Pupil Response as an Index for Perceived Tactile Stimulus Intensity

I. Heiner, 6680909

Utrecht University

Neuroscience and Cognition Master program

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Supervisors: A. F. Ten Brink, C. Strauch, H. C. Dijkerman

Second examiner: S. Gayet

Abstract

The pupil response scales with stimulus intensity, observed in both visual and auditory modalities. It is unknown whether this generalizes to the tactile modality. Here, I investigated in healthy participants the relationship between pupil response and tactile stimulation applied to differently sensitive parts of the body and tactile stimulation of different intensity. In Experiment 1, pupil response was examined after tactile stimulation of the finger, arm, and calf. Participants (*N*=32) showed larger pupil response after stimulation of the finger versus arm and calf, and after stimulation of the arm versus calf. Using vibrotactile stimulation, pupil response after tactile stimulation of differential intensities was examined in Experiment 2 (*N*=20). Pupil response was smaller for weak tactile stimulation compared to medium and strong stimulation. Taken together, the current study showed differential pupil response after tactile stimulation on different body locations and after vibrotactile stimulation of different intensities. Tactile stimulation on more sensitive body locations may thus be perceived as more intense, based on comparable pupil response differences between tactile stimulation on body locations with differential tactile sensitivity and tactile stimulation of differential intensity. The current results indicate that pupil responses convey perceived intensity of tactile stimuli. Pupil response may have the potential to be used as an objective index for tactile sensitivity which is not reliant on verbal response from patients.

Keywords: pupillometry; pupil; touch; tactile sensitivity; tactile stimulation; vibrotactile stimulation

Introduction

Changes in pupil size result from a range of factors, including light level, focal distance, alerting, orienting, and executive functioning (Strauch et al., 2022). An orienting response is a short-lasting response to the perception of a novel external stimulus, which includes pupil response (Strauch et al., 2022). In the visual and auditory domain, the orienting response is affected by the intensity of stimuli, with for example louder auditory stimuli eliciting larger and faster pupil responses (Strauch et al., 2022; Wang et al., 2014). Within the tactile domain, a limited amount of research has been done into the relationship between stimulus intensity and pupil response (Gusso et al., 2021). In the current study, I will investigate whether tactile stimulation on body areas with different tactile sensitivities, and whether different intensities of tactile stimulation affect the pupil orienting response.

Previous research found that stroking velocity modulates pupil response, which suggests that intensity of tactile stimuli also modulates pupil response, possibly as part of the orienting response (Strauch et al., 2022; van Hooijdonk et al., 2019). Changes in pupil size due to the orienting response are linked to the superior colliculus-centered circuit and to the autonomous nervous system pathways (Strauch et al., 2022). The intermediate layers of the superior colliculus may modulate differential pupil response after presentation of stimuli of differential contrast (Wang & Munoz, 2015). The intensity of stimuli of several modalities, including tactile, influences the degree of physiological sympathetic nervous system response (Lee et al., 2020; Loggia et al., 2011; Toyokura, 2006). So, after stimulus presentation of differential intensity, a differential degree of sympathetic nervous system response follows, possibly differentially modulating pupil response.

Differences in tactile sensitivity between body areas may modify the perceived intensity of a tactile stimulus. Tactile sensitivity can be operationalized by the minimal sensitivity with which one consciously perceives tactile stimuli (Weinstein, 1968). Tactile sensitivity differs between body areas due to several factors, including differential mechanoreceptor types and differential mechanoreceptor density across the skin (Delhaye et al., 2018; Weinstein, 1968). Perceived tactile stimulation is followed by a larger pupil response compared to non-perceived tactile stimulation (Gusso et al., 2022). It may be hypothesized that tactile stimulation of body areas with higher tactile sensitivity is perceived as more intense, thus resulting in a larger sympathetic nervous system activation with a subsequent larger orienting response, which includes pupil response (Lee et al., 2020; Strauch et al., 2022).

Although some evidence points to a relationship between stimulus intensity of tactile stimuli and pupil response, no research has been done into the relationship between tactile sensitivity and pupil response (Gusso et al., 2021; van Hooijdonk et al., 2019; Wang & Munoz, 2015). In addition, the current literature regarding tactile stimulation and pupil response is limited by methodological constraints, including non-systematic tactile stimulation (Gusso et al., 2021). However, the relationship between pupil response and stimulus intensity has been studied for painful stimuli. Thermal stimuli which are perceived to be more painful are followed by a larger pupil response (Eisenach et al., 2017). Moreover, due to the close relationship between pupil response and perceived pain, pupil response can used as an index for pain or analgesia level, including for non-responsive and anesthetized patients (López de Audícana-Jimenez de Aberasturi et al., 2023; Sabourdin et al., 2018, 2019; Wildemeersch et al., 2018). Possibly, pupil response may also be used as an index of the perceived intensity of non-painful tactile stimulation.

In the current study it was examined whether differential tactile sensitivity across the body is reflected in how strongly the pupil responds to tactile stimulation (pilot and Experiment 1). It was hypothesized that there is an effect of tactile stimulation on pupil response, that tactile sensitivity differs between body areas, and that pupil response after tactile stimulation scales with tactile sensitivity. In addition, it was examined whether stimulus intensity is reflected in how strongly the pupil responds to tactile stimulation (Experiment 2). It was hypothesized that pupil response after tactile stimulation scales with stimulus intensity.

As an secondary question, it was looked into whether people who self-report to be generally sensitive to touch (e.g. perceiving touch as being intense or overwhelming more easily than others), show larger pupil responses to tactile stimulation compared to people who self-report lower general sensitivity (pilot). Liao and colleagues (2016) described a link between pupil dilation after the presentation of auditory stimuli and the subjective salience of these stimuli, and Tyukhova and Waters (2019) found a relationship between pupil size responses and the subjective evaluation of visual discomfort glare. If the aforementioned relationship between pupil response and perceived salience translates across modalities, subjective sensitivity to sensory stimuli may influence pupil response after tactile stimulation.

To sum up, the current study will evaluate whether pupil response after tactile stimulation reflects the differential tactile sensitivity of multiple body locations. Moreover, the current study will provide insight into the relationship between intensity of tactile stimuli and pupil response. These findings could contribute to knowledge about whether pupillometry can be used as an index for tactile sensitivity, for example in non-responsive patients.

Pilot

Methods

The research and consent procedures were performed in accordance with the standards of the Declaration of Helsinki. Ethical approval was obtained from the Ethics Review Board of the Faculty of Social & Behavioural Sciences (protocol number 23-0229).

Participants

Healthy participants (N = 14) between the ages of 19 and 26 years old (M_{age} = 22.71 years, SD_{age} = 2.27 years), with normal or corrected-to-normal vision participated in the current experiment (2 male, 12 female; all but one right-handed). None of the participants reported impaired or irregular touch sensation or a history of neurological disorders. Participants were compensated monetarily or in course credits. Participants all gave written informed consent.

Apparatus

Pupil size of the left eye was assessed using an Eyelink 1000 tracker (SR Research Ltd., Canada) in a light and sound-attenuated laboratory. Stimuli were presented on an Asus ROG PG278Q monitor, featuring a refresh rate of 99 Hz and a screen resolution of 2560*1440 px, at 67.5 cm distance from eye-position. The participants' head was positioned in a chin and forehead rest. Psychopy version 2021.2.3 (Peirce et al., 2019) was used for the implementation of the experiment. Tactile sensitivity was measured using Von Frey synthetic monofilaments, ranging from 0.008 g to 300 g in force (North Coast Medical Inc., United States of America).

Tactile stimulation was provided using a custom-made tapper box. The current experiment used 6 output channels and tappers. A tapper consisted of a cord with a copper coil at the end. Inside the coil were a small magnet and screw embedded. If the tapper box received an input value corresponding to a tapper value, the magnetic field was reversed. As a result, the screw and the magnet were pushed out of the coil of the tapper, which could be felt if the tapper was on the skin. Due to the tapper box being custom-made, no details about the amount of force of the tactile stimulation or sound levels were available.

Procedure

The first part of the experiment was an online questionnaire to measure subjective sensitivity (Glasgow Sensory Questionnaire), to be filled out at home. Upon arriving at the lab, the order in which the tactile sensitivity task and tactile stimulation task were administered was counterbalanced between participants. The tactile stimulation task was always preceded by a sound detection task. During the tactile stimulation task, the order in which locations were stimulated was counterbalanced using a Latin square design.

Participants had to wear noise-cancelling headphones (Sony WH-1000x M3) playing brown noise to control for the sound that the tappers produced during the sound detection task and the tactile stimulation task. Volume was adjusted to the highest volume that was still perceived as comfortable for the participant.

Sound detection

The sound detection task was conducted to test if the brown noise masked the sound of the tappers being active. This task consisted of 30 trials, which started with a grey screen for 1 s, after which the participant had to respond whether they hear the sound of a tapper or not via keypress. During the grey screen, a tapper could fire, which was randomly sampled from 100 options per participant, with a 40% chance of a trial in which no tapper fired (silent trials) and a 60% chance of a trial in which a tapper fired (tapper trials were evenly distributed across all tappers). The responses in this task were used to determine sensitivity (d’) to distinguish tapper trials and silent trials.

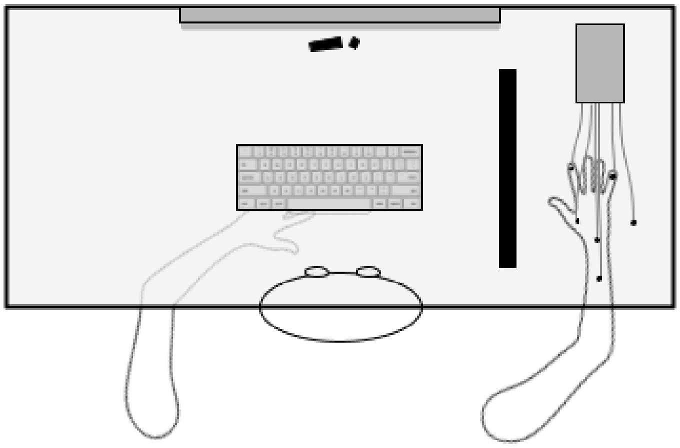
Tactile stimulation

Five tappers were applied on the right arm: on the tip of the index finger, the tip of the small finger, the thenar eminence, the wrist, and the inner forearm (Figure 1). The right arm was laid out in a stretched-out position away from the body on a desk, obstructed from view by a screen, and the left hand was laid on the participants’ lap under the desk (Figure 1). The right arm was hidden from view because having the body part that is touched in the field of view causes visual enhancement of touch, increasing tactile sensitivity (Colino et al., 2017). The sixth tapper was used as a control condition, being laid on a small cushion beside the arm, also obstructed from view.

