

INVESTIGATION OF TEMPORAL AND SPATIAL INTEGRATION OF PAIN INFORMATION IN THE NOCICEPTIVE SYSTEM

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**INVESTIGATION OF TEMPORAL AND SPATIAL
INTEGRATION OF PAIN INFORMATION IN
THE NOCICEPTIVE SYSTEM**

**BY
AHMAD RUJOIE**

DISSERTATION SUBMITTED 2023



AALBORG UNIVERSITY
DENMARK

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by

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AALBORG UNIVERSITY
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Dissertation submitted 2023

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CV

Ahmad received his B.Sc in Electrical Engineering (2011) from IKIU and M.Sc in Biomedical Engineering (2018) from Amirkabir University, Iran. In 2019, he enrolled as a PhD fellow at the Integrative Neuroscience group, the Center for Neuroplasticity and Pain (CNAP), Health Science and Technology Department, Faculty of Medicine, Aalborg University, Denmark. Under the supervision of Ken Steffen Frahm and co-supervision of Ole Kæseler Andersen, he investigated temporal and spatial properties in the nociceptive system using directional discrimination task in healthy individuals. His main area of research involves neuroscience and pain. During his PhD, Ahmad developed a novel laser stimulation system that allows closed-loop temperature control during movable stimuli. The results of his PhD project were presented at the IASP congress on pain (2022) as poster presentations, and published in a peer-reviewed journal.

ENGLISH SUMMARY

The nociceptive system, which is responsible for detecting and responding to noxious or potentially harmful stimuli, plays a critical role in protecting the body from harm and alerting us to potential dangers. The nociceptors are located throughout the body and are particularly innervated in the skin, muscles, and internal organs. To investigate the processing of pain information through the nociceptive system, noxious stimuli are employed to activate myelinated A δ fiber and unmyelinated C fiber nociceptors. Various types of noxious stimuli have been utilized to activate cutaneous nociceptors including mechanical, electrical, pressure, heat, cold and chemical stimuli. Most of these stimulation modalities need to be in mechanical contact with the stimulation site which leads to the activation of mechanoreceptors in addition to nociceptors. Removing the co-activation of mechanoreceptors allows for a more precise investigation of pain processing mechanisms and underlying neural pathways engaged. Over the past 4 decades, laser stimulators have gained attention in pain studies due to their capability to selectively activate cutaneous nociceptors. Lasers also provide non-contact stimuli which allow entirely thermal stimulation without coactivating mechanoreceptors. However, the difficulty of thermal laser stimulation is to make sure uniform activation of cutaneous nociceptors, which is possible by controlling the skin temperature during stimulation. Typically, the intensity of stimulation has been controlled using an open-loop approach (i.e. using fixed laser power), however, some studies implemented closed-loop control for stationary laser stimuli to provide low-temperature variations. While these stationary closed-loop control systems provide valuable information about temporal mechanisms, it is still missing the combined tempo-spatial information. To probe the combined tempo-spatial mechanisms of the nociceptive system, it is necessary to employ a stimulation system that enables displacement of the laser beam across the stimulation area. The moving stimuli allow for the investigation of tempo-spatial acuities such as directional discrimination, which have not been studied in a closed-loop system. Therefore, using a displaceable closed-loop system helps us investigate directional discrimination more accurately and how it can be improved within subjects.

The principal purpose of this PhD project was to investigate the tempo-spatial integration of pain information in the nociceptive system, using directional discrimination. To this end, a novel displaceable closed-loop, temperature-controlled laser stimulation system was developed to provide a more accurate investigation of directional discrimination. The displaceable closed-loop laser stimulation system was implemented, tested and validated (Study I). This stimulation system was then employed to investigate tempo-spatial acuity in the nociceptive system using directional discrimination (Study II). Finally, it was investigated if directional discrimination can be improved by perceptual learning (Study III).

The results of Study I showed that the closed-loop laser stimulation system could reduce the variability of perceived stimuli during a movable stimulus. Study II showed that the variability of directional discrimination decreased using closed-loop stimulation which means the obtained directional discrimination threshold is more reliable compared to open-loop control. Finally, Study III revealed that perceptual learning could improve directional discrimination, indicating that tempo-spatial acuity in the nociceptive system could be improved by learning.

Therefore, the newly developed displaceable closed-loop control system could provide a reliable tool for pain studies to deliver more uniform activation of nociceptors. This control system can be extensively employed in the nociceptive system to have a more accurate investigation of how nociceptive information can be integrated.

DANSK RESUME

Det nociceptive system, er ansvarligt for at opdage og reagere på skadelige eller potentielt skadelige stimuli, spiller en afgørende rolle i at beskytte kroppen mod skade og advare os om potentielle farer. Nociceptorerne er placeret i hele kroppen og innoverer især huden, musklerne og de indre organer. For at undersøge processeringen af smerteinformation gennem det nociceptive system, anvendes ofte smertefulde stimuli til at aktivere myeliniserede A δ -fibre og umyeliniserede C-fibre nociceptorer. Forskellige typer af stimuli er blevet brugt til at aktivere kutane nociceptorer, herunder mekaniske, elektriske, tryk, varme, kulde og kemiske stimuli. De fleste af disse stimuleringsmodaliteter skal være i kontakt med stimulationsstedet, hvilket foruden nociceptor aktivering, også fører til aktivering af mekanoreceptorer.

I løbet af de sidste 4 årtier er laserstimulatorer blevet brugt i smertestudier på grund af deres evne til selektivt at aktivere kutane nociceptorer. Lasere giver tilmed berøringfri stimuli, som tillader termisk stimulering uden aktivering af mekanoreceptorer. Laserstimulering er dog udfordret ift. at sikre ensartet aktivering af kutane nociceptorer, hvilket kræver hudtemperaturen under stimulering kontrolleres. Typisk er intensiteten af stimuli blevet kontrolleret ved hjælp af en såkaldt åben sløjfetilgang (hvori laser effekten er fastholdt under stimulation), dog har nogle studier implementeret en lukket sløjfekontrol for stationære laserstimuli for at reducere temperatur variationer under stimulation, hvori laser effekten justeres for at opretholde den ønskede temperatur. Selvom disse stationære lukkede sløjfesystemer kan give værdifuld information om smertemekanismer, er det stadig uklart, hvordan temporal og spatial smerteinformation bliver integreret i det nociceptive system. For at undersøge de kombinerede temporal og spatielle mekanismer i det nociceptive system, er det nødvendigt at anvende et stimulationssystem, der muliggør forskydning af laserstrålen hen over stimuleringsområdet. Forskydning af stimuli gør det muligt at undersøge temporal og spatielle smertemekanismer såsom retningsdiskrimination, som ikke er blevet undersøgt vha. et lukket sløjfesystem. Det forventes at brugen af et lukket sløjfesystem, der tillader bevægelige stimuli, hjælper os med at undersøge retningsdiskrimination mere præcist samt hvordan den kan forbedres.

Hovedformålet med dette ph.d.-projekt var at undersøge den kombinerede tempo-spatiale integration af smerteinformation i det nociceptive system ved hjælp af retningsdiskrimination. Til dette formål blev et nyt temperaturstyret laserstimuleringssystem, med mulig for at flytte stimulationsstedet hen over huden, udviklet for at give en mere nøjagtig undersøgelse af retningsdiskrimination. Dette laserstimuleringssystem blev implementeret, testet og valideret (Studie I). Derefter blev dette system anvendt til at undersøge den tempo-spatial diskrimination i det nociceptive system (Studie II). Endelig blev det undersøgt, om retningsdiskriminations evnen kan forbedres ved perceptuel læring (Studie III).

Resultaterne af Studie I viste, at laserstimuleringssystemet med lukket sløjfe var i stand til at reducere variabiliteten af stimulationstemperaturen og den oplevede stimulation under en bevægelig stimulus. Studie II viste, at variabiliteten af retningsdiskrimination faldt ved brug af lukket sløjfe stimulering. Slutteligt viste Studie III, at perceptuel læring kunne forbedre retningsdiskrimination, hvilket indikerer, at den tempo-spatiale integration i det nociceptive system kunne forbedres ved læring.

Det nyudviklede temperatur-kontrollerede system kan give smertestudier et pålideligt værktøj til at levere en mere ensartet aktivering af nociceptorer. Dette kontrolsystem kan i vid udstrækning anvendes i det nociceptive system for at få en mere præcis undersøgelse af hvordan nociceptive informationer integreres i det sensoriske system.

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CHAPTER 1. INTRODUCTION

1.1. PAIN AND THE NOCICEPTIVE SYSTEM

Pain is a complex experience that plays a crucial role in the survival and well-being of an individual [1,2]. It serves as a protective mechanism, alerting the body to potential harm and guiding appropriate responses to prevent potential tissue damage [3]. The perception of pain is a process that involves complicated interactions between sensory, cognitive, and emotional factors [4,5]. Understanding the mechanisms underlying pain perception is crucial not only for elucidating fundamental aspects of human physiology but also for developing effective strategies to manage pain-related conditions [4,6].

