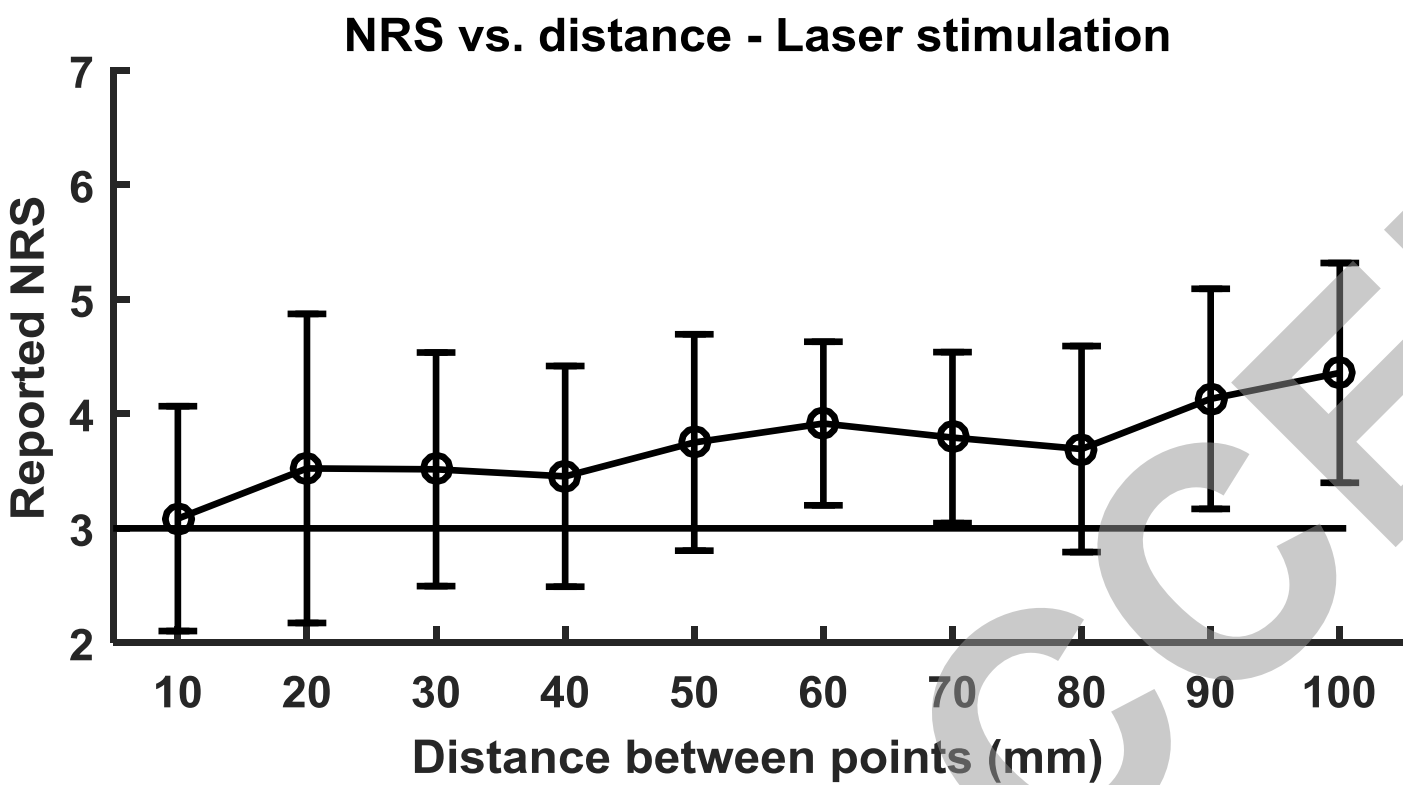
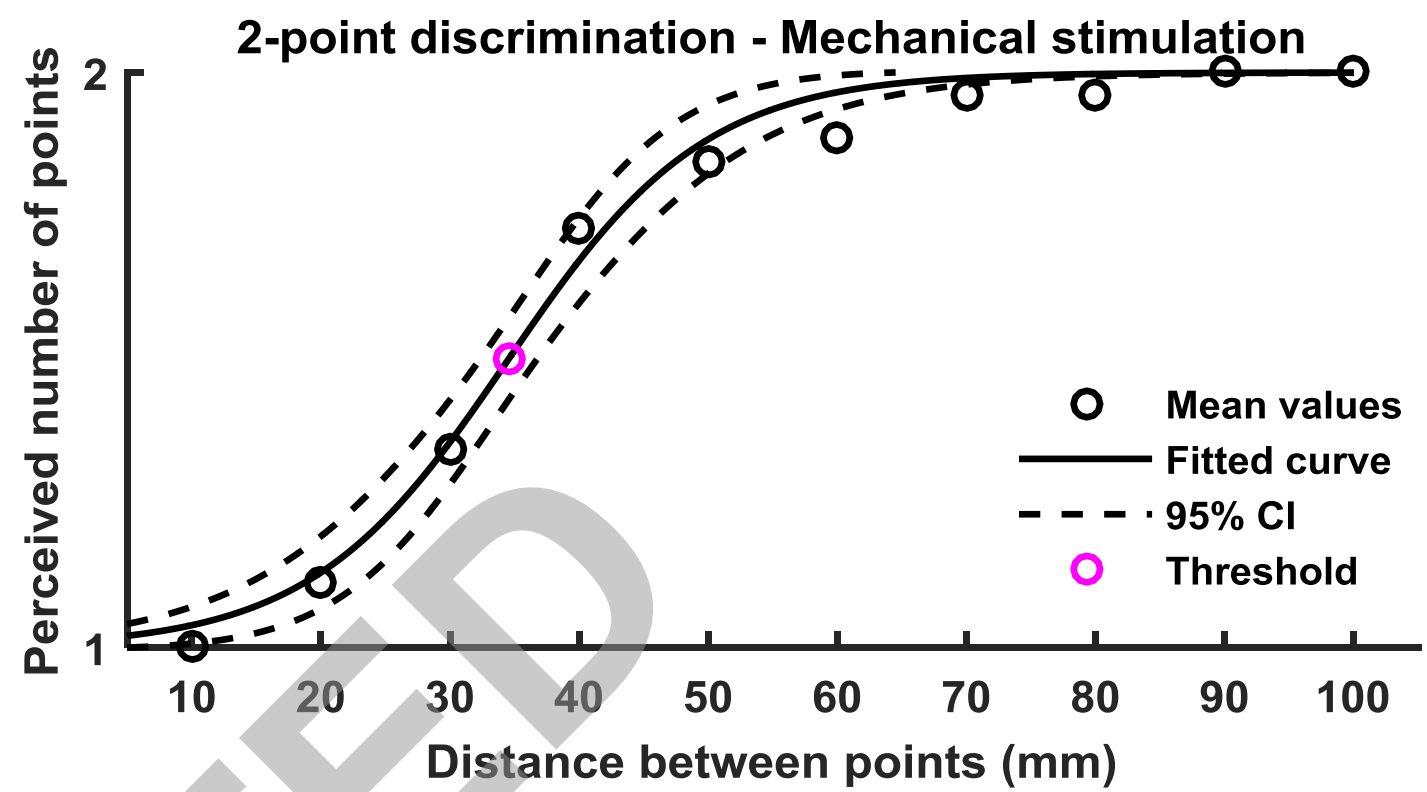