A nine-point calibration and validation of the eye-tracker was performed at the start of each block. The experiment consisted of six blocks, one for each of the five stimulation sites and one for the control tapper. The sequence of a trial is shown in Figure 2. Participants had to look at a fixation cross during the entire trial. A trial started with a 0.5 s baseline period. After this period, a 2.5 s measurement period took place to capture the pupil response. At the start of the measurement period, tactile stimulation occurred. A trial was considered invalid if the participants gazed >3 visual degrees from the center or blinked for >250 ms during the measurement period. Feedback was provided via a red cross (invalid) or grey cross (valid) for 0.5 s, followed by a variable inter-trial interval between 2.5 and 3.5 s. Invalid trials were repeated at the end of each block. Every sixth trial, tactile stimulation took place on a location other than the stimulus location of the current block (out-of-block trials). No pupil response was recorded for these trials and invalid out-of-block trials were not repeated at the end of each block. Each block consisted of 25 trials (excl. out-of-block trials), resulting in 6 blocks of 25 trials.

Figure 1

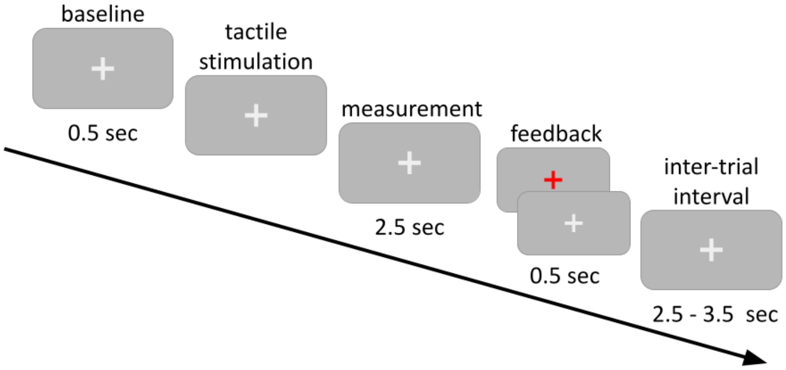
Experimental set-up tactile stimulation in the pilot



Note. Schematic representation of the experimental set-up during the tactile stimulation task. Participants sat at a desk with their head in a chinrest. The monitor was positioned 67.5 cm away from the eyes. Their left arm was on their lap underneath the desk. The right arm was on a desk behind a screen, with 5 tappers attached to their arm and 1 laying beside the arm. Black circles signify sites which were stimulated and of which the tactile sensitivity threshold was measured: small finger, index finger, thenar eminence, wrist, forearm, and control.

Figure 2

Sequence of a trial in the pilot



Note. Participants had to keep their gaze position on a light grey fixation cross presented on a darker grey background. After fixating the center, the trial started with a baseline period of 0.5 s, after which tactile stimulation took place, followed by a post-stimulus period of 2.5 s. A trial was considered invalid if participants gazed >3 visual degrees from the center or blinked >250 ms. Feedback was provided via a red cross (invalid) or grey cross (valid). After feedback, an inter-trial interval of 2.5 to 3.5 s took place. Invalid trials were repeated at the end of each block.

Subjective sensitivity

The Glasgow Sensory Questionnaire (GSQ) was used as a measure of self-reported sensory sensitivity (Robertson & Simmons, 2013). The GSQ covers hyper- and hyposensitivity to tactile, visual, auditory, gustatory, olfactory, vestibular stimulation and proprioception, with possible scores ranging from 0 (low sensory sensitivity) to 168 (high sensory sensitivity) and possible scores on the subscales ranging from 0 (low sensory sensitivity for a specific modality) to 24 (high sensory sensitivity for a specific modality). The current experiment examined both the total score and the tactile subscale. Participants could choose between the original English version of the GSQ

($\alpha = .94$) and a translated Dutch version ($\alpha = .90$), which both had a good reliability (Robertson & Simmons, 2013; Kuiper et al., 2019).

Tactile sensitivity

Tactile sensitivity, defined as the sensitivity with which touch can be perceived, was measured on five locations of the right arm: the tip of the index finger, tip of the small finger, thenar eminence, wrist, and inner forearm (Figure 1; Keizer et al., 2012; Weinstein, 1968). Tactile sensitivity was assessed using 20 von Frey filaments ranging from 0.008 g to 300 g in force, starting with 2.0 g. The experimenter applied tactile stimulation according to a forced choice up one down one staircase procedure, resulting in 5 sub- and 5 suprathreshold reversals, pseudo-randomly mixed with sham trials, in which no stimulus was presented. The participants, while blindfolded, were tasked with reporting whether they felt a stimulus at the given location. The tactile sensitivity threshold was computed as the geometric average of all reversal points, ranging from 0.008 g (high sensitivity) to 300 g (low sensitivity).

Data processing

All data was processed using customized Python (3.9) scripts. Data preprocessing for the sound task and tactile sensitivity task was conducted in Excel (version 2208).

Pupil size data was presented as change from baseline (i.e., last 100 ms of the pre-stimulus baseline period), expressed in arbitrary units over time, downsampled to 100 Hz. Negative values relative to baseline connote pupil constriction, positive values relative to baseline connote pupil dilation.

Statistical tests were performed two-sided, with $\alpha = 0.05$. Assumptions of sphericity and normality for the Repeated Measured ANOVA were checked using Mauchly’s test of sphericity and the Shapiro-Wilk test respectively. When either or both of these assumptions were violated ($\alpha = 0.05$), a non-parametric Friedman’s ANOVA was used and where applicable, non-parametric Wilcoxon signed-rank tests for post-hoc testing. The assumption of homoscedasticity for the

Pearson correlation was checked using Levene's test and when this assumption was violated ($\alpha = 0.05$), a non-parametric Spearman's rank correlation test was used.

Results

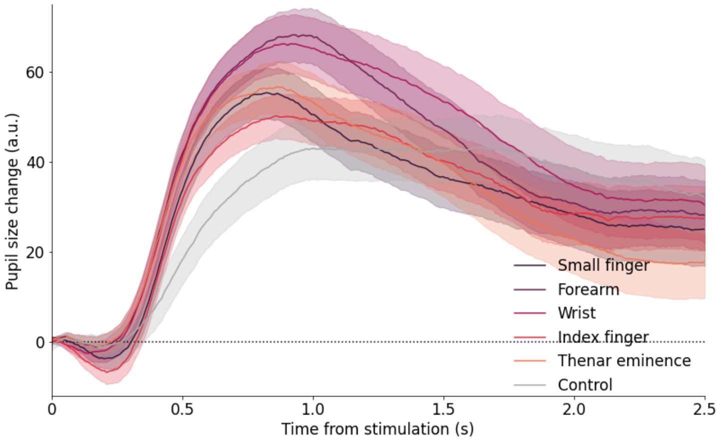
Pupil response

Figure 3 depicts average pupil size over time for all five stimulation locations (small finger, index finger, thenar eminence, wrist, forearm) and the control block. Pupils dilated most around 0.8 s after tactile stimulation for the small finger and 0.9 s for the index finger, thenar eminence, wrist, and forearm. The control location also showed pupil dilation after the tapper fired. Pupil responses of individual participants are depicted in Supplementary Figure A1.

To examine pupil response after tactile stimulation, a linear mixed effect model (LME) was used, in which pupil response was a dependent variable, the stimulation location (and control location), trial number within a block, and block number were fixed predictors, with random intercepts for each participant. Trial number within a block and block number were interaction effects and were added to control for possible habituation effects. An effect of tactile stimulation on pupil response could be seen, with pupil response in stimulus conditions being larger than pupil response in the control condition, Figure 4. Tactile stimulation on the wrist elicited a larger pupil response than tactile stimulation on the small finger, index finger, thenar eminence, and forearm at multiple points during the trial, Figure 5. Pupil response after tactile stimulation on the forearm was larger than after tactile stimulation on the index finger and thenar eminence for a small amount of time during the trial, Figure 5. Participants showed little ability to detect the tapper firing during the sound detection task. Eleven participants were not sensitive in distinguishing tapper trials from silent trials ($d' = 0$), one participant showed a sensitivity to distinguish tapper and silent trials of $d' = 0.08$, and two participants showed an inverse sensitivity to distinguish tapper and silent trials ($d' = -0.25$ and $d' = -1.60$). Changes in pupil size can, therefore, also not (fully) be explained by anticipation or sound of the stimulation.

Figure 3

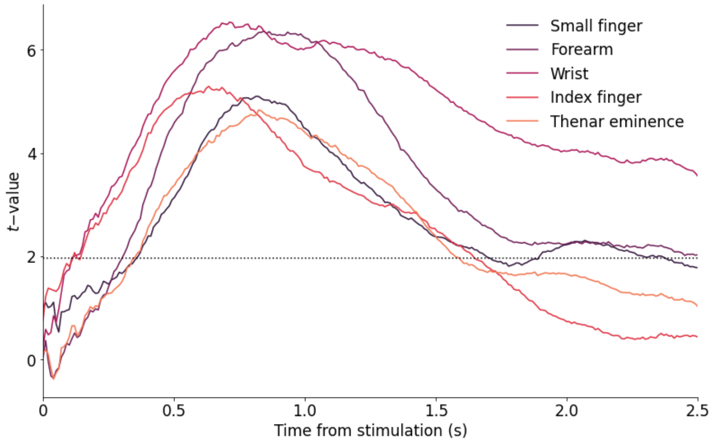
Average pupil response split by stimulus location



Note. Baseline-corrected pupil response over time, averaged per stimulation location and control location. The x-axis depicts time relative to stimulus onset in seconds. The y-axis depicts pupil size change compared to the baseline period, expressed in arbitrary units (a.u.). Positive values on the y-axis indicate pupil dilation, negative values indicate pupil constriction.

Figure 4

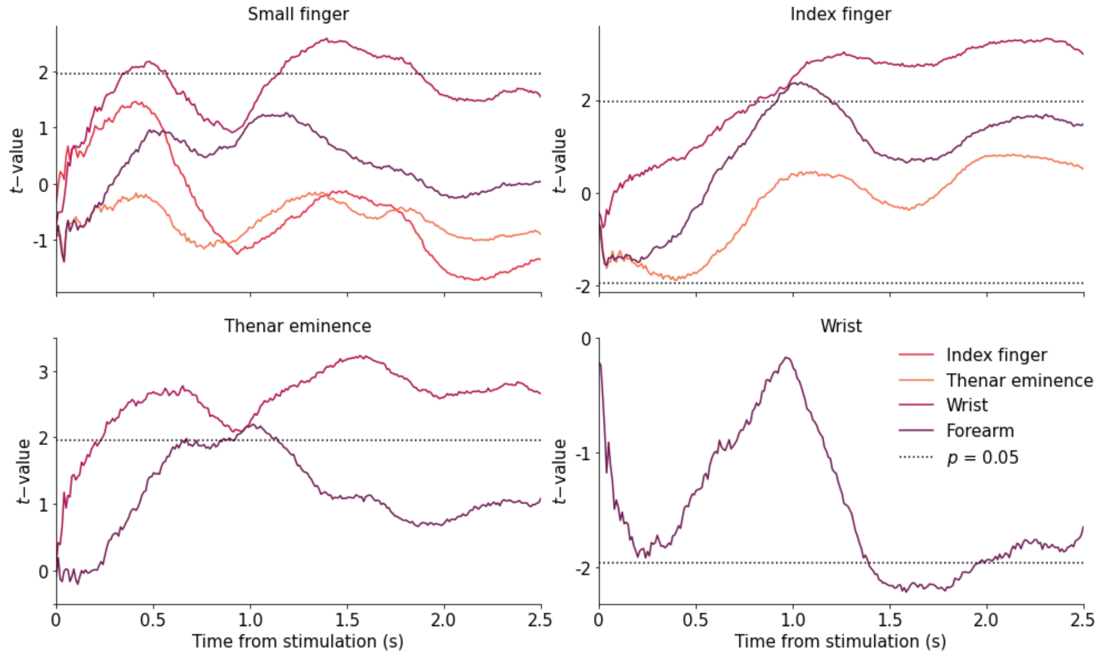
Linear mixed effects model comparing t-values of control and stimulus locations over time



Note. Each line depicts the t-values of the comparison between the control location and a stimulus location on pupil response after tactile stimulation over time, with an interaction of block number and trial number within a block and random intercepts for each participant. The y-axis shows t-values values, with the dotted line depicting $t = |1.96|$, equal to $p = 0.05$. The x-axis shows time relative to stimulus onset in seconds.