Pain can be categorized based on various criteria, including its duration, origin, and underlying mechanisms [3]. Acute pain, typically resulting from tissue damage or injury, serves as a warning signal that prompts individuals to take immediate actions to avoid further harm [7,8]. In contrast, chronic pain persists beyond the expected healing time that lasts more than 3 months [9,10], and is caused by a variety of factors, including medical conditions, injuries, and nerve damage [3]. More than 25% of people suffer from chronic pain world-wide [11,12] which means that it poses a serious threat to the well-being of people. Diagnoses and treatments of chronic pain face challenges due to a limited understanding of how the nervous system process nociceptive information [13]. While different therapies, such as cognitive behavioral therapy (CBT) [14–16] or physical therapy [17–19] are used for minimizing the destructive effects of chronic pain, they still require more knowledge about the underlying mechanism of how pain is perceived.

The nociceptive system is responsible for transducing pain information to the central nervous system (CNS) [3]. Periphery nociceptors respond to external noxious stimuli and convey them into action potentials. The CNS uses these action potentials to process and perceive pain information [20]. When investigating the processing of neural information through the nociceptive system, researchers utilize controlled noxious stimuli to activate these nociceptors [21]. Investigation of the nociceptive system helps to better understand chronic pain and the mechanisms related to it [3].

1.2. TEMPORAL AND SPATIAL PROCESSING IN THE NOCICEPTIVE SYSTEM

The information being processed in the nociceptive system includes multiple characteristics. Two important characteristics of perceived and integrated information are temporal and spatial characteristics [22–26] which provide insights into the neural mechanisms underlying the perception of pain [27,28] and can be used to evaluate the

effectiveness of pain therapies [29,30]. Moreover, information about the location and duration of nociceptive stimuli is important for organizing functional defensive or orienting responses such as withdrawal reflex [31–33]. To investigate temporal and spatial properties, several studies focused on perceived intensity. The studies on the temporal properties of the nociceptive system [31,34–37] show that with more repetition of the stimuli, the perceived intensity is higher due to the temporal summation of pain (TSP). Other studies which focused on spatial properties of the nociceptive system [38–41] found that increasing the stimulation size causes more intense perceived stimuli due to spatial summation of pain (SSP).

Another way to investigate spatial properties is testing the spatial acuity in the nociceptive system. The ability to accurately distinguish between two closely spaced noxious stimuli applied to the skin is referred to as spatial discrimination [42]. Spatial discrimination can be measured via different psychophysical tests, such as point localization and two-point discrimination [41,43–45]. Point localization is a measure used in pain studies to assess the accuracy of localizing a noxious stimulus applied to the body. This is typically done using a pinprick (or other sharp objects) and noxious heat to apply a painful stimulus to a specific location on the skin, and the subject is asked to indicate the exact stimulation location [23]. Another metric to determine spatial discrimination is two-point discrimination. In the two-point discrimination test, two points are applied to the skin surface with different distances between them, and the subject is asked to report whether they feel one or two points. The distance at which the subject can reliably distinguish between one or two points is taken as a measure of spatial discrimination [46].

Spatial discrimination is an important measure in pain studies, as it can be impaired in conditions such as chronic pain or nerve damage [47–49]. It provides insight into the neural mechanisms underlying the perception of pain and can be used to evaluate the effectiveness of pain treatments [41,43,46]. However, spatial discrimination lacks the temporal information which is integrated in the nociceptive system, and that calls for a more complete metric that includes both temporal and spatial information.

1.3. DIRECTIONAL DISCRIMINATION

As mentioned in Section 1.2, the integration of combined tempo-spatial properties in the nociceptive system has not been extensively studied. This is important because a complex representation of pain includes the combined tempo-spatial information. In order to investigate the combined tempo-spatial properties in the nociceptive system, a displaceable stimulus can be applied [50]. For this purpose, some psychometric tasks have been proposed. One task is known as graphesthesia which includes identifying numbers drawn on the skin [51]. Using the graphesthesia task, it was shown that discrimination precision in the nociceptive system is poorer than in the tactile system [51]. However, the graphesthesia task cannot quantify the tempo-spatial acuity in the nociceptive system, meaning that it cannot determine a discrimination threshold.

Another psychometric task is directional discrimination which is simpler than graphesthesia. Directional discrimination is the ability to discriminate the orientation of a moveable stimulus [50]. Directional discrimination employs the combined tempo-spatial information and can quantify spatial acuity in the nociceptive system [50,52,53]. Since directional discrimination investigates both spatial and temporal information simultaneously, it can be viewed as the integration of several nociceptive information compared to measures that solely rely on spatial (e.g. two-point discrimination and point localization) or temporal information (e.g. repetitive stationary stimuli). Therefore, directional discrimination could be a complex measure of pain that improves our understanding of underlying pain mechanisms.

Previous studies mainly investigated directional discrimination in two directions (distal and proximal) [50,52,53]. When there are just two directions, some limitations arise to this approach, both from psychometrical and psychological points of view. From the psychometrical point of view, the probability of guessing correctly in two directions is 50% by chance. Therefore, it will be hard to determine whether the subjects perform the task correctly or by chance alone. From the physiological point of view, different spatial resolutions exist in orthogonal orientations due to the shape of the receptive fields (RF) [41,54] and the engagement of dermatomes [20] in the stimulated area. Some studies have shown that spatial acuity along the lateral-medial direction is better (i.e. more accurate) than along the distal-proximal direction on the volar forearm [54] or on the back of the hand [41]. Therefore, using more than two directions can be beneficial to probe directional discrimination in a more precise and comprehensive way.

1.4. PERCEPTUAL LEARNING

An impairment of temporal properties of pain perception has been showed in chronic pain patients [55]. Spatial acuity is similarly impaired both during chronic pain and experimental pain [24,56]. Additionally, it has been shown that capsaicin impairs directional discrimination [52]. Therefore, it is interesting to see if the tempo-spatial mechanisms could be reversed, i.e. improved. The potential for enhancing directional discrimination has not been explored within the nociceptive system. Enhancing directional discrimination can improve the tempo-spatial acuity in the nociceptive system. Researchers can also gain additional knowledge about the underlying mechanisms of pain by investigating the ways to manipulate directional discrimination. Such manipulations could represent the neuroplasticity of the nociceptive system. By modulating the directional discrimination, this neuroplastic mechanism can help chronic pain patients [21–23]

One way to improve directional discrimination could be via perceptual learning. Perceptual learning refers to improvement of the sensory perception through repetition, experience, and practice [57]. It is referred to as perceptual because it involves changes in sensory information processing by the CNS [58]. During

perceptual learning, feedback is provided to inform the person about their performance which can help them adjust and learn [59].

Perceptual learning has been studied in tasks such as discrimination of orientation [60–63], tactile [64–66], thermosensory [58], auditory [67–69], and visual [70–72]. According to our knowledge, there is only one study that investigated perceptual learning in the nociceptive system [59]. The study showed that perceptual learning could improve the discrimination of the intensity and spatial location of nociceptive stimuli [59]. However, perceptual learning has not been studied for directional discrimination.

1.5. LASER STIMULATION

Various methods of stimulation have been employed to activate cutaneous nociceptors, with the most frequently utilized modalities being mechanical (using pin prick or punctate stimuli), noxious heat and cold stimuli (delivered by a thermode) [21]. These stimulation modalities are in contact with the skin, therefore they coactivate mechanoreceptors as well [21]. To avoid this, researchers have used laser stimulators in pain studies [73,74]. Lasers allow non-contact cutaneous stimulation which is therefore purely thermal stimulation without the coactivation of mechanoreceptors [75,76]. This type of stimulation selectively activates unmyelinated C and myelinated A δ fiber nociceptors [77] and also non-nociceptive C fiber warmth afferents. Laser stimulation has also been shown to improve the delivered spatial resolution of a stimulus [78].

There are several types of laser stimulators that have been used in pain studies including argon ion [79,80], copper vapor [75,77], yttrium-aluminum-garnet (YAG) [76,81], and diode [82,83]. However, the most frequently used laser is the carbon dioxide (CO₂) laser [77,84–86].

The penetration depth of a laser stimulator is dependent on wavelength [87]. The CO₂ laser beam is superficially absorbed up to 50 μ m from the skin surface [88] (wavelength: 10600 nm [75]). As the nociceptors are not entirely located at the surface of the skin [3], low-penetrating lasers such as CO₂ lasers rely on passive conduction to transmit the thermal energy to the nociceptors. This reliance on passive conduction can result in overheating of the superficial skin layers. In contrast, the diode laser beam penetrates deeper from 2 mm up to 2.5 mm into the human skin [89] (wavelength: 800–980 nm [90]). Therefore, diode lasers which allow for more penetration depth, directly heat the nociceptors and do not require passive conduction to activate the receptors, which avoid overheating the superficial layers [91].

