Changing the size of a mirror-reflected hand does not affect pain perception: a repeated measures study on healthy human participants

Running head: Mirror visual feedback and pain perception

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Significance: An adaptive phase with visuo-motor feedback enhances the perceptual experience of embodiment of a reflection of a hand and a painful stimulus does not weaken the experience. This should be considered when using visual feedback techniques for pain management.

ABSTRACT

Background: Studies suggest that observing magnified and minified body parts using mirrors, lenses and virtual reality may affect pain perception. However, the direction of effect varies between studies. The aim of the present study was to evaluate the effect of observing a normal-sized, magnified, and minified reflection of a hand on perceptual embodiment and contact-heat stimuli. Methods: Participants (n=46) observed a normal-sized, magnified and minified reflection of the hand and a no-reflection condition while performing synchronised finger movements for 3 min (adaptive phase). Measurements of embodiment were taken before adaptive phase, pre and post-contact-heat stimuli. Results: There were no differences in pain threshold nor tolerance between reflection and no-reflection conditions. Altering the size of the reflection of the hand did not affect estimates of pain threshold nor tolerance. The temperature for warm detection threshold was lower when participants were observing the magnified reflection of the hand compared with the no-reflection condition. Perceptual embodiment of the reflection of the hand was stronger after an adaptive phase with visuo-motor feedback, and the painful stimuli did not weaken the perceptual experience. Conclusion: observing a reflection of the hand in front of a mirror did not alter heat pain threshold nor tolerance when compared with a no-reflection condition, and altering the sized of the reflection did not affect pain perception. Researchers and clinicians using visual feedback techniques may consider including an adaptive phase with visuo-motor feedback to ensure facilitate embodiment of the viewed body part.

Key words

Mirror visual feedback, pain, pain threshold, perceptual embodiment, size distortion

INTRODUCTION

Mirror visual feedback, whereby a mirror is placed in a position so that the patient can view a reflection of a body part, is commonly used to relieve pain in limbs (Boesch et al., 2016; Thieme et al., 2016). The painful limb is hidden behind the mirror, whilst the non-painful limb is placed in front of the mirror creating the illusion of having two healthy, moving limbs (Ramachandran et al., 1995). It is also used in the rehabilitation of conditions where body parts feel large, heavy or swollen (e.g., complex regional pain syndrome) (Boesch et al., 2016; Thieme et al., 2016).

Generally, a normal-sized reflection is used, and in an attempt to improve efficacy, lenses and virtual reality have been used to minify the appearance of painfully swollen limbs and magnify painfully withered limbs (Wittkopf and Johnson 2016). Moseley et al., (2008) found that minifying the appearance of a chronic painful arm alleviated movement-induced pain, and magnifying the appearance of the arm exacerbated movement-induced pain. Experiments investigating pain-free individuals exposed to painful stimuli have been used to explore the factors influencing the response to visual distortion of the size of limbs. Mancini et al., (2011) found that magnifying a mirror reflection of the hand reduced contact-heat pain whereas minifying the reflected hand increased pain. In contrast, Johnson and Gohil (2016) used cold-pressor pain and found no difference in pain measures when magnifying and minifying reflections of the hand.

It is possible that the inconsistency in study findings is due to inter- and intra-participant variability in the embodiment of the viewed limb (Foell et al., 2014). Embodiment is the subjective experience of ownership and agency of body parts, and it seems to be an important aspect of the analgesic effect of visual feedback techniques (Foell et al., 2014; McCabe 2011). Martini et al., (2014) studied 32 healthy participants and found higher heat pain thresholds only when participants reported ownership over a virtual arm. Recently, we found that an adaptive phase in which participants watch a reflection of the hand while performing synchronised finger movements with both hands over a 3-min period enhanced the embodiment of the reflected hand (Wittkopf et al., 2017). In the present experiment we were interested in analysing if a painful stimulus applied to the hand hidden behind the mirror and after an adaptive phase could weaken the embodiment of the reflection of the hand. We were also interested in replicating previous studies, by investigating the effect of magnifying and minifying the reflection of the hand in front of the mirror on heat pain threshold. The aim of the present study was to evaluate the effect of observing a normal-sized, magnified, and minified reflection of a hand on perceptual embodiment and on contact-heat stimuli applied to the hand hidden behind the mirror. We hypothesized that there would be differences in contact-heat measures between a normal-sized reflection of the hand compared with magnified and minified reflections, but the direction could not be anticipated because of the inconsistences in previous research.