Figure 5

Linear mixed effects model comparing t-values of stimulus location over time



Note. Each subplot depicts a stimulus location compared with other stimulus locations on pupil response over time after tactile stimulation, with lines also depicting stimulus location. The y-axis shows t-values, with the dotted line depicting $t = |1.96|$, equal to $p = 0.05$. The x-axis shows time relative to stimulus onset in seconds.

Tactile sensitivity

Tactile sensitivity thresholds per stimulation location, assessed using Von Frey monofilaments, are depicted in Supplementary Figure A2. Tactile sensitivity differed between stimulation locations (χ^2 (26) = 26.25, $p < 0.001$), as tested using Friedman's ANOVA. Post-hoc pairwise comparisons indicated both wrist and forearm were less sensitive than small finger, index finger, and thenar eminence, and that small finger was more sensitive than index finger (Supplementary Table A1).

Subjective sensitivity and tactile sensitivity

Subjective sensitivity scores, assessed using the Glasgow Sensory Questionnaire (GSQ), ranged from 81 to 110 for the total score (*M* = 93.57, *SD* = 8.52), and between 9 to 15 for the tactile subjective sensitivity sub score (*M* = 11.14, *SD* = 1.70). No significant Spearman correlations, corrected for multiple tests using a Holm-Bonferroni correction, were found between total subjective sensitivity score and tactile subjective sensitivity score with the stimulation locations. Because the subjective sensitivity was not related to tactile sensitivity for all stimulation locations, subjective sensitivity was not taken into account when constructing the linear mixed effects model to explain pupil size after tactile stimulation.

Interim discussion

The aim of the pilot was to investigate whether differential tactile sensitivity across the body would be reflected in the strength of pupil response after tactile stimulation and whether pupil response after tactile stimulation is related to subjective sensitivity.

Pupil response in the control condition was different from stimulus conditions, but still increased compared to baseline pupil size, possibly pointing to other factors than tactile stimulation contributing to pupil response in the current experiment. Therefore, we decided to discontinue the pilot before including 14 participants. Despite that participants could not reliably detect the sound of a tapper firing in the sound detection task, a possibility was that the sound of the tapper was still loud enough to cause a pupil orienting response in the control condition. If so, the pupil response in all conditions could have been influenced by the auditory signal of the tappers (Strauch et al., 2022). Another possibility is that there was a pupil response in the control condition the tactile stimulus was always presented after a fixed baseline period of 0.5 s, possibly causing a preparatory pupil dilation (Dragone et al., 2018; Jennings et al., 1998). To address these issues, Experiment 1 controlled for the effects of the auditory signal by using earplugs in combination with headphones and use a more sound-dampening cushion in the control condition, making the sound of the tapper in the control condition more comparable with the sound of the tapper in the stimulation conditions. To control for effects of expectancy, Experiment 1 had a variable baseline period between 0.5 and 2.5 s.

Moreover, based on the effect sizes of the differences in tactile sensitivity and the possibility of the feeling tactile stimulation spreading to other nearby stimulation locations, new stimulation locations were chosen to investigate in Experiment 1: small finger, forearm, and calf, based on the effect sizes of the largest differences in tactile sensitivity in the current experiment, tactile sensitivity reports of Weinstein (1968), and the distance between these locations.

No relationship was found between general subjective sensitivity and tactile sensitivity or tactile subjective sensitivity and tactile sensitivity, except for the thenar eminence. Therefore, subjective sensitivity will not be measured in Experiment 1.

Experiment 1

Methods

The research and consent procedures were performed in accordance with the standards of the Declaration of Helsinki. Ethical approval was obtained from the Ethics Review Board of the Faculty of Social & Behavioural Sciences (protocol number 23-0229). Methods were the same as in the pilot, only deviations are described.

Participants

In total, 32 healthy participants (7 male, 24 female, 1 non-binary; all but 3 right-handed) between the ages of 18 and 29 years old (M_{age} = 23.56 years, SD_{age} = 2.78), with normal or corrected-to-normal vision participated. None of the participants reported impaired or irregular touch sensation or a history of neurological disorders. Participants were compensated monetarily or in course credits. Participants all gave written informed consent.

Apparatus

The apparatus was the same as that used in the pilot.

Procedure

The procedure was the same as in the pilot, only the Glasgow Sensory Questionnaire was not administered.

Sound detection

The sound detection task was the same as in the pilot, with the only being that the sound detection task consisted of 10 silent trials and 20 tapper trials.

Tactile stimulation

On the right side of the body, 3 tappers were applied to the tip of the small finger, forearm, and calf. The fourth tapper was used as a control condition, being laid on a small cushion beside the arm. The right arm was hanging down, the left hand was laid on the lap under the desk. All stimulation locations were obstructed from view to prevent visual enhancement of touch increasing tactile sensitivity (Colino et al., 2017). A foam ring was placed at the base of the small finger to prevent the small finger from touching the ring finger.

A nine-point calibration and validation of the eye-tracker was performed at the start of each block. The experiment consisted of four blocks, one for each of the three stimulation sites and one control block. Participants had to fixate a cross throughout the trial. A trial started with a variable baseline period of 0.5 to 2.5 s, which was set to 1.5 to 2.5 s after the first 14 participants to ensure a stable pupil baseline measure. Next, tactile stimulation occurred and the pupil response was captured for 1.5 s. A trial was considered invalid if the participants gazed >3 visual degrees from the center or blinked for >200 ms during the measurement period. Feedback was provided via a red cross (invalid) or grey cross (valid) of 0.5 s, followed by an inter-trial interval of 1.5 s. Invalid trials were repeated at the end of each block. Every sixth trial, tactile stimulation took place on a location other than the stimulus location of the current block (out-of-block trials), to prevent possible habituation or expectation effects. Each block consisted of 25 trials (excluding out-of-block trials), resulting in 4 blocks of 25 trials.

Tactile sensitivity

The procedure to measure tactile sensitivity was the same as in the pilot, with the only deviation being that tactile sensitivity was measured on three locations of the right side of the body: the tip of the small finger, forearm, and calf.

Data processing

All data was processed using customized Python (3.9) scripts. Data preprocessing for the sound task and tactile sensitivity task was conducted in Excel (version 2208).

Pupil size data was presented as change from baseline (i.e., last 50 ms of the pre-stimulus baseline period), expressed in arbitrary units over time, downsampled to 100 Hz. Negative values connoted pupil constriction, positive values pupil dilation. Pupil response was defined as pupil size derivative, indicating whether there was a change in the amount of increase (positive derivative) or decrease (negative derivative) in pupil size, or no in- or decrease of the degree of change (derivative of 0). Pupil response was presented in arbitrary units over time. Pupil response data was filtered using a lowpass Butterworth filter, with a critical frequency of 18 Hz and an order of 3. Only the first peak in pupil response was interpreted.

Statistical tests were performed two-sided, with $\alpha = 0.05$. Assumptions of sphericity and normality for the Repeated Measured ANOVA were checked using Mauchly's test of sphericity and the Shapiro-Wilk test respectively. When either or both of these assumptions were violated ($\alpha = 0.05$), a non-parametric Friedman's ANOVA was used and where applicable, non-parametric Wilcoxon signed-rank tests for post-hoc testing. The assumption of homoscedasticity for the Pearson correlation was checked using Levene's test and when this assumption was violated ($\alpha = 0.05$), a non-parametric Spearman's rank correlation test was used.

Results

To examine pupil response after tactile stimulation, a linear mixed effects model (LME) was used. The best model was determined by using the Bayesian Information Criterion (BIC), with the dependent variable being pupil response, or maximum amplitude of pupil response, with the independent variables being stimulus location, trial number within a block, and block number, with random intercepts for each participant. Trial number within a block and block number were included in the model to control for possible habituation effects. See Figure 6a for the average pupil size after tactile stimulation and Figure 6b for the pupil response after tactile stimulation.