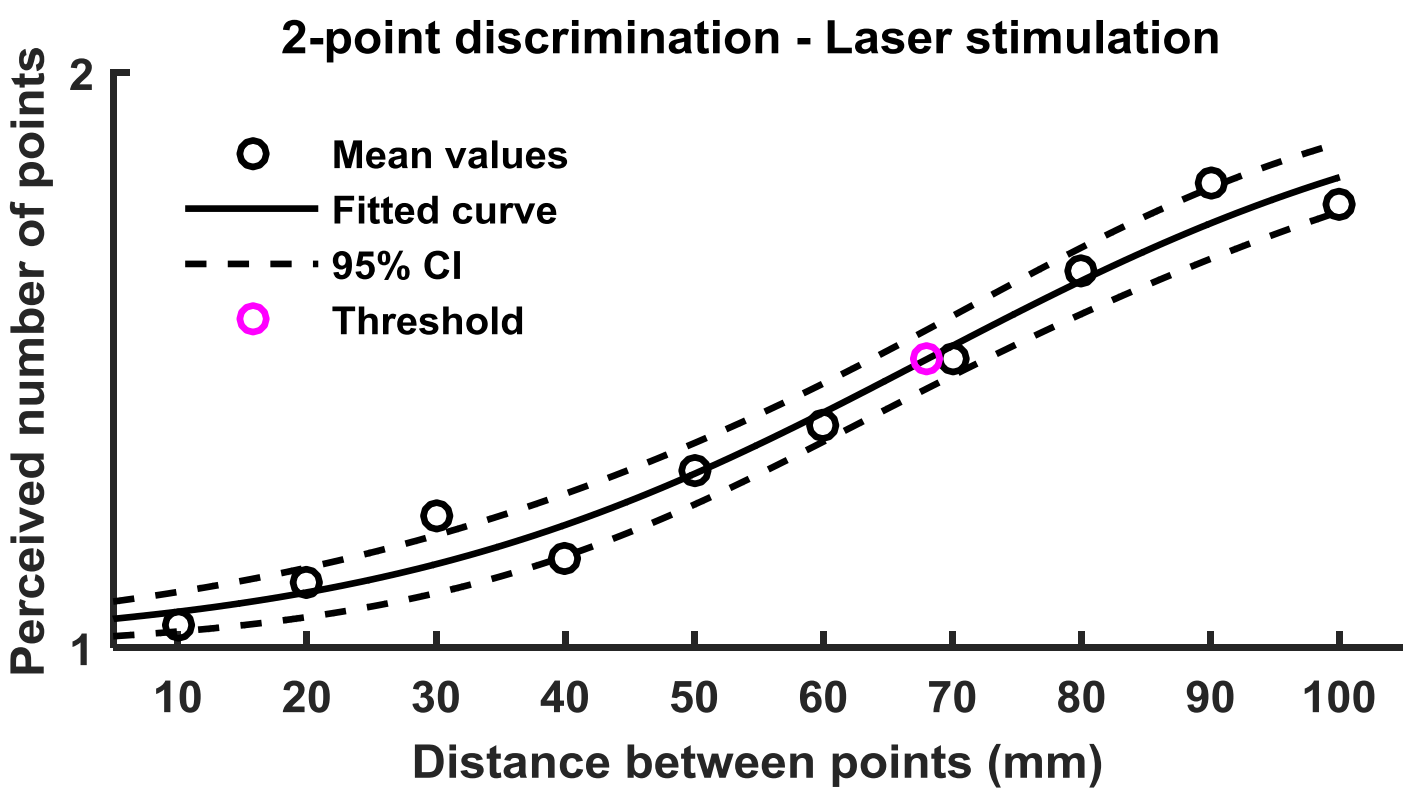


NRS vs. answer correctness - Laser stimulation



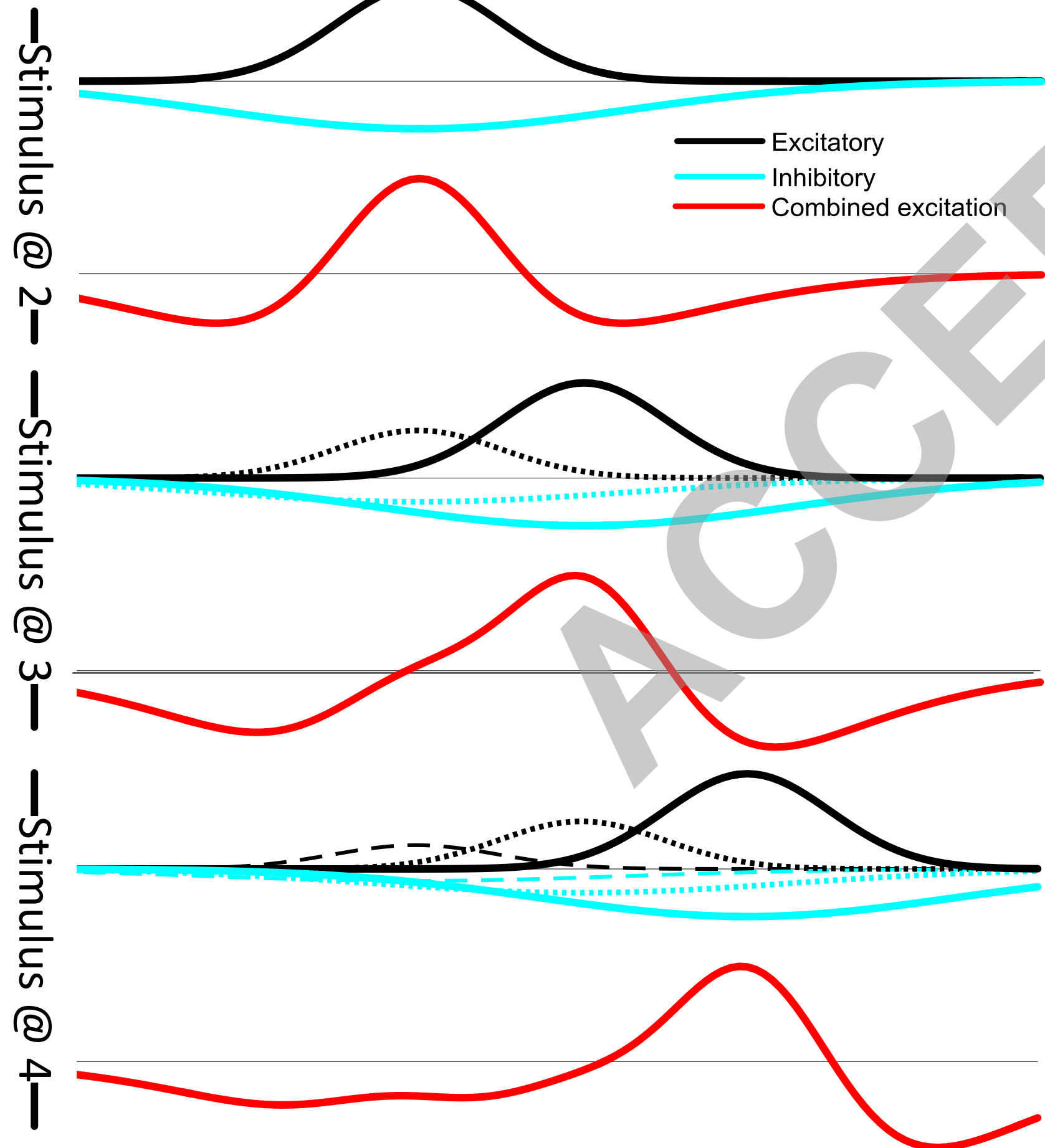