One of the challenges of using laser stimulation is the uniform receptor activation to investigate the nerve system more accurately. This is done through the assessment of skin temperature and adjusting the laser power. The skin temperature is measured

because of two reasons: first, to ensure preventing tissue damage by overheating the skin, and second, to adjust the laser power to selectively activate the thermal receptors. Skin temperature can be monitored by a radiometer [74,92], an infrared (IR) thermometer [93] or an IR camera [21,51]. When the skin temperature is evaluated, the stimulation intensity can be adjusted. This has been mainly done in an open-loop fashion, in which laser stimulation has constant power during the stimulation [51,94,95]. While easy to implement, it has a major flaw that is not adaptive to the skin temperature during stimulation. To overcome this shortcoming, closed-loop control systems have been used [74,96–99] to get feedback to adjust the laser power dynamically. Using this strategy, the skin temperature remains closer to the target temperature during the stimulation with less variation.

While a closed-loop system has been used in studies with stationary stimulation [92,100–103], there is no study with closed-loop movable stimuli (however, there are studies with open-loop movable stimuli [50–53,104]). Using a movable diode laser stimulation, which implements a closed-loop control system, allows for more precise delivery of stimulations, and hence, more accurate investigation of directional discrimination.

1.6. AIMS AND HYPOTHESES

The main goal of this PhD project was to investigate the combined tempo-spatial integration of pain information in the nociceptive system which was done using the directional discrimination task. The neuroplasticity of the nociceptive system was also investigated in the nociceptive system using perceptual learning. To this end, three studies were designed and implemented.

1.6.1. STUDY I

The aim of Study I was to implement and test a new diode laser stimulation system that allows a movable cutaneous stimulation with a closed-loop temperature control system. The hypothesis of this study was that using a closed-loop control system leads to more precise skin stimulation compared to the open-loop control. To test the system, the variability of the skin temperature during moveable stimuli was monitored and compared to the desired temperature.

1.6.2. STUDY II

The aim of Study II was to investigate directional discrimination in healthy subjects by using the temperature-controlled stimulation system developed in Study I. The hypothesis was that using a closed-loop control system can provide less variable (i.e. more accurate), and thus better investigation of combined temporal and spatial integration of the nociceptive information compared to the open-loop system. To this end, the directional discrimination task was used in four different directions (distal,

proximal, lateral, medial) and different displacement lengths. It was hypothesized that directional discrimination is more accurate in the lateral-medial directions compared to the distal-proximal directions.

1.6.3. STUDY III

The aim of Study III was to investigate if perceptual learning in the nociceptive system can improve directional discrimination. In this way, the neuroplasticity of the nociceptive system can be explored. For this purpose, test subjects were asked to perform the directional discrimination task before and after perceptual learning. The developed temperature-controlled stimulation system was used to assess directional discrimination as an evaluation of perceptual learning. Similar to Study II, different displacement lengths and directions were used to deliver noxious stimuli. It was hypothesized that directional discrimination is improved following perceptual learning.

CHAPTER 2. METHODS

2.1. SYSTEM DEVELOPMENT

To deliver thermal stimulations to the skin, a 20 W, 970 nm diode laser (DL-20; IPG Laser, Germany) with continuous-wave (CW) operation mode was employed (Figure 2-1). To record the temperature of the skin, an IR camera (FLIR SC645, Sweden) with a sampling frequency of 25 Hz was used. To displace the laser beam over the skin, the IR camera and the focusing lens of the diode laser were installed on a cartesian robot. The robot could operate in three directions (X, Y and Z-axis) with an accuracy of 0.01 mm. The laser beam was perpendicular to the stimulation site during all stimulations. In order to have a laser spot of 5 mm on the skin surface, the gap between the focusing lens and the skin was kept at 13 cm during stimulations. LabVIEW 20 was used for software control on a PC that communicated with the cartesian robot, IR camera and diode laser (Figure 2-1).

2.1.1. TEMPERATURE CONTROL

To maintain a stable and precise temperature during the displaceable stimuli, a closed-loop temperature control system was developed. This control system adjusted the laser power based on the temperature recordings from the IR camera, using a proportional-integral-derivative (PID) controller (Figure 2-1). Based on the image update frequency (25 Hz) the controller worked in 50 ms time windows. The PID gains differed for each displacement velocity based on the trial-and-error method (detailed values in [105]).

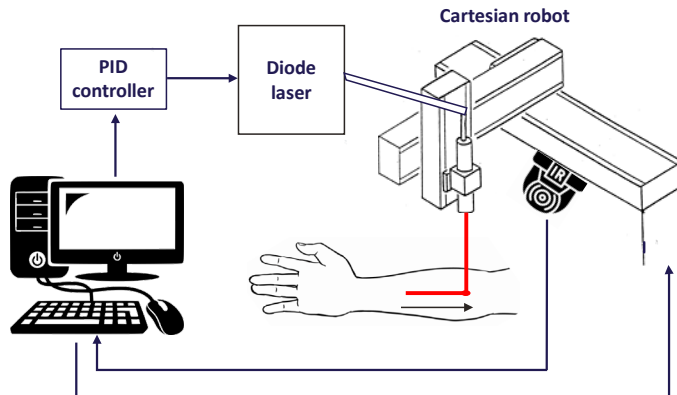


Figure 2-1 - The stimulation system; Laser's focusing lens and the IR camera were installed on the cartesian robot to move across the skin. The IR camera measured the skin temperature continuously. The laser power was regulated by a proportional-integral-derivative (PID) controller based on the temperature measured by the IR camera.

In order to achieve the fastest rise time while avoiding any overshoot, an initial boost stimulation was implemented with the aim of reaching stimulation target vicinity at the beginning of each stimulation [74]. The objective of the boost stimulation was to minimize the gap between the initial skin temperature and the desired temperature. The boost used maximum laser power in a few numbers of stimulation loops before enabling the PID controller (Figure 2-2).

To validate closed-loop control, an additional open-loop control mode was used for comparison. In open-loop control, the laser power was adjusted individually for each subject. In order to do so, prior to the experiment, a small number of stimulations were applied on the stimulation area to find an appropriate laser power to reach the desired target temperature. The obtained power was then fixed for the rest of the experiment.

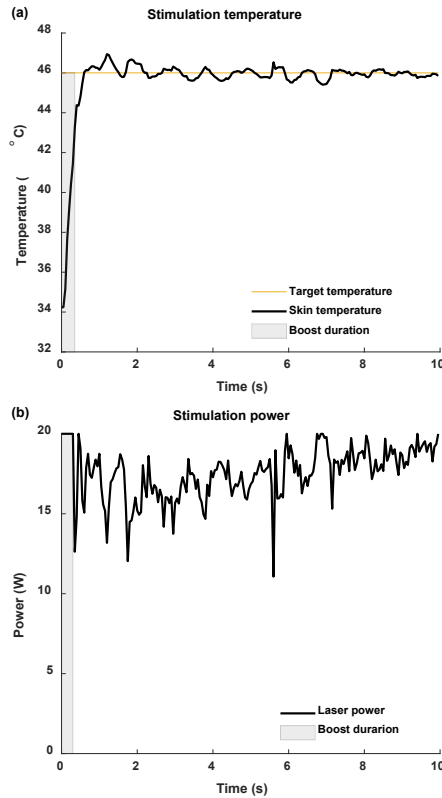


Figure 2-2 - The system performance during closed-loop control. a) The orange line shows the target temperature, and the black line shows the skin temperature at the location where the laser beam was radiating. b) The black line indicates the laser power during stimulation. The gray area shows the boost duration that the laser shots were delivered to the skin with maximum power to decrease the gap between skin the temperature and the target temperature. Following that period, the laser power was adjusted by the PID controller onwards.

2.1.2. BEAM TRACKING

Small variations in the distance between the skin and the IR camera during the laser beam displacement cause the position of the beam varying slightly within the image frame. To find the exact location of the laser spot on the skin, the full thermographic image was used to detect the pixels showing a temperature increase above a predefined threshold due to the laser radiation. A matrix method was designed to find the laser spot in the IR image during stimulation, explained with details in [105]. The maximum temperature of the laser beam spot was used as PID controller feedback.

2.2. EXPERIMENTS

For all three studies, the subjects gave written informed consent, according to the Declaration of Helsinki, prior to the experiment. The local ethics committee approved all the procedures of the experiments (ref. no. N-20200087). For the safety of all the subjects, as well as the experimenter, safety eye goggles were used throughout the experiment. To ensure that all the responses were only based on the perception of the stimulus, the subject's goggles were blinded. Another safety consideration for the subjects was that if the skin temperature exceeded 50 °C at any point in the experiments, the laser switched off automatically. Also, for the laser stimulation, any hair growth on the stimulation site (which was varied slightly within the right volar forearm between the stimulations) was removed before the experiment.