METHODS

Study design

A within-subject repeated-measures design was used to evaluate the effect of observing a normal-sized, magnified, and minified reflection of the hand, and a no-reflection condition on the perception of contact-heat stimuli applied to the real hand, which was hidden behind the mirror. Each participant took part in one experiment that measured warm detection threshold, heat pain threshold, and heat pain tolerance under four conditions:

- Normal-sized reflection of the left hand using a 46 cm diameter flat mirror.
- Magnified reflection of the left hand using a 46 cm diameter concave mirror (1.3 magnification).
- Minified reflection of the left hand using a 46 cm diameter convex mirror (0.7 magnification).
- No reflection control condition using the back of the flat mirror.

Participant recruitment, Screening and Enrolment

A sample size calculation was conducted using α of 0.001, β of 0.95 and an effect size of 0.56 based on a previous study (Mancini et al., 2011) resulting in a target of 36 participants. To account for differences in study designs and for the possibility of participant withdrawal/dropout we set target recruitment at 46 participants. We sought unpaid volunteers aged 18 years or above via announcements in lectures in our university. Volunteers were requested not to take part in the study if they did not consider themselves healthy, had a long-term illness, were currently seeking medical care, were experiencing pain or sensory disturbances, taking any medication either prescribed or purchased over the counter, were known to be pregnant, had a dermatological condition or were unable to see clearly at a distance of up to one meter. There was no restriction on gender, ethnicity nor body mass index although this was recorded. Volunteers expressing interest received a participant information pack and were given 48 hours to consider participation before a formal invitation to attend a study visit was made. During the study visit, volunteers were formally screened for eligibility and then provided written consent. Participants were reminded that they could withdraw consent at any time and without reason.

Ethical approval was received from the Research Ethics Committee of Leeds Beckett University.

Experimental procedure

Each participant attended our research laboratory for one experimental visit lasting no longer than two hours. Each experiment was conducted by the principal investigator (PW: 27 years old, female, physiotherapist, Brazilian national) who is fluent in English. All instructions were read verbatim from a crib sheet to ensure that all participants received standardised information. Measurements of the participant's height, weight and left hand size (from wrist perpendicular to the scaphoid to the tip of the middle finger) were taken. Then participants were familiarised to each of the thermal tests which involved applying the thermode to the skin covering the carpal bones of the left hand.

During the experiment participants were seated with both arms resting on a desk, flexed at the elbows. The right hand was placed behind the mirror, in a cardboard box at a distance of 25 cm behind a mirror attached to the outer left hand wall of the box (Figure 1). Both hands were kept in a neutral position with fingers straight in line with the palms and with skin covering the carpal bones in contact with a real thermode (right hand) or a fake thermode (left hand). A black cloth was draped across the participant's right shoulder and upper arm and the left hand was placed 25 cm in front of the mirror. Initially, a cardboard sheet covered the reflective surface of the mirror.

[Please insert figure 1 here - Photograph of experimental set-up]

Outcome measures were taken for each of the three mirror conditions presented in a counter-balanced manner between participants with measurements for the no-reflection condition taken last as a manipulation check to control for visual cues provided by the reflective surface of the mirror. Participants could not see the right hand during the no-reflection condition. The opaque grey colour of the back of the flat mirror occluded the view of the right hand. During the adaptive phase participants, were asked to gaze at a white stripe placed on the surface of the back of the flat mirror.

[Please insert Figure 2 here –Time course of experiment]

At the start of each cycle the mirror was uncovered and participants were instructed to look at the reflection of their left hand and provide verbal responses to the following questions read verbatim from the embodiment questionnaire:

1. It feels like I am looking directly at my right hand rather than at a reflection of the hand (associated with Ownership of the reflection)
2. It feels as if the reflection of the hand is my real hand (associated with Ownership of the reflection).