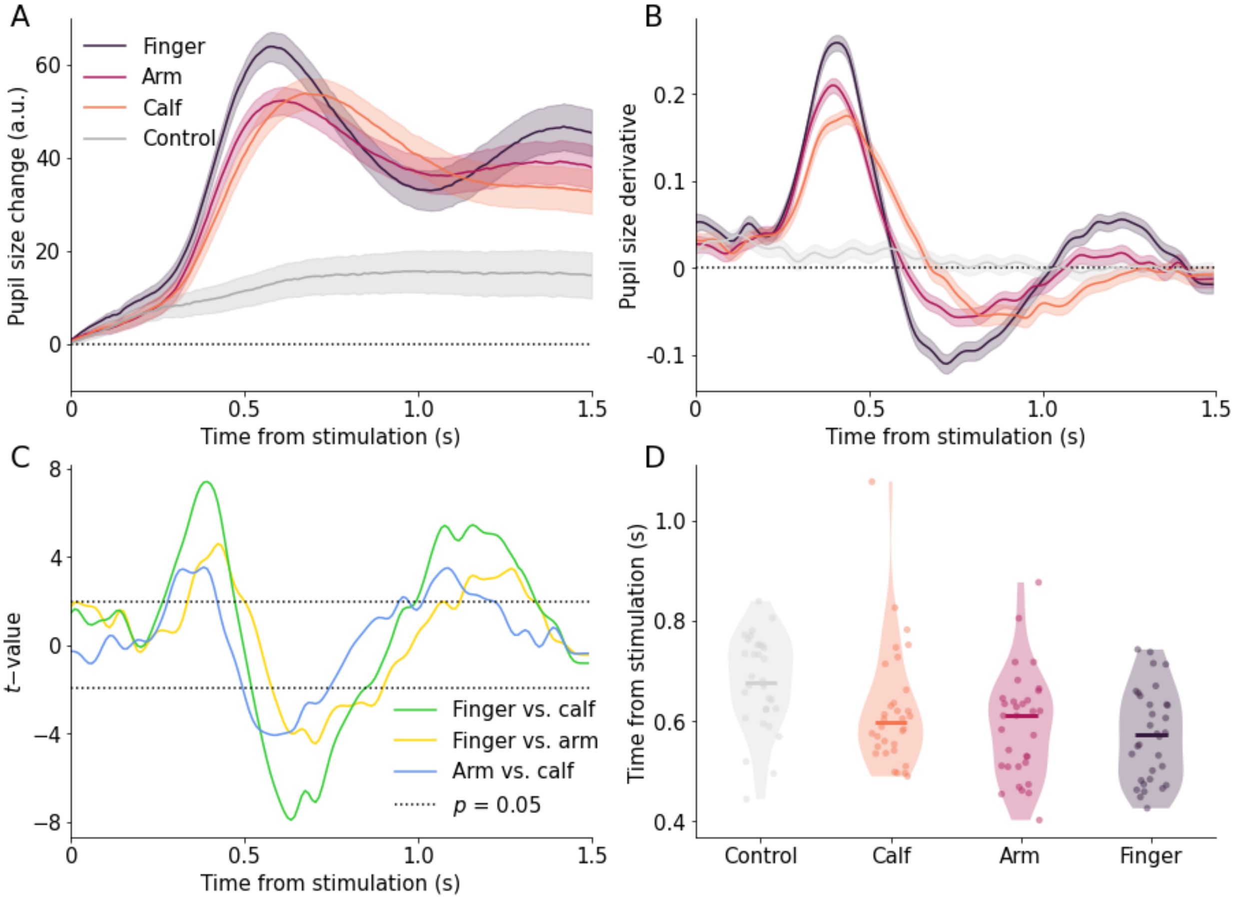
Pupil response

Tactile stimulation on the finger elicited a larger pupil response than tactile stimulation on the arm and calf and tactile stimulation on the arm elicited a larger pupil response than tactile stimulation on the calf, Figure 6c. In addition, an effect of tactile stimulation could be seen, with tactile stimulation on all three locations being significant predictors of pupil response compared to the control condition (Supplementary Figure B2). Moreover, based on the sound detection task, most participants were not sensitive in distinguishing tapper trials from silent trials (*d'* = 0), one participant showed a sensitivity to distinguish tapper and silent trials of *d'* = 0.44, and three participants showed an inverse sensitivity to distinguish tapper and silent trials (*d'* = -0.15, *d'* = -0.68, and *d'* = -1.04). Changes in pupil size can, therefore, also not (fully) be explained by anticipation or sound of the stimulation. See Supplementary Figure B1 for pupil response per participant.

Differences in pupil response after tactile stimulation of different locations might be explained by differences in pupil response latency, Figure 6d. Time to maximum pupil response, compared using Friedman's ANOVA, differed between the three stimulation locations ($\chi^2(1596) = 7.76$, $p = 0.021$), meaning that differences in pupil response after tactile stimulation may (partially) be explained by differences in latency of the pupil response. Post-hoc pairwise testing showed that maximum pupil response was later in for calf than for finger ($W = 139053$, $p = 0.037$, $r = -0.10$), but no differences in time to maximum pupil response between calf and arm and between arm and finger ($W = 145287$, $p = 0.200$, $r = -0.06$; $W = 151355$, $p = 0.564$, $r = -0.02$ respectively; Holm-Bonferroni correction was applied on p values).

Figure 6

Pupil response compared between stimulus locations over time



Note. A). Baseline-corrected pupil traces over time, averaged per stimulation location and control location. The y-axis depicts pupil size change compared to the baseline period, expressed in arbitrary units (a.u.), with positive values indicating pupil dilation and negative values indicating pupil constriction. Error bands show one standard error above and below average. B) Pupil response derivative traces over time, averaged per stimulation location. The y-axis depicts the change in the amount of pupil size increase (positive values) or decrease (negative values) compared to the previous time point. Error bands show one standard error above and below average. C) Linear mixed effects model for pupil response comparing t-values between stimulus locations over time. Each line depicts the t-values of the comparisons between stimulus locations on pupil response after tactile stimulation over time, with an additive effect of block number and trial number within a block and random intercepts for each participant. The dotted line depicts

$t = |1.96|$, equal to $p = 0.05$. D) Time to maximum pupil response, averaged per participant and split between stimulation locations. Horizontal line depicts median score.

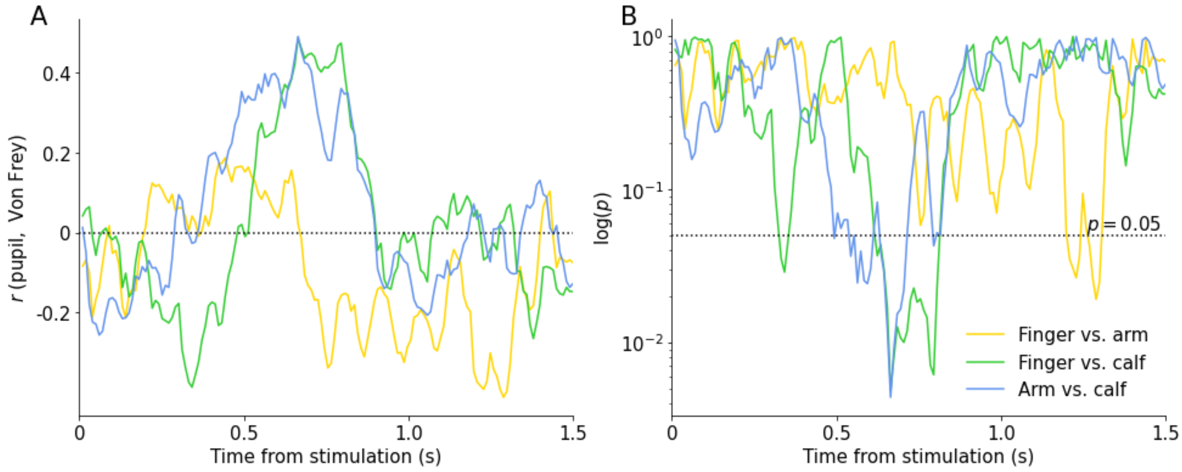
Tactile sensitivity and pupil response

Differences in pupil response after tactile stimulation on different locations may be driven by differential tactile sensitivity of the stimulation locations. Tactile sensitivity thresholds per stimulation location, assessed using Von Frey monofilaments, are depicted in Supplementary Figure B3. Tactile sensitivity differed between stimulation locations (χ^2 (62) = 44.43, $p < 0.001$), compared using Friedman's ANOVA. Post-hoc pairwise comparisons indicated that tactile sensitivity for all locations differed significantly from each other (finger and arm: $W = 1$, $p < 0.001$, $r = -0.86$; finger and calf: $W = 1$, $p < 0.001$, $r = -0.87$; arm and calf: $W = 65$, $p < 0.001$, $r = -0.66$; Holm-Bonferroni correction was applied on p values).

When comparing tactile sensitivity and pupil response differences between stimulation locations, averaged between participants, over time, the Spearman correlation for the difference between finger and calf was significant between 0.32 s and 0.34 s and between 0.61 s and 0.79 after tactile stimulation, the Spearman correlation for the difference between arm and calf was significant at 0.48 s, 0.53 s, between 0.55 s and 0.69 s, and at 0.78 s after tactile stimulation, and the Spearman correlation for the difference between finger and arm was significant between 1.19 s and 1.22 s and between 1.25 s and 1.28 s, Figure 7. Thus, for a small amount of time after tactile stimulation there is a relationship between pupil response differences and tactile sensitivity differences, but not in the timeframe of interest (i.e., the first peak in pupil response).

Figure 7

Relationship between tactile sensitivity and pupil response differences



Note. A) Spearman correlation for the differences between stimulation locations for pupil response and tactile sensitivity thresholds over time, averaged per participant. B) Spearman correlation logarithmically transformed p-value for the differences between stimulation locations for pupil response and tactile sensitivity thresholds over time, averaged per participant. The dotted line depicts $p = 0.05$.

Interim discussion

The aim of Experiment 1 was to investigate whether differential tactile sensitivity across the body is reflected in the strength of pupil response after tactile stimulation, using an improved experimental design as compared to the pilot. Indeed, after tactile stimulation on the finger, a larger pupil response followed compared to the arm and calf. Additionally, a clear effect of tactile stimulation was seen for pupil response. Differences in pupil responses after tactile stimulation were partially explained by a difference in latency, which suggests the difference in pupil response is mainly due to a difference in amplitude. While differences in pupil response were found between stimulation locations, tactile sensitivity as measured by Von Frey monofilaments, the minimal amount of force that is perceived, were only related to each other for a small portion of time after tactile stimulation, and not within the timeframe of interest (i.e., the first peak in pupil response).

When examining pupil size over time in the control condition, no clear in- or decrease in pupil size was seen and participants consistently could not distinguish trials in which a tapper did and did not fire, which suggests that the sound or expectancy effects of Experiment 1 were reduced or resolved.

Thus, the current experiment found differences in pupil response after tactile stimulation on different locations of the body. These differences could be explained by a difference in tactile sensitivity or perceived intensity of the stimulus. However, these differences could also (partly) be explained by differences inherent to the different body locations used e.g. different types of receptors on the skin, differential representation in the brain, or differences in proportion of soft tissue and bone. Experiment 2 therefore examined pupil response after tactile stimulation of varying intensities on a single location on the body, which gave the possibility to test the hypothesis described in Van Hooijdonk and colleagues (2019) that the strength of tactile stimulation modulates pupil response. This leads to the following research question: Is differential stimulus intensity reflected in how strongly the pupil responds to vibrotactile stimulation? In addition to the main research question, explorative tests were done to examine possible relationships between conscious perception of differences in vibrotactile stimulus intensity.

Experiment 2

Methods

The research and consent procedures were performed in accordance with the standards of the Declaration of Helsinki. Ethical approval was obtained from the Ethics Review Board of the Faculty of Social & Behavioural Sciences (protocol number 23-1738).

Participants

In total, 20 healthy participants (6 male, 14 female; all but 1 right-handed; the author IH was one of the participants) between the ages of 18 and 30 years old (M_{age} =24.55 years, SD_{age} = 2.87), with normal or corrected-to-normal vision participated. None of the participants reported impaired or irregular touch sensation or a history of neurological disorders. Participants were compensated monetarily or in course credits. Participants all gave written informed consent.

Apparatus

The apparatus to measure pupil response was the same as that used in the pilot and Experiment 1. Tactile stimulation was provided using a tactor (Dancer Design, United Kingdom), a miniature electromagnetic solenoid-type stimulator. In the current experiment, the tactor provided vibrotactile stimulation at 40 Hz. No information about absolute vibration intensities of the stimulus was available.

Procedure

The tactile stimulation task always preceded the discrimination task. Participants had to wear earplugs to control for the sound the tactor produced. The tactor was applied on the tip of the small finger on the right hand, obstructed from view to prevent visual enhancement of touch increasing tactile sensitivity (Colino et al., 2017). A foam ring was placed at the base of the small finger to prevent the small finger from touching the ring finger.

Tactile stimulation

Four different levels of stimulus intensity were provided in the current task: 0, 20, 50, and 100% intensity. The intensity of the vibrotactile stimuli is exponentially related to the perceived intensity of vibrotactile stimuli, which is why the current intensity levels were chosen (Stevens, 1959).