2.2.1. STUDY I

Eight healthy subjects (4 males, aged 26.6 ± 4.2 years) participated in the study. Seven of the subjects were Caucasian (4 males and 3 females) and one subject was Asian (female).

To validate the system, the skin temperature during different stimulation parameters, including velocity (5, 10 and 12 mm/s), intensity (high 46 °C and low 42 °C), displacement length (20 and 100 mm), direction (distal and proximal), were recorded in both open-loop and closed-loop control modes. All the conditions were repeated twice (in total 96 trials, all randomly presented).

The subjects were asked to rate the intensity of the perceived pain on a Numerical Rating Scale (NRS) between 0 and 10. Value 0 is defined as no perception of the stimulus, 3 as pain threshold, and 10 as maximum imaginable pain.

2.2.2. STUDY II

Twenty healthy subjects were recruited for this study (9 females, aged 24.1 ± 4.5 years). Eighteen subjects were Caucasian (7 females) and two subjects were Asian (2 females).

The experiment consisted of two blocks. For each block stimulation either closed-loop or open-loop control mode was applied. The laser beam was displaced across the skin in the distal, proximal, lateral and medial directions with a velocity of 10 mm/s. Five displacement lengths (20, 40, 60, 80 and 100 mm) over the distal/proximal directions and three displacement lengths (20, 40 and 60 mm) over the lateral/medial directions were used. The reason that longer lengths were not used in the lateral-medial direction was due to the limited width of the volar forearm. Each stimulation condition was repeated twice (a total of 64 trials, all randomly presented).

Subjects were asked to indicate the direction of the stimulus (e.g. the distal direction). The correctness of the indicated directions was considered as the accuracy of their discriminative performance. The subjects were also asked to rate the degree of certainty in percentage, and the intensity of the pain perception (i.e. NRS). The degree of certainty is a measure to observe how the subjective confidence of the subjects change with the experimental manipulations [106,107]. The degree of certainty showed how much confidence the test subjects had in their discriminative performance.

2.2.3. STUDY III

Twenty-eight healthy volunteers participated in this study (14 females, aged 26.8 ± 5.8 years). Twenty subjects were Caucasian (9 females), three subjects were Hispanic (1 female) and the remaining five were Asian (4 females).

The experiment was a crossover study over two days, with 48 hours in-between each day. Subjects were divided into two groups. Group 1 received unsupervised training (no feedback) on day 1 and supervised training (feedback) on day 2. Group 2 received supervised training on day 1 and unsupervised training on day 2. The subjects were randomly assigned to each group. Each day included three sessions: baseline, training and test. In the baseline session, subjects were asked to discriminate the stimuli with no feedback. In the training session, during supervised training, the subject had auditory feedback after each response. For the unsupervised training, there was no feedback. In the test session, subjects were asked again to discriminate the stimuli (with no feedback). There was a 30-minute break between successive sessions, and each included 40 laser stimulations.

The stimulations were delivered in four directions (distal, proximal, lateral and medial), with five displacement lengths (distal/proximal direction: 20, 40, 60, 80, 100 mm and lateral/medial direction: 20, 30, 40, 50, 60 mm). Each stimulus was repeated twice. All the conditions and repetitions were presented in random order. Subjects were asked to indicate the direction of the stimuli (i.e. discriminative performance). Additionally, subjects were asked to rate the level of certainty of their discriminative performance. The degree of certainty was measured to investigate if perceptual learning can also increase the subjects' response confidence.

2.3. DATA ANALYSIS

Data analysis and statistical tests were performed using custom scripts in MATLAB 2020b.

2.3.1. SKIN TEMPERATURE

The first 500 ms of each stimulation (i.e. rise time) was removed from all the temperature analyses. The actual skin temperature in 50 ms time windows during the stimulations was recorded and compared to the target temperature. The difference between the target and the actual temperature was considered as the stimulation error. The root mean squared error (RMSE) and standard deviation (SD) were calculated for all time windows of each stimulation. The RMSE reflected the accuracy of the system, and the SD showed the variability of skin temperature due to the stimuli.

2.3.2. DIRECTIONAL DISCRIMINATION THRESHOLD

The directional discrimination threshold (DDT) using noxious stimuli reflects the tempo-spatial acuity in the nociceptive system. The DDT shows the length at which subjects can correctly discriminate 50% of the stimuli direction (out of 4 possible options). To obtain DDT, first, the percentage of correct indicated directions for each displacement length was calculated. Then, a sigmoidal model was fitted to the results, using logistic regression. The displacement length at which the discriminative performance is 50% correct on the model is then considered as DDT.

2.3.3. STATISTICS

Linear mixed models (LMM) were used for all the statistical evaluations [108]. Fixed factors were the factors that were controlled and manipulated in each study (as listed in Table 2-1) and the random factor was each subject to account for individual differences within the group. P-values of less than 0.05 were considered statistically significant.

Table 2-1 - The dependant and independent variables across the three studies.

Study	Dependent(s)	Independent(s)
I	RMSE, SD, NRS	Control mode, length, direction, velocity, intensity
II	Performance, NRS, Certainty	Control mode, length, direction
III	Performance, Certainty	Session, supervision, length, direction, group

CHAPTER 3. RESULTS

3.1. STUDY I

3.1.1. TEMPERATURE CONTROL

Delivering thermal stimuli with open and closed-loop control modes revealed that more than 90% of the closed-loop stimulation had an error of less than 1 °C which was a significant improvement compared to open-loop control. Table 3-1 shows the details of the temperature absolute error in open and closed-loop control modes.

Table 3-1 - The absolute error of stimulation temperature in three different ranges of less than 1 °C, between 1 to 2 °C and greater than 2 °C

Absolute error	The percent of stimulation duration (%)	
	Open-loop control	Closed-loop control
< 1 °C	47.4	90.4
1 – 2 °C	33.7	6.7
> 2 °C	18.9	2.9

For the RMSE of temperature, there was a significant effect of control mode, with closed-loop showing smaller RMSE compared to open-loop (LMM, $t = 3.63$, $p < 0.001$) (Figure 3-1). The model also revealed a significant interaction between control type and intensity, as RMSE decreased with lower intensity in closed-loop control (LMM, $t = -2.23$, $p < 0.05$). Another significant interaction was between control type and velocity, in which the RMSE decreased with a decrement of velocity in closed-loop control (LMM, $t = -2.96$, $p < 0.01$) (Figure 3-1). No effect of displacement length (LMM, $t = 0.58$, $p = 0.56$) and direction (LMM, $t = 0.71$, $p = 0.47$) was found on RMSE.

The SD of the stimulation temperature was significantly smaller in closed-loop control compared to open-loop control (LMM, $t = 15.31$, $p < 0.001$) (Figure 3-1). There was a significant decrease of the SD with a decrement in intensity (LMM, $t = 9.47$, $p < 0.001$), decrement in displacement length (LMM, $t = 5.10$, $p < 0.001$), and direction (LMM, $t = 3.58$, $p < 0.001$). Control type and velocity showed significant interaction on the SD (LMM, $t = -9.81$, $p < 0.001$) in which the SD decreased with a decrement of velocity in closed-loop control (Figure 3-1).

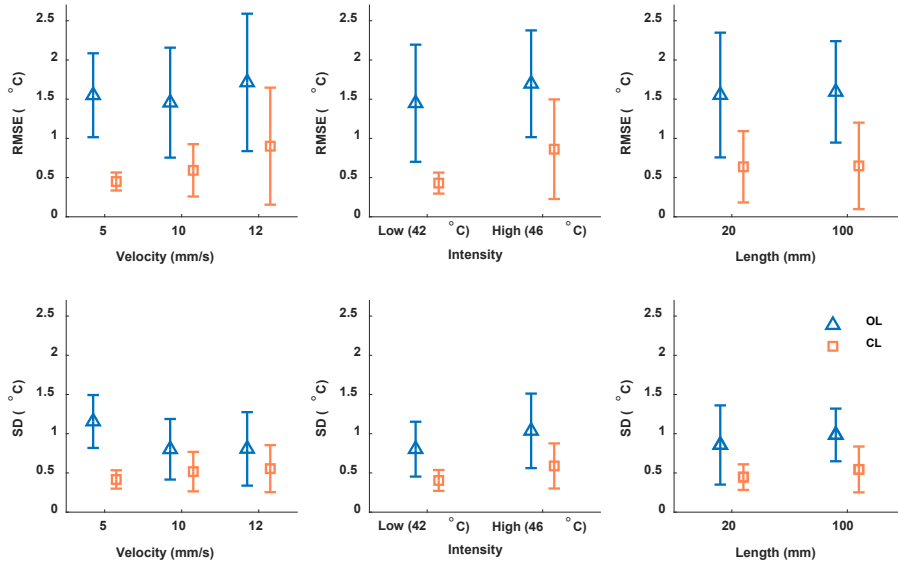


Figure 3-1 - The root mean square error (RMSE) (top panels) and SD (bottom panels) of the stimulation velocities (left panels), intensities (middle panels) and lengths (right panels). In closed-loop control (orange error bars), the RMSE and SD increased with stimulation velocity (LMM, p -value < 0.001) and intensity (LMM, p -value < 0.001). RMSE and SD decreased significantly in all the measures during closed-loop control compared to the open-loop control (blue error bars) (LMM, p -value < 0.001). CL: closed-loop control, OL: open-loop control.