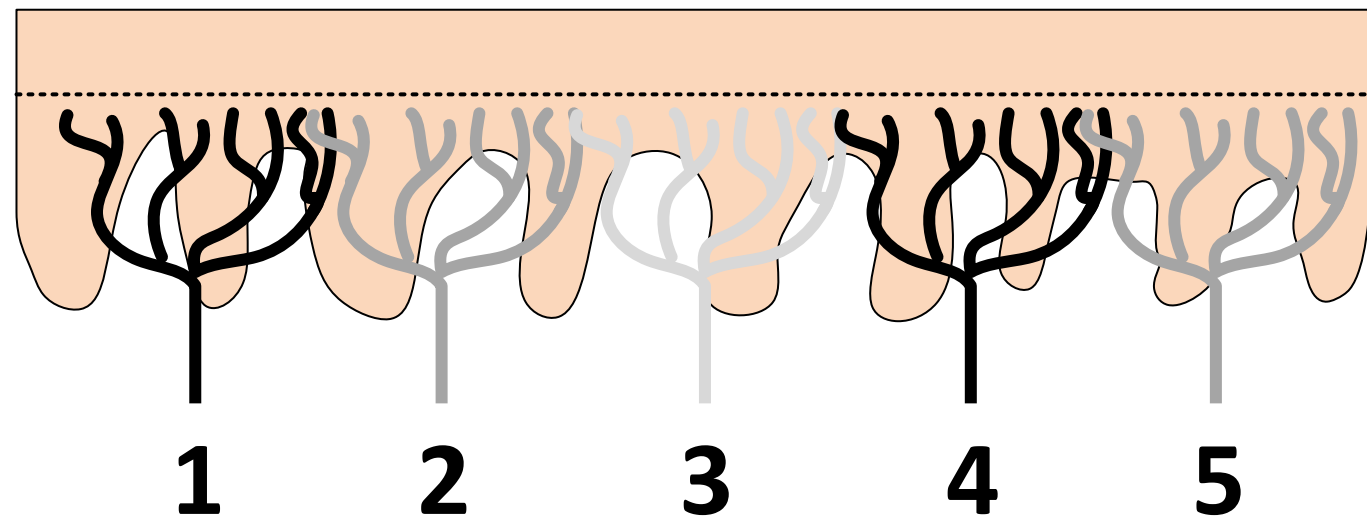
NRS vs. answer correctness - Mechanical stimulation





With lateral inhibition

Stimulus →



Without lateral inhibition

Stimulus →