A nine-point calibration and validation of the eye-tracker was performed at the start of each block and when deemed necessary. The experiment consisted of two blocks, containing 100 trials each. To ensure a balanced presentation of the different stimulus intensities, stimulus intensities were randomized within clusters of 20 trials. Participants had to fixate a central cross throughout the trial. A trial started with a variable baseline period of 1.5 to 2.5 s. Next, tactile stimulation occurred for 80 ms and the pupil response was captured for 1.58 s after stimulus onset. A trial was considered invalid if the participants gazed >3 visual degrees from the center or blinked for >200 ms during the measurement period. Feedback was provided via a red cross (invalid) or grey cross (valid) of 0.5 s, followed by a variable inter-trial interval of 1.5 s. Invalid trials were repeated at the end of each block.

Discrimination of stimulus intensity

The discrimination task was conducted to test whether participants could distinguish the different stimulus intensities used in the tactile stimulation task. This task consisted of 30 trials, which started with the presentation of a vibrotactile stimulus for 80 ms, a break of 1 s, and the presentation of a vibrotactile stimulus of a different intensity for 80 ms. The order of the stimulus intensities was randomized between participants. Consecutively, the participant had to respond via keypress whether they perceived the first of the second vibration to be stronger, and via mouse click how certain they were of this choice on a scale from 0 (not sure at all) to 100 (completely certain).

Data processing

All data was processed using customized Python (3.9) scripts. Data preprocessing for the discrimination task was conducted in Excel (version 2208). Stimulus intensity was regarded as an ordinal variable (20%: weak intensity, 50%: medium intensity, 100%: strong intensity).

Pupil size data was presented as change from baseline (i.e., last 50 ms of the pre-stimulus baseline period), expressed in arbitrary units over time, downsampled to 100 Hz. Negative values connoted pupil constriction, positive values pupil dilation. Pupil response was defined as pupil size derivative data, indicating whether there was a change in the amount of increase (positive derivative) or decrease (negative derivative) in pupil size, or no in- or decrease of the degree of change (derivative of 0). Pupil response was presented in arbitrary units over time. Pupil response data was filtered using a lowpass Butterworth filter, with a critical frequency of 18 Hz and an order of 3. Only the first peak in pupil response was interpreted.

Statistical tests were performed two-sided, with $\alpha = 0.05$. To examine pupil response and the derivative of pupil response after tactile stimulation, a linear mixed effects model (LME) was used. An effect of trial number was added to the LME to account for possible habituation effects and a random intercept was added for participants to account for possible differences between participants.

Assumptions of sphericity and normality for the Repeated Measured ANOVA were checked using Mauchly's test of sphericity and the Shapiro-Wilk test respectively. When either or both assumptions were violated ($\alpha = 0.05$), a non-parametric Friedman's ANOVA was used and where applicable, non-parametric Wilcoxon signed-rank tests for post-hoc testing. The assumption of homoscedasticity for the Pearson correlation was checked using Levene's test and when this assumption was violated ($\alpha = 0.05$), a non-parametric Spearman's rank correlation test was used.

Results

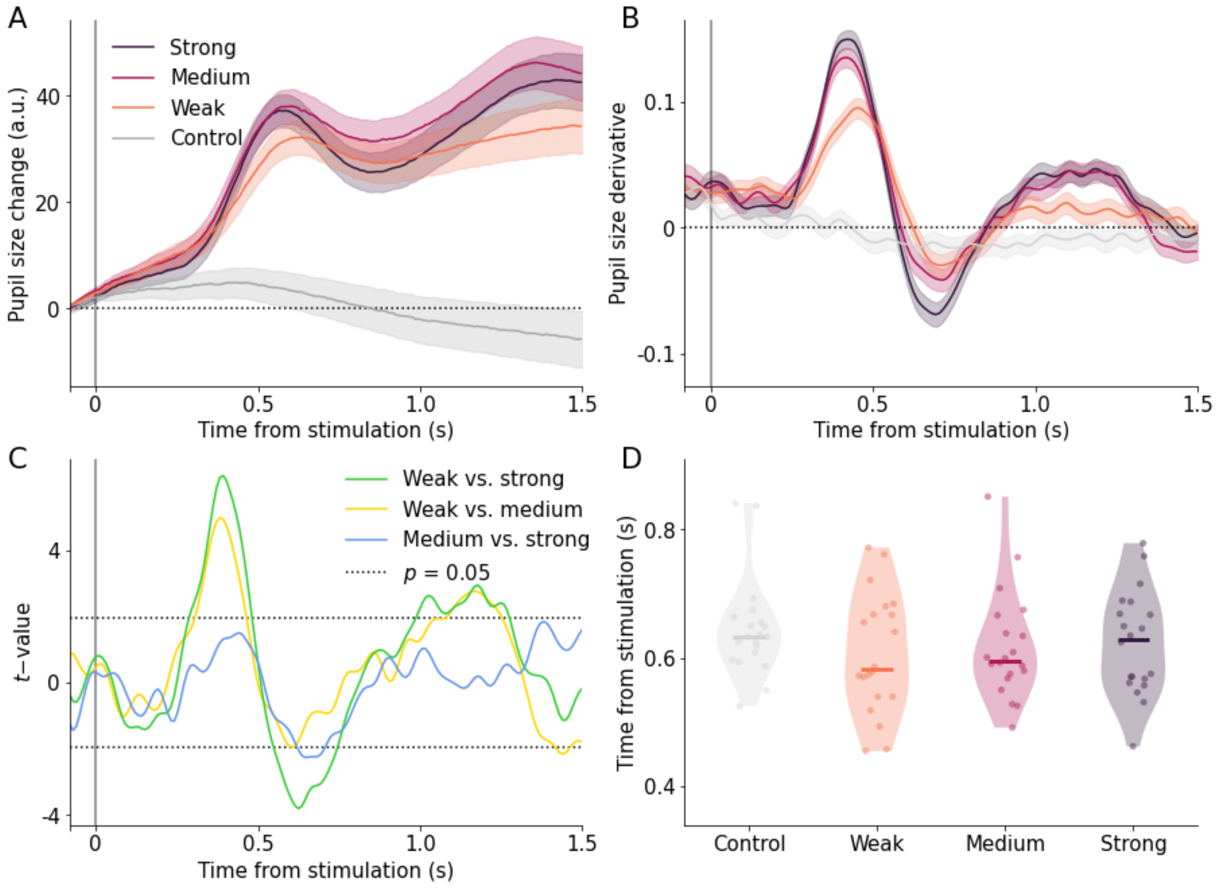
Pupil response

See Figure 8a and 8b for the average pupil size and pupil response split by stimulus intensity. Tactile stimulation of weak intensity elicited a smaller pupil response than tactile stimulation of medium and strong intensity (see Figure 8c for LME model t-values of differences between stimulation locations over time). In addition, a general effect of tactile stimulation on pupil response could be seen, with tactile stimulation of all three intensities being significant predictors of pupil response compared to the control condition (See Supplementary Figure C2 for LME model t-values of differences between control and stimulation locations over time).

Differences in pupil response after tactile stimulation of different intensities might be explained by differences in pupil response latency. Time to maximum pupil response, as compared using Friedman's ANOVA, did not differ between the three stimulation locations ($\chi^2(1998) = 0.58$, $p = 0.748$), meaning that differences in pupil response after tactile stimulation cannot be explained by differences in latency of the pupil response (Figure 8d).

Figure 8

Pupil response compared between stimulus locations over time



Note. A) Baseline-corrected pupil traces over time, averaged per stimulation intensity and control. The y-axis depicts pupil size change compared to the baseline period, expressed in arbitrary units (a.u.), with positive values indicating pupil dilation and negative values indicating pupil constriction. Error bands show one standard error above and below average. B) Pupil response derivative traces over time, averaged per stimulation intensity. The y-axis depicts the change in the amount of pupil size increase (positive values) or decrease (negative values) compared to the previous time point. Error bands show one standard error above and below average. C) Linear mixed effects model for pupil response comparing t-values between stimulus intensities over time. Each line depicts the t-values of the comparisons between stimulus intensity on pupil response after tactile stimulation over time, with an additive effect of trial number and random intercepts for each participant. The dotted line depicts $t = |1.96|$, equal to $p = 0.05$. D) Time to maximum pupil response, averaged per participant and split between stimulation intensities. Horizontal line depicts median score.

Discrimination of stimulus intensity

Due to issues during data collection, no data on the discrimination task was available for participant 2 and this participant was excluded for the consecutive analyses.

Differences in pupil response after tactile stimulation of differential intensities may be driven by differences in the ability to consciously discriminate the differences in intensity of tactile stimulation. Correct answers on the discrimination task are depicted in Supplementary Figure C3a. To check whether the number of correct answers was different from chance level (5 out of 10 correct answers), a Wilcoxon signed-rank test was used for the weak versus medium and weak versus strong intensity conditions and a one sample t-test for the medium versus strong intensity condition. The number of correct answers was above chance level for all intensity conditions (weak versus medium: $W = 0$, $p < 0.001$, $r = -0.92$; weak versus strong: $W = 1$, $p < 0.001$, $r = -0.92$; medium versus strong: $t(18) = 5.22$, $p < 0.001$, $d = 3.92$). The number of correct answers differed between conditions ($\chi^2(38) = 26.63$, $p < 0.001$), as tested using a Friedman's ANOVA. Post-hoc pairwise comparisons indicated that the number of correct answers in the medium versus strong intensity condition was lower than the weak versus strong intensity condition ($W = 0$, $p = 0.002$, $r = -0.82$) and the weak versus medium intensity condition ($W = 0$, $p = 0.001$, $r = -0.85$), but no differences were seen between the weak versus medium intensity condition and the weak versus strong intensity condition ($W = 6$, $p = 0.655$, $r = -0.10$; Holm-Bonferroni correction was applied on p values).

Moreover, there were also differences in the certainty the participants had about their response on the forced-choice task based on the Friedman's ANOVA ($\chi^2(38) = 25.62$, $p < 0.001$), comparing weak versus medium, weak versus strong, and medium versus strong stimulus intensities, Supplementary Figure C3b. Post-hoc pairwise comparisons showed that the certainty of response in the medium versus strong intensity condition was lower than the weak versus strong intensity condition ($W = 5$, $p < 0.001$, $r = 0.83$) and the weak versus medium intensity condition ($W = 1$, $p < 0.001$, $r = 0.87$), but no differences between the weak versus medium intensity condition and the weak versus strong intensity condition ($W = 44$, $p = 0.124$, $r = 0.38$; Holm-Bonferroni correction was applied on p values), which is in line with the results regarding correct answers.

To summarize, participants were worse at distinguishing tactile stimulation of medium and strong intensity compared to weak and medium and compared to weak and strong, and were less certain about their answers.

Discrimination and pupil response

As an explorative analysis, the relationship between the ability to discriminate between two stimulus intensities and the difference in pupil responses was examined. Due to the limited variation in scores on the discrimination task and certainty task for the weak versus medium intensity and weak versus strong intensity conditions, only the medium versus strong intensity condition was used in comparisons with pupil response.

When comparing number of correct answers and pupil response differences between medium and strong intensities, averaged between participants, over time, the Spearman correlation was significant between 0.07 s and 0.09 s after tactile stimulation. When comparing certainty of answers and pupil response differences between medium and strong intensities, averaged between participants, over time, the Spearman correlation was significant at 1.30 s, 1.31 s, 1.41 s, and 1.42 s after tactile stimulation, Supplementary Figure C4. Thus, there seems to be no relationship between tactile discrimination and pupil response differences between medium and strong stimulation intensities.