3.1.2. PERCEIVED INTENSITY

The NRS increased significantly with increasing displacement length (LMM, $t = 14.14$, $p < 0.001$) and intensity (LMM, $t = 16.45$, $p < 0.001$) (Figure 3-2). The NRS was also significantly higher in the proximal direction compared to the distal direction (LMM, $t = 2.64$, $p < 0.01$). Stimulation velocity had no effect on the NRS (LMM, $t = -0.42$, $p = 0.67$).

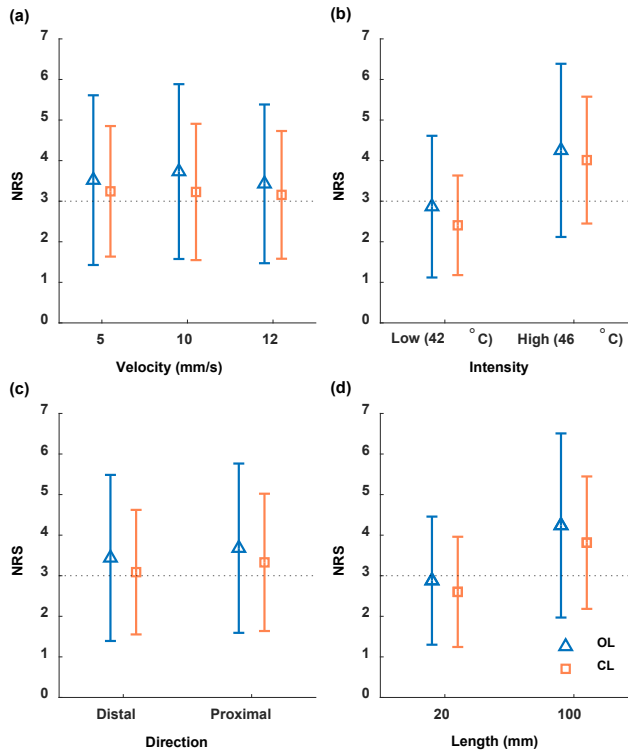


Figure 3-2 - The numerical rating scale (NRS) for closed-loop control (orange error bars) and open-loop control (blue error bars) in different displacement velocities (a), stimulation intensities (b), directions (c) and lengths (d). The dotted line shows the arbitrary value that the noxious stimuli were perceived as painful. The NRS increased with increasing intensity (LMM, p -value < 0.001) and length (LMM, p -value < 0.001). For closed-loop control, the variance of NRS was significantly smaller than for open-loop control (LMM, p -value < 0.001). CL: closed-loop control, OL: open-loop control.

3.2. STUDY II

3.2.1. DIRECTIONAL DISCRIMINATION

The detailed results of the DDT for each direction are shown in Table 3-2. This table shows that the DDT decreased in closed-loop control mode, as well as less variation, compared to open-loop control.

The discriminative performance was significantly higher during the closed-loop control (65%) than during the open-loop control (59%) (LMM, $t = 2.40$, $p < 0.05$) (Figure 3-3). The discriminative performance was also significantly increased with

increasing displacement length (LMM, $t = 2.08$, $p < 0.05$). There was an interaction between length and direction on the discriminative performance (LMM, $t = 2.15$, $p < 0.05$). The significant interaction revealed that the discriminative performance increased with increasing length, in the distal-proximal direction compared to the lateral-medial direction.

Table 3-2 - Directional discrimination threshold (DDT) with 95% CI in each direction, as well as combined distal-proximal and lateral-medial directions.

Direction	Closed-loop		Open-loop	
	DDT (mm)	95% CI (mm)	DDT (mm)	95% CI (mm)
Proximal	30.9	21.9 – 39.8	44.4	34.0 – 54.8
Distal	33.4	27.9 – 38.9	24.2	-10.7 – 59.3
Medial	13.8	6.8 – 20.7	43.7	-112.4 – 199.9
Lateral	29.7	18.4 – 41.0	30.6	-98.2 – 159.6
Distal-proximal	31.9	24.9 – 39.0	37.2	21.4 – 52.9
Lateral-medial	26.1	20.7 – 31.4	36.4	13.5– 59.3

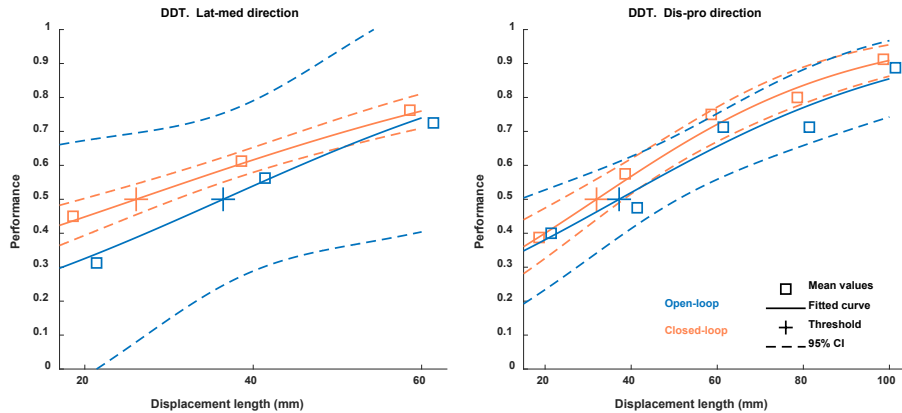


Figure 3-3 - Directional discrimination in directions of lateral-medial (left) and distal proximal (right). Squares represent average discriminative performance for all subjects. Sigmoidal curves fitted to the discriminative performance are shown in straight lines to determine the directional discrimination threshold (DDT) shown as crosses, with 95% confidence intervals (CI) shown as the dotted lines. In open-loop control (orange lines), the DDTs are smaller with a narrower 95% CI than in open-loop control (blue lines). The discriminative performance was higher in close-loop control (LMM, $p\text{-value} < 0.05$) and also increased with increasing length (LMM, $p\text{-value} < 0.05$). Notice the narrower CIs during closed-loop stimulation.

3.2.2. DEGREE OF CERTAINTY

Increasing displacement length significantly increased the degree of certainty (LMM, $t = 15.67$, $p < 0.001$) (Figure 3-4). Additionally, in the lateral-medial directions, the degree of certainty was higher compared to the distal-proximal directions (LMM, $t = 4.66$, $p < 0.001$). However, there was no significant difference in the degree of certainty in relation to the control mode (LMM, $t = 1.12$, $p = 0.26$).

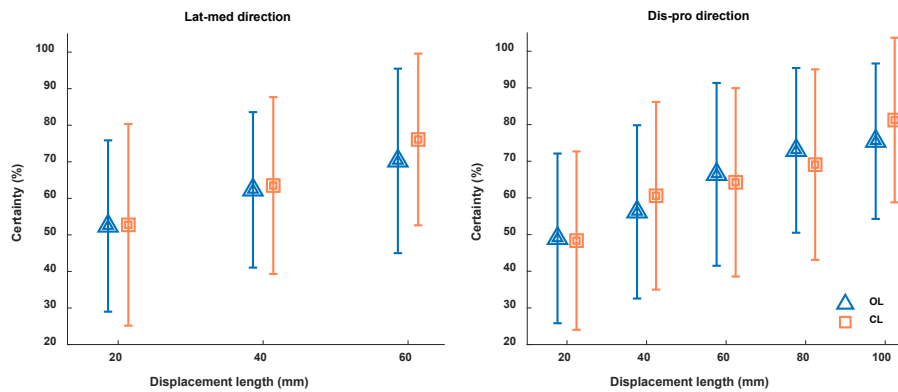


Figure 3-4 - The degree of certainty in displacement directions of lateral-medial (left) and distal-proximal (right). The degree of certainty increased with increasing displacement length in both closed-loop control (orange error bars) and open-loop control (blue error bars) (LMM, p -value < 0.001). The degree of certainty was also significantly higher in the lateral-medial directions compared to the distal-proximal directions (LMM, p -value < 0.001). CL: closed-loop control, OL: open-loop control.