This was followed by an 'adaptive phase' to facilitate embodiment of the reflection of the hand using a method previously described by Wittkopf et al., (2017). Participants were asked to look at the reflection of their left hand for 30 seconds, followed by clenching and unclenching of both fists in synchrony with a metronome (60 beats per minute) for 60 seconds. This was followed by keeping both hands still for 30 seconds followed by 60 seconds of touching each fingertip with the thumb in synchrony with a metronome (60 beats per minute).

Measurements of embodiment and of proprioceptive drift were taken at two time points: immediately after the 'adaptive phase' and after the thermal tests. Thermal tests consisted of three measurements of warm detection threshold, three measurements of heat pain threshold, and one measurement of heat pain tolerance. Participants estimated the size of the reflection of their left hand by stating numbers on a ruler placed parallel to the mirror that were in line with the reflection of the tip of their middle finger and the mark on their wrist. Participants then rested for five minutes before commencement of the next measurement cycle (Figure 2).

Outcome measures

Thermal tests

The thermal tests for warm detection threshold, heat pain threshold, and heat pain tolerance were performed using a TSA-II Neurosensory Analyser (Medoc Ltd, Israel) using the method of ascending limits and adapted from the recommendations of the German Research Network on Neuropathic Pain (DFNS) for standardized Quantitative Sensory Testing (Rolke et al., 2006; Yarnitsky and Ochoa 1990). Baseline temperature was set at 32°C and increased at a rate of 1°C/sec. There were three measurements of warm detection threshold followed by three measurements of heat pain threshold with 5 seconds rest between each repetition and 10 seconds rest between each type of measurement. Only one measurement for heat pain tolerance was taken due to hazards associated with skin burns resulting from repeated exposure to excessively high temperatures. The maximum temperature of the stimulus was limited to 50.5°C.

Participants were instructed as follows:

- Warm detection threshold: "Please say stop as soon as you feel the slightest change of temperature to ´warm´. Then the thermode will cool down to its starting temperature. This procedure will start in a few seconds and will be repeated a total of 3 times."
- Heat pain threshold: "The temperature of the skin will increase to ´warm´ and a few moments later to ´hot´. Eventually a painful component will be added to the sensation of ´hot´, and it may change in quality from ´hot´ to, for example, ´burning´ or ´stinging hot´. Please say stop immediately at the first ´burning´ or painful sensation. Then the thermode will cool down to its starting temperature. This procedure will start in a few seconds and will be repeated a total of 3 times."
- Pain tolerance: "The temperature of the skin will increase to ´warm´ and a few moments later to ´hot´. Eventually a painful component may be added to the sensation of ´hot´, and it will change in quality from ´hot´ to, for example, ´burning´ or ´stinging hot´. Please say stop immediately when this ´burning´ or ´stinging hot´ sensation becomes unbearably painful. Then the thermode will cool down to its starting temperature."

Proprioceptive drift

Participants were asked to indicate when the position of a pen being moved by the investigator along the top of the cardboard box in the left-right axis coincided with proprioceptive awareness of the middle finger of the hand hidden behind the mirror. The distance (cm) between the perceived location and actual location of the hidden hand was measured using a measuring tape and recorded as proprioceptive drift.

Embodiment

An embodiment questionnaire was adapted from those used in previous studies by ourselves and others (Lewis and Lloyd 2010; Longo et al., 2008; Medina et al., 2015; Wittkopf et al., 2017). Participants were instructed to rate their agreement on a Likert scale: strongly disagree, disagree, undecided, agree, and strongly agree (coded into numbers for data analysis) to each of the following statements:

1. It feels like I am looking directly at my right hand rather than at a reflection of the hand (associated with Ownership of the reflection).

2. It feels as if the reflection of the hand is my real hand (associated with Ownership of the reflection).
3. It feels as if the reflection of the hand is part of my body (associated with Ownership of the reflection).
4. It feels as if my right hand is in the same location as the reflection of the hand (associated with Location of body part).
5. It feels as if I could move the reflection of the hand without having to move my left hand (associated with Agency of the reflection).
6. It feels as if I move my right hand the reflection of the hand will move too (associated with Agency of the reflection).
7. It feels like I cannot tell where my right hand is (associated with Deafference).
8. My right hand feels unusual (associated with Deafference).