Interim discussion

The aim of Experiment 2 was to investigate whether differential tactile stimulus intensity was reflected in the strength of pupil response after tactile stimulation. As hypothesized, pupil response was smaller for weak stimulus intensities compared to medium and strong stimulus intensities. When comparing pupil response in conditions in which tactile stimulation was provided, versus a control condition in which no stimulation was provided, a clear effect of tactile stimulation was seen.

When participants were asked to distinguish between the different stimulus intensities, they were less able to distinguish medium and strong stimulus intensities than other stimulus intensity pairs and were less certain of their answers. Participants were almost always able to distinguish weak from medium or strong stimulus intensities, and were in general highly certain about their ability to distinguish these specific stimulus intensity pairs. These results are in line with the non-linear relationship between stimulus intensity and perceived stimulus intensity (Stevens, 1959). No relationship was found between the ability to distinguish different intensities of tactile stimulation and pupil response or between the certainty of answers and pupil response, which might be explained by the large variation in pupil response between participants (Supplementary Figure C3).

General discussion

The current results showed differences in pupil response after tactile stimulation on body locations with differential tactile sensitivity. In addition, the intensity of vibrotactile stimuli was reflected in the strength of pupil response after tactile stimulation.

The current results indicate that tactile stimulation on more sensitive body locations was followed by a larger pupil response than tactile stimulation on less sensitive body locations. Tactile sensitivity was different between different body locations with differential tactile sensitivity, but there was no direct relationship between pupil response differences and differences in tactile sensitivity. One alternative explanation for the differences in pupil response could be that tactile stimulation is processed differently on glabrous (non-hairy) and non-glabrous (hairy) skin, due to factors including differential mechanoreceptor types and densities across the skin and thickness of the skin (Delhaye et al., 2018; McGrath et al., 2014). The glabrous skin of the finger has a higher density of mechanoreceptors and a thick epidermis compared to the non-glabrous skin of the arm and calf, possibly resulting in differential response after tactile stimulation. Moreover, the current results indicate that the intensity of tactile stimulation is reflected in pupil response, which is similar to the finding of Van Hooijdonk and colleagues (2019) who reported differential pupil response after tactile stimulation of differential intensity (i.e., slower or faster stroking). In addition, the current results are also in line with research on painful stimuli, in which it was found that an increase in pupil response after presenting noxious thermal stimuli of increased intensity or for longer periods of time (Drummond & Clark, 2023; Eisenach et al., 2017).

The pattern of pupil response differences was similar for tactile stimulation on different body locations and tactile stimulation of differential intensity. This suggests that tactile stimulation on a body location with high tactile sensitivity is perceived or processed as a more intense stimulus. A possible mechanism could be differential activation of the salience network. Pupil dilation is related to activity in the salience network, so differential pupil response may be modulated by differential activation of the salience network (Schneider et al., 2016).

Future directions

Further research could implement a wider range of vibrotactile stimulus intensities to elicit a wider range of pupil responses to investigate whether pupil response to tactile stimulation of differential intensity shows a gradual relationship with pupil response. In addition, further research could implement a range of vibrotactile stimulus frequencies to stimulate different types of mechanoreceptors in the skin (Delhaye et al., 2018). Moreover, the current study could be replicated using a longitudinal design, to elucidate the within-participant variability in pupil response after tactile stimulation to get insight in the applicability of pupillometry as a reliable measure for tactile sensitivity. To investigate at which level tactile stimulation is processed and whether conscious perception of tactile stimulation affects pupil response, pupil response could be measured after tactile stimulation on numbed skin, using local anesthesia. Resulting insights might be used to investigate residual processing of touch in patients who do not experience touch sensation to predict recovery or outcomes of rehabilitation therapy. Pupil response scales with the intensity of electrical stimuli when patients are under general anesthesia, but no research has been done into pupil response after non-noxious tactile stimulation on a body location under local anesthesia (Sabourdin et al., 2018; Wildemeersch et al., 2018). In addition, investigating pupil response after tactile stimulation in patients with hypersensitivity may also provide information on the relationship between stimulus intensity and perceived stimulus intensity.

Limitations

The reliability of the results of Experiment 1 was impacted by the device used to provide tactile stimulation. Due to the variable and not controllable stimulus intensity and timing, and the sound levels of this device, several precautions had to be taken when designing the experiment. To account for variable stimulus intensity between different tappers, a block design was implemented in the tactile stimulation task of Experiment 1 using a single tapper for each body location. This resulted in order effects on pupil response after tactile stimulation, which were corrected for in the statistical analysis. To account for the sound levels of the device, participants had to wear headphones playing brown noise during the tactile stimulation task in Experiment 1, the sound of which may have influenced pupil response (Wang et al., 2017). Despite possible influences of sound levels of the tactile stimulation device and headphones playing noise, a distinction in pupil response was apparent between control and stimulus conditions for Experiment 1. Future studies could provide tactile stimulation at multiple body areas in a mixed design, using a device that provides more consistent and controllable tactile stimulation, for example the tactor (Dancer Design, United Kingdom). The reliability of the results of Experiment 2 was impacted by the use of vibrating stimuli of different intensities. A larger intensity vibration may spread to nearby locations on the skin, thus not only activating mechanoreceptors on the intended location on the skin, but also activating mechanoreceptors on nearby locations. As a result, differences in pupil response between intensity conditions may not solely be the result of more intense tactile stimulation, but also of more widespread tactile stimulation.

Conclusion

To conclude, in the current study it was found that the tactile sensitivity at different body locations and differential stimulus intensity were reflected in pupil response, which may indicate that pupil response conveys perceived stimulus intensity. Tactile stimulation on body locations with high tactile sensitivity may be perceived or processed as an intense tactile stimulus, based on pupil response measurements in the current study. Pupil response may have the potential to be used as an objective index for tactile sensitivity which is not reliant on verbal response from patients.

Plain language summary

The pupil responds to many influences, including the presentation of new stimuli. For visual and auditory stimuli, research has shown that the larger the intensity of the stimulus, the more the pupil responds afterwards. For touch processing, a limited amount of research has been done on the relationship between intensity of touch and pupil response, but there is evidence that an increase in intensity of a tactile stimulus is followed by a larger pupil response. For example, in one study in which they stroked the back of the hand of participants, it was found that the faster the stroking was, the larger the pupil response would be. Tactile sensitivity can be defined as the minimal sensitivity to perceive touch, which is different for different people and differs between different locations on the body. Based on previous research, it may be hypothesized that touching a more sensitive body location is perceived as more intense and results in a larger pupil response. In the current study, pupil response after touching body locations with different sensitivities was measured, and pupil response after providing touch of different intensities.

Pupil response was measured using an eye-tracker, and tactile stimulation was provided using an automated device. In Experiment 1, participants did two tasks: they looked at the center of the screen while tactile stimulation was provided to small finger, forearm, and calf and the following pupil response was measured, and they had to report whether they felt small hairs, which had different weights, touching their skin. The goals of these tasks were to measure pupil response after touch on different body locations and to measure tactile sensitivity on these body locations, respectively. Pupil response was larger after touch was provided to the finger than to the arm or calf and pupil response was larger after touch was provided to the arm than the calf.

In Experiment 2, participants had to look at the center of the screen while tactile stimulation of different intensities was provided to the small finger and the following pupil response was measured, and they had to distinguish tactile stimulation of different intensities. The goals of these tasks were to measure pupil response after tactile stimulation of different intensities and to assess whether participants could distinguish these intensities. Pupil response was smaller after weak touch was provided than after medium or strong touch. In addition, people were able to distinguish weak from medium and strong stimuli, but they were not able to distinguish medium and strong stimuli.

If the results from Experiment 1 and 2 are combined, this would suggest that that providing touch at a more sensitive location is perceived or processed as a more intense stimulus. In the current study, it is shown that pupil response has the potential to be used as an index for tactile sensitivity, which could possibly be applied for measuring tactile sensitivity in patients.

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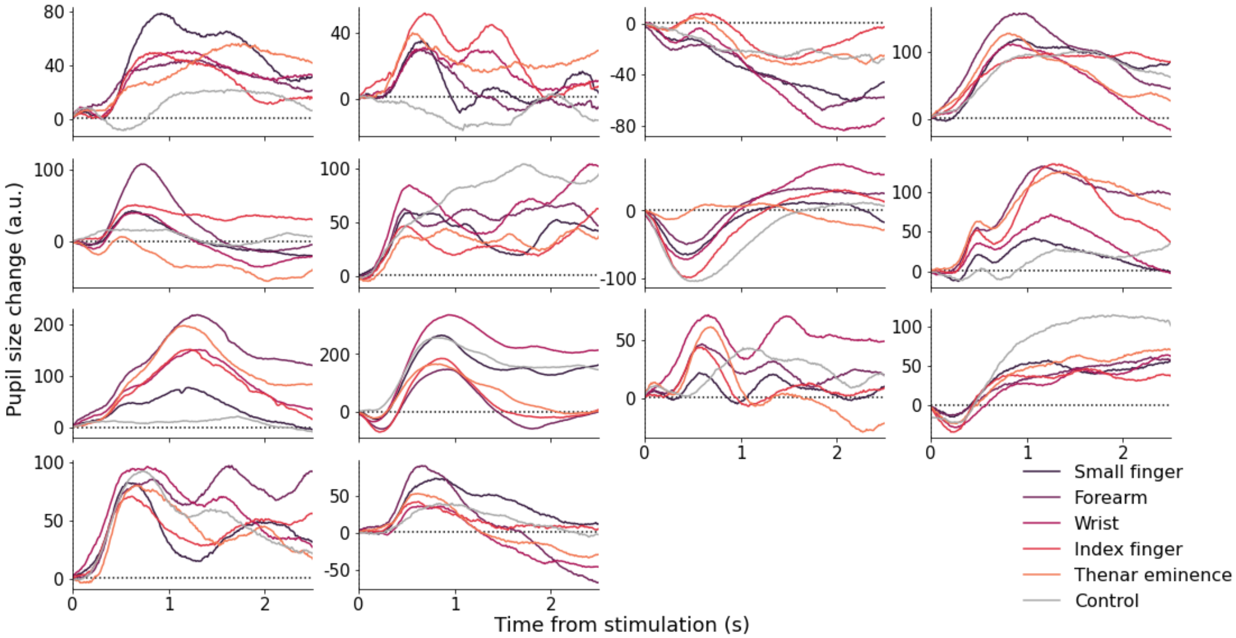
Appendix A

Supplementary Figures and Tables Pilot

Figure A1

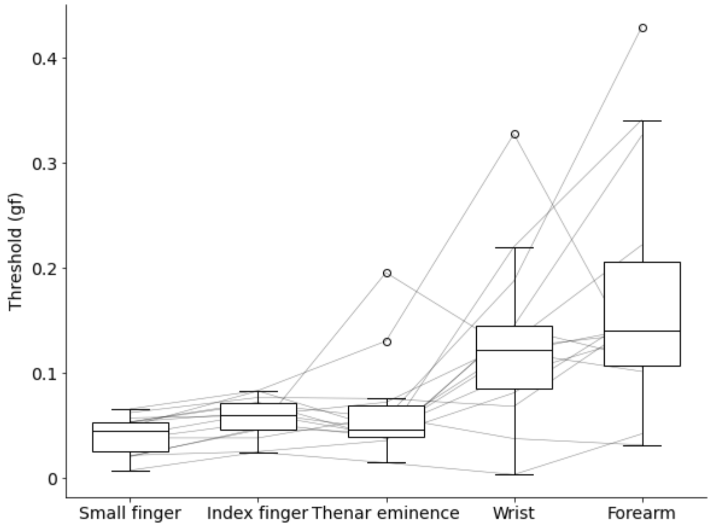
Average pupil response split by stimulation intensity and participant



Note. Each subplot depicts the baseline-corrected pupil traces over time, averaged per stimulus location and control location. The x-axis depicts time after stimulus onset in seconds. The y-axis depicts pupil size change compared to the baseline period, expressed in arbitrary units (a.u.). Positive values on the y-axis indicate pupil dilation, negative values indicate pupil constriction.