3.2.3. PERCEIVED INTENSITY

The results showed that the NRS increased significantly during closed-loop control (78.1% as painful) compared to open-loop control (66.7% as painful) (LMM, $t = 10.68$, $p < 0.001$) (Figure 3-5). The NRS also increased with increasing displacement length (LMM, $t = 19.47$, $p < 0.001$), and direction (LMM, $t = 4.40$, $p < 0.001$), with the lateral-medial direction being perceived as more intense than in the distal-proximal direction.

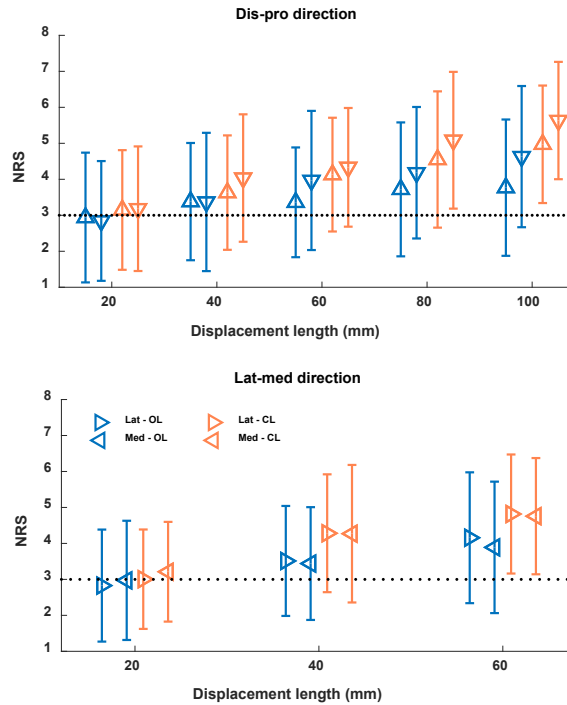


Figure 3-5 - The NRS in displacement directions of the distal-proximal (top) and the lateral-medial (bottom) direction. The dotted line shows the value that the noxious stimuli were perceived as painful. The NRS increased with increasing displacement length in both closed-loop control (orange error bars) and open-loop control (blue error bars) (LMM, p -value < 0.001). The NRS was also significantly higher in the lateral-medial directions compared to the distal-proximal directions (LMM, p -value < 0.001). CL: closed-loop control, OL: open-loop control.

3.3. STUDY III

3.3.1. THE IMPACT OF LEARNING ON DISCRIMINATIVE PERFORMANCE

There was no difference in the discriminative performance in the baseline session compared to the test session for both groups on the first day (Figure 3-6, left panels). However, there was a significant increase (LMM, $t = 3.15$, $p < 0.01$) in the discriminative performance in the baseline of the second day compared to the baseline of the first day for group 2. On the second day, there was a significant increase (LMM, $t = 2.49$, $p < 0.05$) in the discriminative performance from baseline to test sessions (Figure 3-6, top right panel) for group 1.

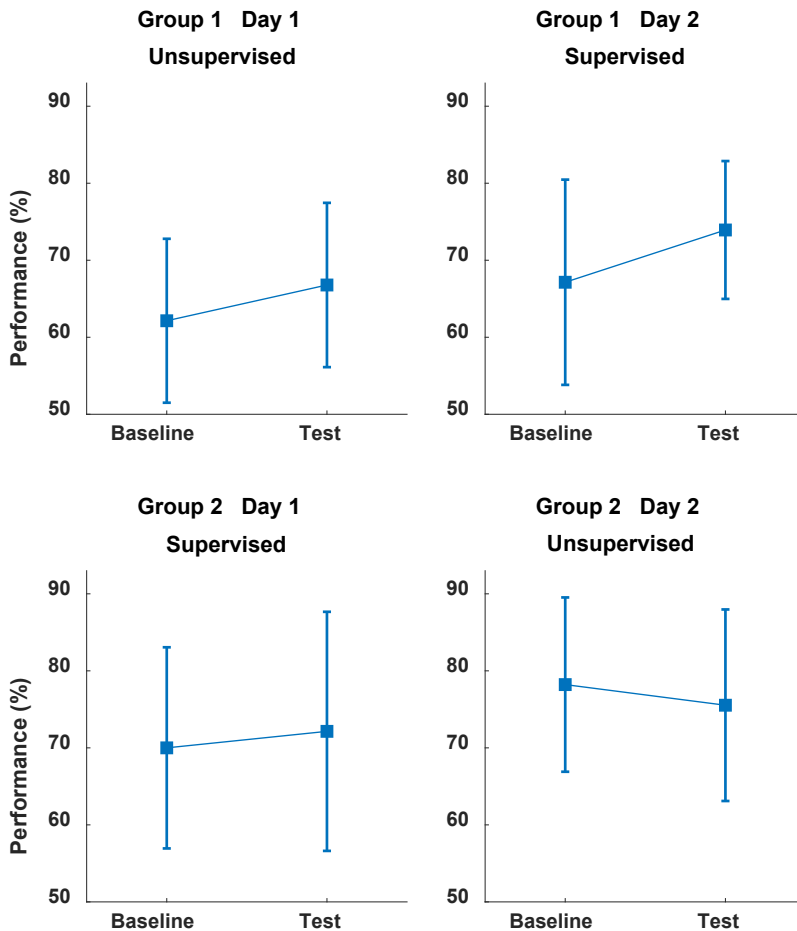


Figure 3-6 - The discriminative performance in the baseline and test sessions for both days (Day 1 and Day 2) and both groups (Group 1 and Group 2); top left) Group 1 Day 1 (unsupervised training), bottom left) Group 2 Day 1 (supervised training), top right) Group 2 Day 1 (supervised training) and bottom right) Group 2 Day 2 (unsupervised training). For group 2, the discriminative performance increased in the baseline of the second day compared to the baseline of the first day (LMM, p -value < 0.01). For group 1, the discriminative performance increased from baseline to test on the second day (LMM, p -value < 0.05).

For the supervised training, there was a significant increase in discriminative performance in the test session compared to the baseline session (LMM, $t = 2.39$, $p < 0.05$) (Figure 3-7, left panel). However, for the unsupervised training, there was no effect of training on the discriminative performance (LMM, $t = 0.52$, $p = 0.598$) (Figure 3-7, right panel).

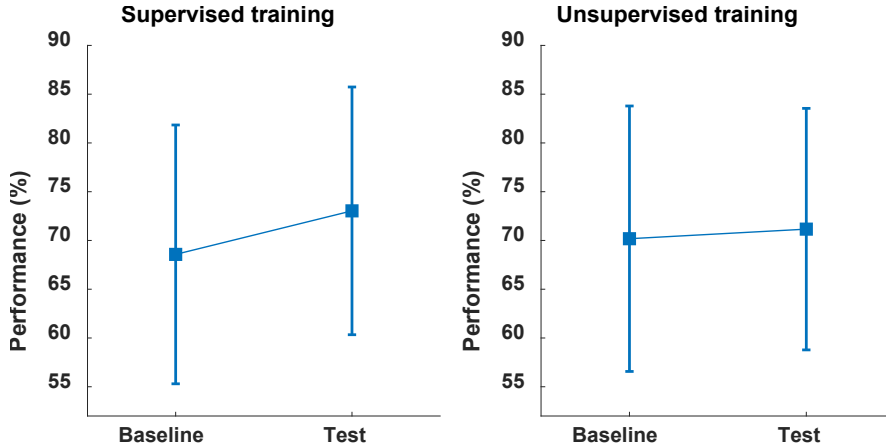


Figure 3-7 - The discriminative performance in the baseline and test sessions for supervised (left) and unsupervised (right) training. The discriminative performance increased in the test session compared to the baseline session for the supervised training (LMM, p -value < 0.05).

In the test session, the results showed no difference in discriminative performance in relation to the direction (LMM, $t = -0.76$, $p = 0.442$) and group (LMM, $t = -0.40$, $p = 0.684$). However, there was a significant increase in the discriminative performance due to the supervised training (LMM, $t = -3.27$, $p < 0.01$) and displacement length (LMM, $t = 14.49$, $p < 0.001$). The interactions between training and group (LMM, $t = 3.08$, $p = 0.002$) and between direction and length (LMM, $t = 4.98$, $p < 0.001$) were significant. The significant interaction between training and group revealed that in group 1 (i.e. those who had the unsupervised training first) the discriminative performance increased after the supervised training. In group 2 (i.e. those who had the supervised training first) the discriminative performance increased after the unsupervised training. The interaction between direction and length indicated that increasing the length improves the discriminative performance in the lateral-medial direction more than in the distal-proximal direction.