Figure A2

Scores on tactile sensitivity task split per stimulation location



Note. Boxplot depicting tactile sensitivity threshold values in grams of force (gf), assessed using Von Frey monofilaments, for the small finger, index finger, thenar eminence, wrist, and forearm. All five stimulation locations are depicted on the x-axis. Higher values on the y-axis (threshold; gf) indicate a lower tactile sensitivity. Each box depicts the interquartile range, with the median being depicted as the horizontal line in the box. The whiskers depict the minimum and maximum values, with the exception of outliers. Outliers, y-axis values that are 1.5 times the interquartile range higher or lower than the upper or lower quartile respectively, are depicted as circles.

Table A1

Post-hoc pairwise comparisons of tactile sensitivity between stimulation locations

		W-value	Adj. p-value	Pearson’s r
Small finger	Index finger	0.0	0.010*	-0.87
	Thenar eminence	16.0	0.118	-0.55
	Wrist	3.0	0.005*	-0.83

	Forearm	1.0	0.002*	-0.86
Index finger	Thenar eminence	45.0	0.670	0.13
	Wrist	7.0	0.014*	-0.76
	Forearm	1.0	0.002*	-0.86
Thenar eminence	Wrist	13.0	0.043*	-0.66
	Forearm	11.0	0.034*	-0.70
Wrist	Forearm	22.0	0.118	-0.51

Note. A post-hoc Wilcoxon signed-ranks test to examine differences in tactile sensitivity thresholds between all stimulation locations. Tactile sensitivity thresholds were obtained using von Frey filaments. Holm-Bonferroni correction was applied to the p-value compensate for multiple tests. * $p < 0.05$, ** $p < 0.001$.

Appendix B

Supplementary Figures and Tables Experiment 1

Table B1

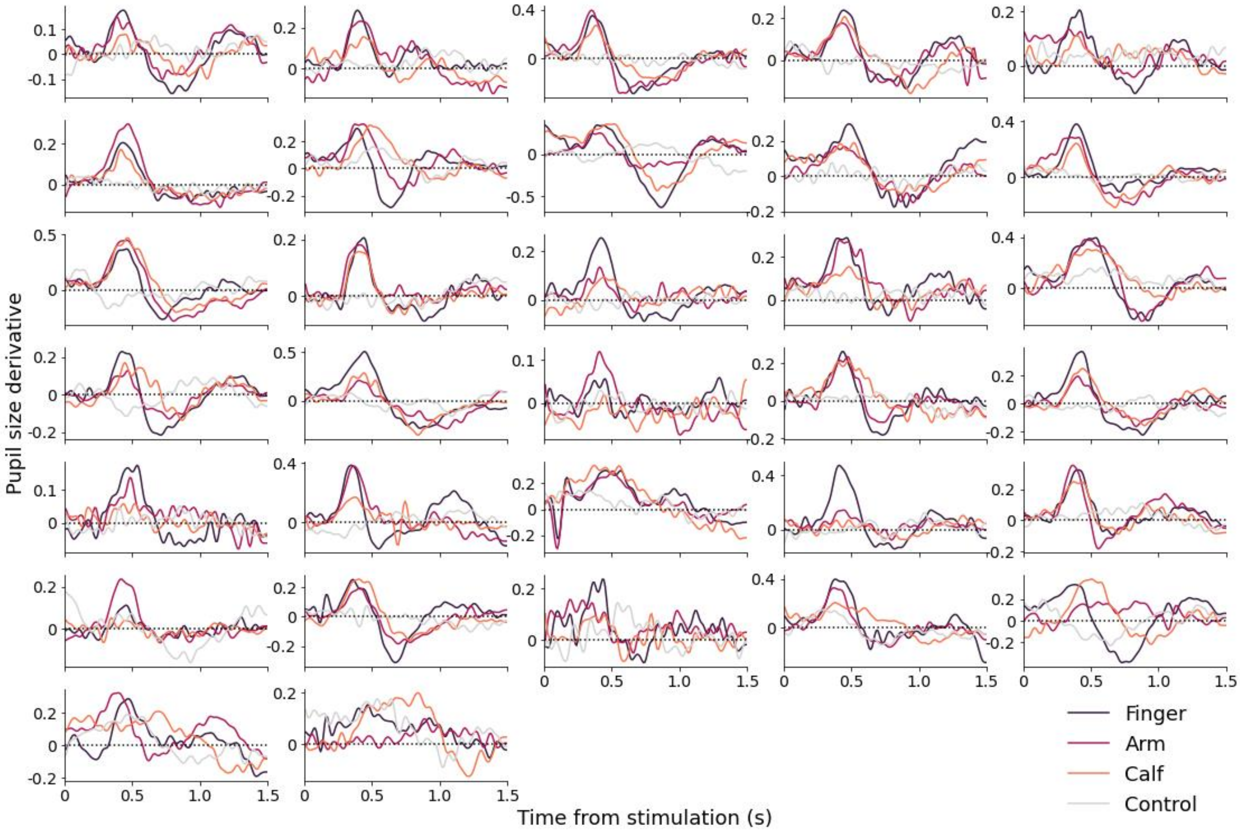
Linear mixed effects model for pupil response

	$t > \|1.96\|$
Block number	[0.33 s; 0.76 s] , [0.95 s; 1.46 s]
Trial number within block	[0 s; 0.67 s] , [0.77 s; 0.99 s]

Note. Each interval depicts the timepoints within the trial at which the parameters block number and trial number within block were significant predictors of pupil response. Alpha level is set at $\alpha = 0.05$, corresponding to $t = |1.96|$.

Figure B1

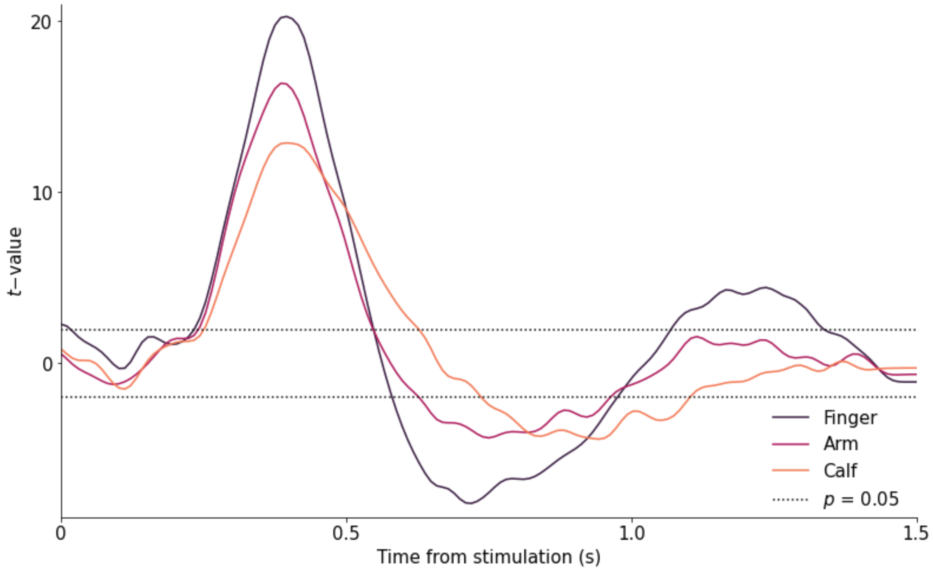
Average pupil response split by stimulation intensity and participant



Note. Pupil response over time, averaged per stimulation location. The x-axis depicts time after stimulus onset in seconds. The y-axis depicts the change in the amount of pupil size increase (positive values) or decrease (negative values) compared to the previous time point.

Figure B2

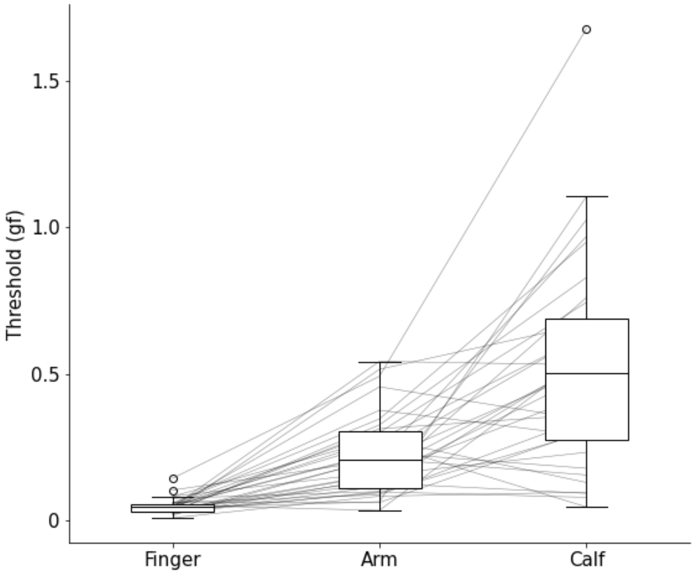
Linear mixed effect model for pupil response comparing t-values of control and stimulus locations over time



Note. Each line depicts the t-values of the comparison between the control location and a stimulus location on pupil response derivative after tactile stimulation over time, with an additive effect of block number and trial number within a block and random intercepts for each participant. The y-axis shows t-values values, with the dotted line depicting $t = |1.96|$, equal to $p = 0.05$. The x-axis shows time relative to stimulus onset in seconds.

Figure B3

Scores on tactile sensitivity task split per stimulation location



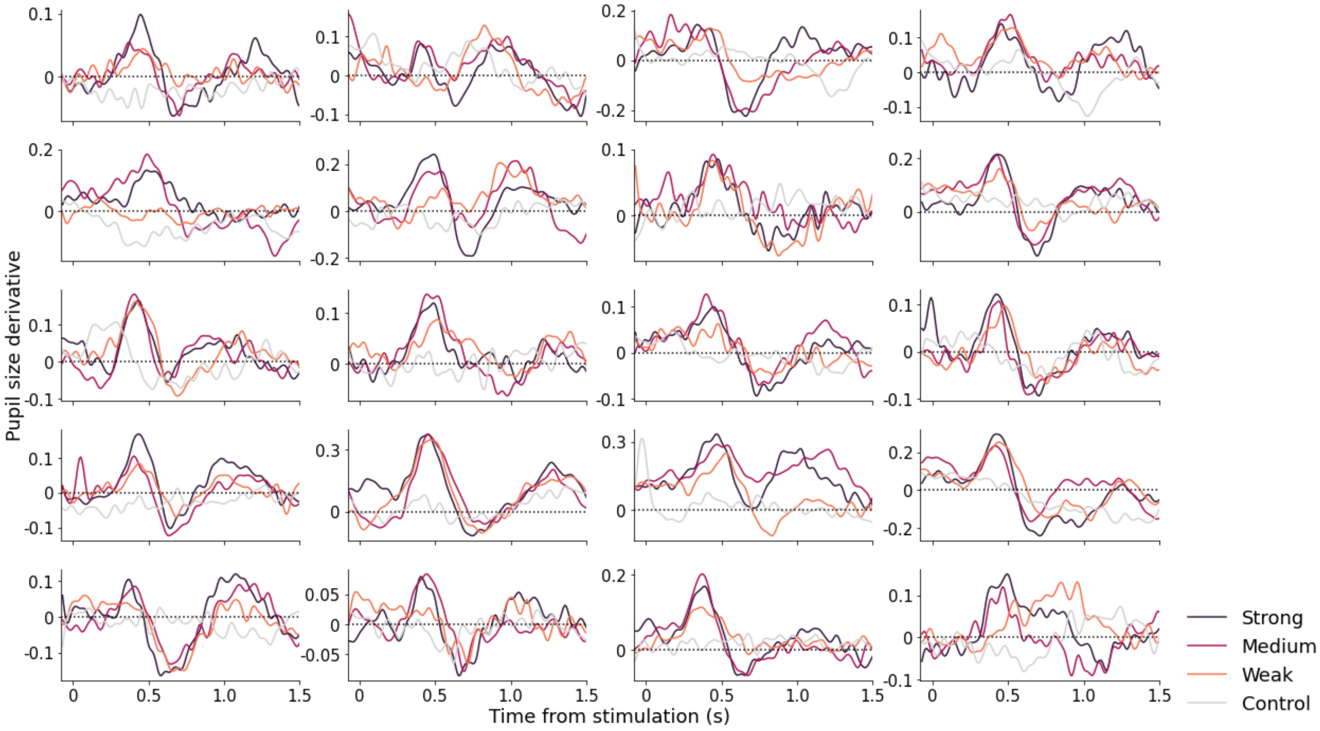
Note. Boxplot and line plot depicting tactile sensitivity threshold values in grams of force (gf), assessed using Von Frey monofilaments, for the finger, arm, and calf. All three stimulation locations are depicted on the x-axis. Higher values on the y-axis (threshold; gf) indicate a lower tactile sensitivity. Each box depicts the interquartile range, with the median being depicted as the horizontal line in the box. The whiskers depict the minimum and maximum values, with the exception of outliers. Outliers, y-axis values that are 1.5 times the interquartile range higher or lower than the upper or lower quartile respectively, are depicted as circles. Each line depicts tactile sensitivity thresholds for one participant.

Appendix C

Supplementary Figures Experiment 2

Figure C1

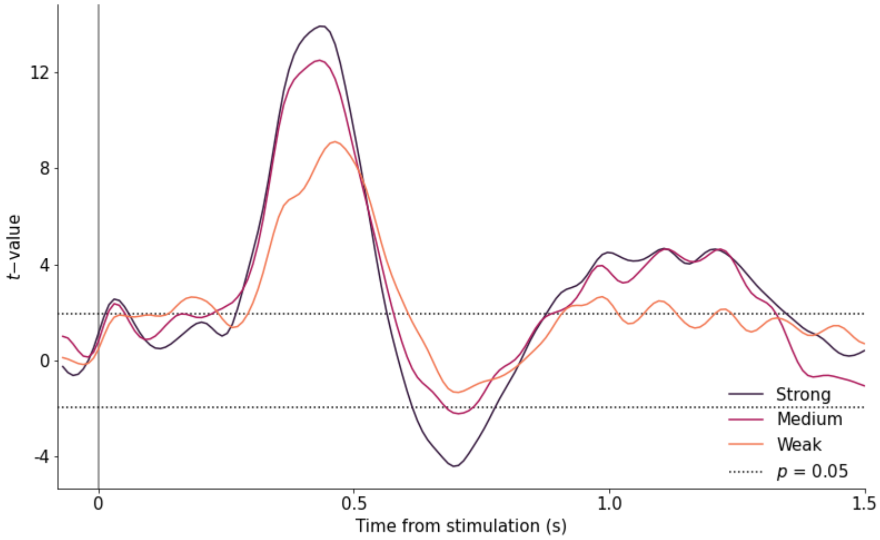
Average pupil response split by stimulation intensity and participant



Note. Pupil response over time, averaged per stimulation intensity. The x-axis depicts time after stimulus offset in seconds. The y-axis depicts the change in the amount of pupil size increase (positive values) or decrease (negative values) compared to the previous time point.

Figure C2

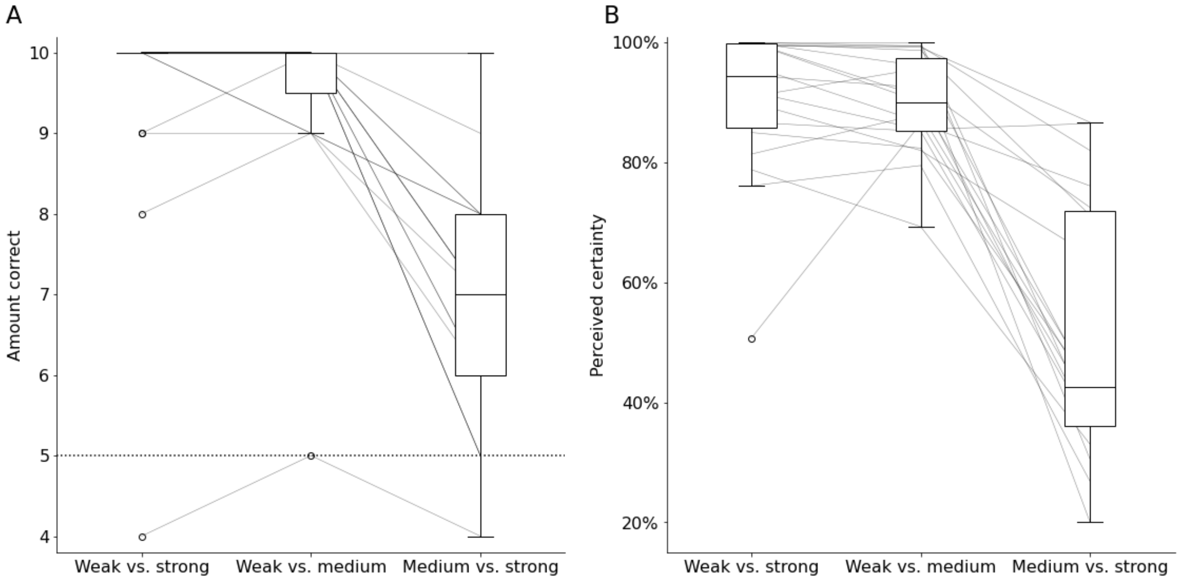
Linear mixed effect model for pupil response derivative comparing t-values of control and stimulus intensities over time



Note. Each line depicts the t-values of the comparison between the control location and a stimulus intensity on pupil response derivative after tactile stimulation over time, with an additive effect of trial number and random intercepts for each participant. The y-axis shows t-values values, with the dotted line depicting $t = |1.96|$, equal to $p = 0.05$. The x-axis shows time relative to stimulus offset in seconds.

Figure C3

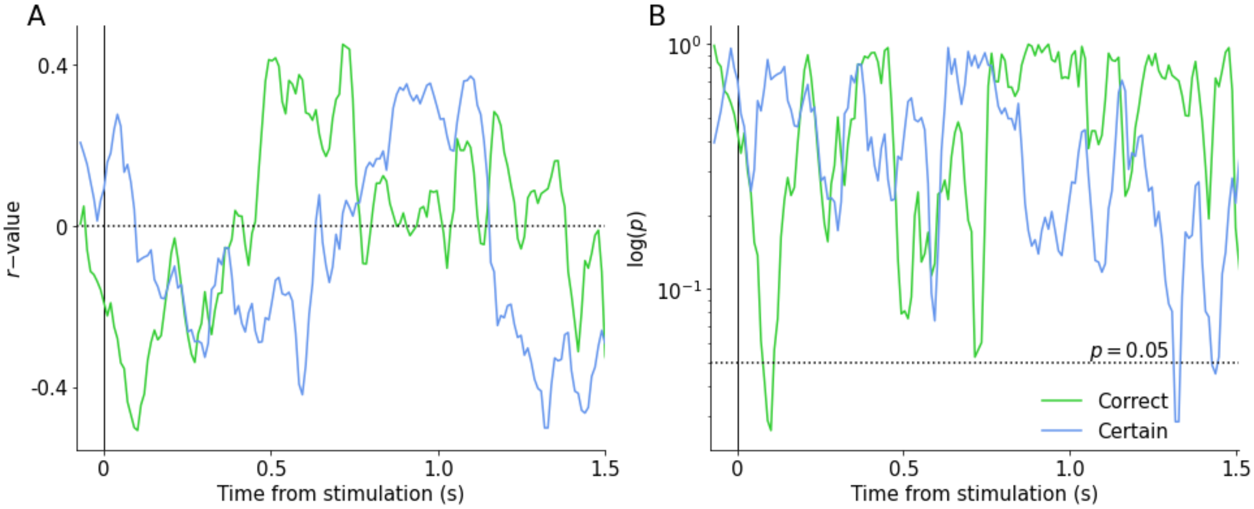
Scores on discrimination task split per stimulus intensity comparison



Note. A) Boxplot and line plot depicting the number of correct answers on the discrimination task. All three stimulus intensity comparisons are depicted on the x-axis. Each box depicts the interquartile range, with the median being depicted as the horizontal line in the box. Chance level lies at *y* = 5. Not all categories show a box, as a result of little variation in scores. B) The average of perceived certainty answers are depicted on the y-axis in percentages. For both figures, a boxplot and line plot depicted the number of correct answers or perceived certainty scores for the discrimination task. All three stimulus intensity comparisons are depicted on the x-axis. Each box depicts the interquartile range, with the median being depicted as the horizontal line in the box. The whiskers depict the minimum and maximum values, with the exception of outliers. Outliers, y-axis values that are 1.5 times the interquartile range higher or lower than the upper or lower quartile respectively, are depicted as circles. Each line depicts the certainty of answers for one participant

Figure C4

Relationship between tactile discrimination and pupil response differences



Note. A) Spearman correlation for the differences between stimulation intensities medium and weak for pupil response and tactile discrimination over time, averaged per participant. Lines depict number of correct answers and certainty of answers. B) Spearman correlation logarithmically transformed p-value for the differences between stimulation locations for pupil response and tactile sensitivity thresholds over time, averaged per participant. Dotted line depicts $p = 0.05$.