3.3.2. THE IMPACT OF LEARNING ON THE DEGREE OF CERTAINTY

The results showed that the degree of certainty increased in the test session compared to the baseline session (LMM, $t = 2.54$, $p < 0.05$) after the supervised training (Figure 3-8, left panel). However, for the unsupervised training, there was no change in the degree of certainty in the test session compared to the baseline session (LMM, $t = 0.49$, $p = 0.621$) (Figure 3-8, right panel).

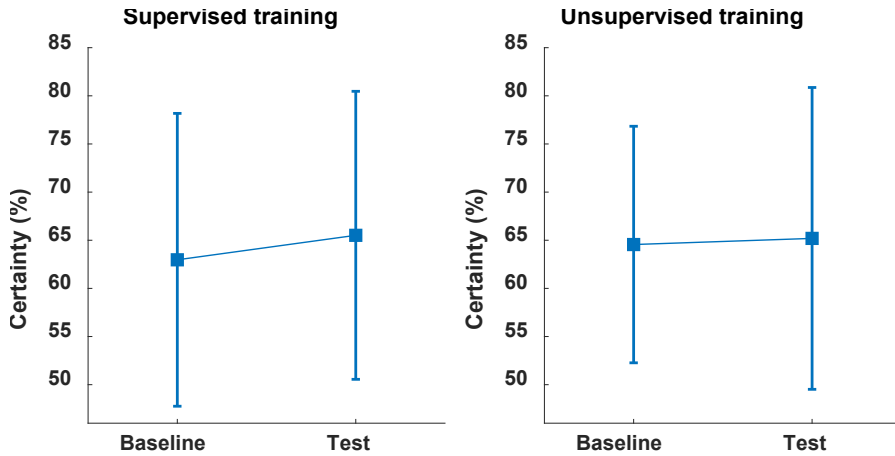


Figure 3-8 - The degree of certainty in the baseline and test sessions for supervised (left) and unsupervised (right) training. The degree of certainty increased in the test session compared to the baseline session for the supervised training (LMM, p -value < 0.05).

3.3.3. THE IMPACT OF LEARNING ON DIRECTIONAL DISCRIMINATION

Investigation of the DDT in the lateral-medial direction showed that in the supervised training, better discriminative performance leads to the psychometric curves are different, resulting in a lower DDT in the test session. However, the DDT did not change after the unsupervised training (Figure 3-9, left panel). For the distal-proximal direction, the DDT increased between baseline and test sessions for both supervised and unsupervised training (Figure 3-9, right panel). Unexpectedly, this showed that the DDT increased after the trainings.

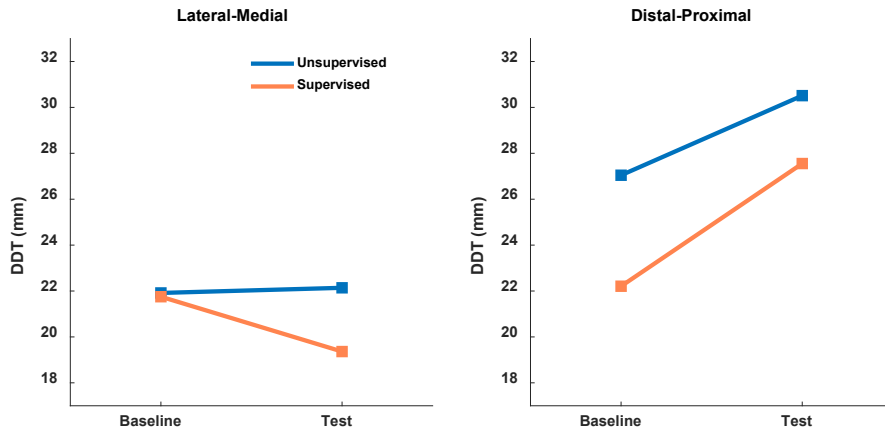


Figure 3-9 - The directional discrimination thresholds (DDT) in the baseline and the test sessions for the lateral-medial direction (left) and the distal-proximal direction (right). The orange lines show the supervised training, and the blue lines show the unsupervised training.

In the lateral-medial direction, the DDT decreased for the supervised training in the test session. However, the DDT did not change after the unsupervised training. For the distal-proximal direction, the DDT increased between baseline and test sessions for both supervised and unsupervised training.

CHAPTER 4. DISCUSSION

In this PhD project, a displaceable temperature-controlled laser stimulation system was developed to decrease the variability of skin temperature during the moving laser stimulations. Applying closed-loop temperature control resulted in more accurate and less variable stimulation temperature compared to open-loop control. The variation of DDT also decreased in the closed-loop temperature control system compared to open-loop control. Delivering precise stimuli to the skin provided a more accurate probe of directional discrimination. By investigation of directional discrimination, the integration of combined tempo-spatial information in the nociceptive system was studied. In addition to exploring tempo-spatial properties, the neuroplasticity of the nociceptive system was also investigated by perceptual learning and directional discrimination. The results showed that perceptual learning (following supervised training) improved directional discrimination in the nociceptive system and increased the degree of certainty in the subjects.

4.1. PERFORMANCE OF THE DEVELOPED SYSTEM

The results indicated that the absolute error during closed-loop control was less than open-loop control (Table 3-1). During closed-loop control, the average stimulation temperature could track the desired temperature better than in open-loop control (detailed in [105]). Additionally, RMSE and SD of stimulation in closed-loop control were significantly smaller compared to open-loop control (Figure 3-1). These results indicate that the overall performance of the closed-loop control system was better than open-loop control in terms of temperature control.

During stationary thermal laser stimuli, the temperature sensing during stimulation is limited to a small area on the skin surface when there is no beam displacement. However, using a moving laser stimulation across the skin, creates a major challenge as the temperature will vary during stimulation due to some physiological reasons. Variations in size and density of hair follicles [109] and skin tone are some of the reasons that may result in temperature differences during laser stimulation as the photon absorption changes [110]. The results also revealed that during the displaceable stimuli, the average skin temperature showed low SD, indicating the skin temperature stayed identical during movement (especially for the slower velocities).

There are few studies in the literature that have compared open-loop vs. closed-loop laser stimulation systems. One example is a study where in vitro, stationary stimulations were delivered to pig skin in which closed-loop control (between 50 and 55 °C), and open-loop control (up to 60 °C) were used to maintain the stimulation temperature for 5 min [102]. The moving stimulations were delivered to the human skin to achieve fixed target temperatures (42 °C and 46 °C) with shorter stimulation durations (1.66 - 20 s). The final results showed better performance than what was

reported in [102] in terms of temperature control for all conditions. Another study used temperature-controlled photocoagulation (TCPC) system [111]. In this study, pig tissue welding was performed in vivo using both open-loop and TCPC systems. The results of that study indicated a temperature deviation between 70 °C to 100 °C for open-loop control and a set point temperature of ± 4 °C during closed-loop control. Overall, they showed more accurate temperature control using a closed-loop system compared to an open-loop [111], which further supports the findings in Section 3.1.1. However, the movable temperature-controlled stimulation system had a smaller temperature deviation compared to the achieved results of invasive stimulations in [111] for different conditions.

4.2. DIRECTIONAL DISCRIMINATION

The ability to discriminate the spatial location of nociceptive stimuli has been shown to be connected to the primary somatosensory cortex (SI) [112]. The SI neurons that are engaged in the stimulated area have small RF that can determine the sensitivity to spatial discrimination and the intensity of the stimulus [113]. In addition to SI, the secondary somatosensory cortex (SII) is also linked to spatial discrimination [114–117], to encode intensity and perceive pain [118]. Several studies also have suggested that SI and SII are involved during TSP [119,120]. Another brain area that is linked to nociceptive processing, is the anterior cingulate cortex (ACC), which is responsible for the affective processing of pain [121]. Therefore, SI, SII and ACC are engaged in the processing of tempo-spatial information to perceive noxious stimuli.

A lower temperature variation of the skin was shown during closed-loop control compared to the open-loop control (Figure 3-1). Lower variation of the skin temperature in closed-loop control leads to more uniform activation of nociceptors [105]. Therefore, the afferent information delivered to the CNS will have better contrast, which may lead to lower DDT during closed-loop control. This was tested in this PhD project, where DDT was measured in both closed-loop control and open-loop control systems. The results showed that the closed-loop control system decreased the DDT and the variation of the DDT (Figure 3-3, Table 3-2) compared to the open-loop control system.

The DDT was lower in the lateral-medial direction compared to the distal-proximal direction (Figure 3-3, Table 3-2). This means that subjects had better discriminative performance in the lateral-medial direction which led to lower DDT, as well as lower variation in the discriminative performance. There are several possible reasons for this observation. The first reason could be due to the engagement of more dermatomes in the lateral-medial direction compared to the distal-proximal direction. Longer stimuli in the lateral-medial direction probably cover several different dermatomes [20], as opposed to the distal-proximal direction which covers a single dermatome. The second reason could be due to the oval shape of the RF of the primary afferents [122] which are oriented in the longitudinal direction [41]. It has been suggested that this

can lead to differences in discrimination thresholds in orthogonal directions [41]. This finding was in line with other studies that showed spatial discrimination is better in the lateral-medial direction compared to the distal-proximal direction in the forearm [54] and in the back of the hand [41].

4.3. PERCEPTUAL LEARNING IN DIRECTIONAL DISCRIMINATION

The results showed that the discriminative performance increased after supervised training, indicating the effectiveness of perceptual learning in the improvement of directional discrimination (Figure 3-6). This was in line with a previous study on perceptual learning that showed improvement in spatial discrimination after training with feedback (supervised training) [59]. The results also showed that supervised training was more effective than unsupervised training reflecting that repetition alone (unsupervised training) was not as effective as providing feedback (supervised training).

Previous studies have suggested that feedback leads to effective training due to the engagement of attention [123] and a specific memory trace of the task [124]. Providing feedback would help the subjects to re-evaluate their discriminative performance which may lead to effective training. This was also supported by the results of the degree of certainty (Section 4.4).

Investigation of the order effects on training showed that the supervised training improved the discriminative performance in group 1 (day 2) (Figure 3-7, left panel). Also, the results showed that group 2 had increased discriminative performance after the unsupervised training (day 2) (Figure 3-7, right panel). Due to the order of training, group 2 went through the supervised training on day 1, and thus the improved discriminative performance on day 2 following the unsupervised training could be due to the effects of the supervised training on day 1. Mancini et al. also showed similar findings that the effects of the supervised training were present several hours after the training [59]. The design of Study III did not allow for the investigation of the longevity of the supervised training systematically. However, based on the findings of this study, it is speculated that the effects of supervised training could last for up to 48 hours.

As mentioned in Section 4.2, SI and SII are involved in directional discrimination. Similarly, studies have shown that SI and SII regions are active during perceptual learning and can have structural and functional changes [58,125]. Similarly, one study has shown that ACC is affected during stimulus-response learning [126]. Based on the improvement of discriminative performance due to perceptual learning, it can be considered that perceptual learning changes the plasticity of SI, SII and ACC regions to improve directional discrimination.

The DDT in the lateral-medial direction decreased after the supervised training. This was according to the hypothesis that training can increase the discriminative performance and thus decrease the DDT. However, the opposite happened in the distal-proximal direction and both trainings increased the DDT when the baseline vs test sessions were compared. This was not in line with the hypothesis and more work is required to investigate this observation.

4.4. DEGREE OF CERTAINTY

In addition to directional discrimination, the effects of the control system and perceptual learning were also investigated on the degree of certainty. The results showed that the control mode did not affect the degree of certainty. While closed-loop control increased the discriminative performance, the degree of certainty was unchanged. Therefore, more accurate stimuli (closed-loop control) do not improve subjects' confidence in their discriminative performance.

One possible reason that the degree of certainty was unchanged during discriminative performance could be due to a lack of feedback to the subjects [106,127], which was addressed by the investigation of perceptual learning. This was supported by the results which showed that the degree of certainty improved after the supervised training (Figure 3-8, left panel), but not after unsupervised training (Figure 3-8, right panel). These findings showed that providing feedback to the subjects is important for increasing the degree of certainty. Similar to Section 4.2, ACC has been suggested to engage confidence [128] and certainty [129]. Therefore, the increased degree of certainty after training could be due to the involvement of ACC (as discussed in Section 4.3).

4.5. PERCEIVED INTENSITY

The NRS was investigated on the volar forearm. The pain sensitivity is the same in different areas of the volar forearm areas, due to evenly distributed density of nociceptors [130]. Therefore, stimulating different sites of the forearm, would not change the NRS within a single stimulation.

The results showed that during closed-loop control, the NRS was significantly higher than in open-loop control. In open-loop control, the laser intensity was fixed, which may not have been sufficient to reach the target temperature, resulting in a lower perceived intensity. However, the variance of the NRS was significantly smaller than during open-loop control (Figure 3-2). This can be due to the uniform activation of the cutaneous sensory receptors by closed-loop control.

The results also showed that the NRS increased with increasing displacement length which is in line with previous studies [50,53]. This increased NRS could be due to covering a larger stimulation area that recruited more neurons, resulting in SSP

[38,40,131]. Another reason for increased NRS could be due to TSP. During longer displacement lengths, the longer duration of stimuli may lead to temporal summation [37,132], which causes the stimuli to be perceived as more intense.

The NRS did not change with stimulation velocity. This was conflicting with a previous study which showed that the NRS decreased with increasing velocity [53]. In the previous study, a CO₂ laser was used to deliver stimuli. Since the penetration of the CO₂ laser is superficial, most of its energy is absorbed by water within the epidermis [80,133]. Therefore, the generated heat is conducted passively to the receptor terminals [134]. In this PhD project, a diode laser was used as the stimulator. Diode lasers have high penetration and therefore, there is little difference between the skin surface and receptor temperature [99]. This means that the receptor temperature does not rely on the velocity of the stimulation and does not need passive heat conduction.

4.6. LIMITATIONS

There was a limitation in reaching the higher temperature target during the velocity of 12 mm/s (i.e. the fastest velocity). For this velocity during closed-loop control and high intensity, the system was not able to reach the target temperature. The reason for this limitation was due to insufficient power of the diode laser to reach the noxious temperature (46 °C). Therefore, for this stimulation system, the maximum recommended velocity is 10 mm/s, if noxious temperatures are required. Using a more powerful diode laser would allow faster noxious stimuli.

The shape of the forearm caused a stimulation limitation. The space in the lateral-medial direction in the forearm is shorter than the space in the distal-proximal direction. Therefore, stimuli longer than 60 mm were not feasible in the lateral-medial direction. This can be the reason why the discriminative performance was higher in the distal-proximal direction, as longer stimuli (80 and 100 mm) were delivered. As discussed in Section 4.2, the longer stimuli are easier to perceive correctly.

CHAPTER 5. SUMMARY AND CONCLUSIONS

The aim of this PhD project was to investigate the tempo-spatial integration of pain information in the nociceptive system, using directional discrimination. By combining the tempo-spatial information, it was attempted to offer a perspective on the complex nature of pain perception. Directional discrimination has potential implications for personalized pain management strategies based on the location, the duration and the direction of perceived pain. This could pave the way for the development of targeted interventions designed to improve pain management, particularly in cases of chronic pain.

To this aim, a displaceable diode laser stimulator system was developed which allowed closed-loop control of the moving stimuli for more precise investigation of combined temporal and spatial integration of nociceptive information. The findings indicated that using this closed-loop control system could reduce the variability of perceived stimuli during the displaceable stimuli. This control is essential for obtaining robust and reliable insights into the complexities of pain perception. Researchers may find inspiration in this approach to design experiments with more precise control of stimulation in various physiological processes.

The developed system was employed to investigate directional discrimination in the nociceptive system. The findings showed that using a closed-loop temperature control system decreased the variation of DDT compared to open-loop control. This demonstrated that the developed displaceable temperature-controlled laser stimulation system enhances the precision of the investigations of combined tempo-spatial integration in the nociceptive system. Using this technological advancement could be a pivotal step forward in the field of pain research.

Besides directional discrimination, the neuroplasticity of the nociceptive system was explored using directional perceptual learning. The findings demonstrated that perceptual learning could improve directional discrimination in the nociceptive system. Perceptual learning also increased the degree of certainty in the subjects, which could reflect that the subjects also perceived the usefulness of training. The impact of perceptual learning on neuroplasticity revealed promising insights. It demonstrated that perceptual learning could enhance the tempo-spatial acuity and improve discriminative abilities within the nociceptive system. This suggests that interventions focused on perceptual learning hold the potential for advancing pain management strategies.

In conclusion, the displaceable temperature-controlled system introduced in this PhD project serves as a powerful tool for exploring the complicated interactions that

underlie pain perception. By using methods such as directional discrimination, a person's unique sensory processing profile may be better understood, which may help improving pain management and enhancing the overall quality of life for individuals dealing with chronic pain conditions. The results of this PhD project highlight the plasticity of the nociceptive system and the potential for targeted interventions to reshape pain perception. Future research can go deeper into the neural mechanisms underpinning perceptual learning and explore the clinical applications of these discoveries. One of the applications of perceptual learning can be applying it in different therapies such as CBT. Perceptual learning principles have the potential to be incorporated into such therapies to help individuals with their chronic pain.

Lastly, in this PhD project, the effects of perceptual learning remained after 2 days. However, for observing longer effects of modulating neuroplasticity within the nociceptive system, a more comprehensive examination is warranted. How sustained changes in sensory perception affect individuals over time is an intriguing question. Future research might follow individuals who participate in perceptual learning interventions to assess the lasting effects on pain perception and associated functions.

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