Data analysis

The mean of the 3 measurements for warm detection threshold and for heat pain threshold was calculated for each participant and used in analysis. Data from thermal tests, proprioceptive drift and estimation of hand size were asymmetrical and not normally distributed so the data were transformed using the two-step approach (Templeton 2011). A repeated measures analysis of variance (ANOVA) was conducted on warm detection threshold, heat pain threshold and heat pain tolerance. The within-subjects factor was Condition (four levels: normal-sized, magnified, minified, and no-reflection) and between-subjects factor was Order (six levels: normal-sized, minified, magnified; minified, magnified, normal-sized; magnified, normal-sized, minified; magnified, minified, normal-sized; normal-sized, magnified, minified; minified, normal-sized, magnified). A 4 x 2 repeated measures factorial ANOVA was conducted on proprioceptive drift data. The within-subjects factors were Condition (four levels: normal-sized, magnified, minified, and no-reflection) and Time point (two levels: pre-thermal tests and post-thermal tests). The between-subjects factor was Order (six levels: normal-sized, minified, magnified; minified, magnified, normal-sized; magnified, normal-sized, minified; magnified, minified, normal-sized; normal-sized, magnified, minified; minified, normal-sized, magnified). If a significant interaction was detected and power was greater than 0.80, simple effects analyses would be conducted to determine the direction of the interaction. A Greenhouse–Geisser correction was used if Mauchly's test showed that sphericity could not be assumed. A Friedman's ANOVA was conducted on data from the embodiment questionnaire. Factors were Condition (three levels: normal-sized, magnified and minified) and Time point (Three levels for the first two statements: baseline, pre-thermal tests, and post-thermal tests; two levels for the other six statements: pre-thermal tests and post-thermal tests). The Wilcoxon signed-rank test was used for pairwise comparisons. Results were interpreted according to the level of statistical significance $p \leq 0.05$, power ≥ 0.80, and effect size reported as partial eta squared (η_p^2). Post hoc power analysis were conducted using alpha = 0.05. Adjustments were made for multiple comparisons using the Bonferroni correction. Analyses were conducted with SPSS version 22.0 and G*Power 3.1 (Faul et al., 2007).

RESULTS

Characteristics of study sample

Forty-six right-handed volunteers expressed interest in the study and all started and completed the experiment (mean ± SD: age = 21.43 ± 6.59 years, min = 18 years, max = 47 years; weight = 67.26 ± 15.38 Kg; height = 1.66 ± 0.075 m, female n = 40).

Estimation of the size of reflection of the left hand

The mean ± SD of the real size of the left hand of the participants was 18.06 ± 1.02 cm. The main effect of condition was statistically significant [$F(2.32,104.41) = 121.38$, $p < 0.001$, $\eta_p^2 = 0.730$, power = 1.0]. Pairwise comparisons showed that participants estimated the size of the minified reflection of the hand (12.28 ± 3.26 cm) to be smaller than the estimation of the size of the normal-sized reflection of the hand (17.47 ± 3.10 cm, $p < 0.001$) and the real size of their hand ($p < 0.001$). Participants estimated the size of the magnified reflection of the hand (22.73 ± 3.44 cm) to be larger than the estimation of the size of the normal-sized reflection of the hand ($p < 0.001$) and the real size of their hand ($p < 0.001$). There was no difference between estimation of the size of the normal-sized reflection of the hand compared with the real size of their hand ($p = 0.715$). The estimation of the size of magnified reflection was larger than the minified reflection ($p < 0.001$).

Thermal tests

Warm detection threshold

The main effect of condition was statistically significant [$F(3,120) = 6.54$, $p < 0.001$, $\eta_p^2 = 0.141$, power = 0.97]. Pairwise comparisons showed that temperatures for warm detection threshold were lower when participants were observing the magnified reflection of the hand compared with the no-reflection condition ($p < 0.001$, Figure 3, Table S1). There were no other statistically significant differences. The condition x order interaction was not statistically significant [$F(15,120) = 1.44$, $p = 0.139$, $\eta_p^2 = 0.153$, power = 0.76].

Heat pain threshold

The main effect of condition rendered a significant p value, but a small effect size and power smaller than the threshold (≥ 0.80). Therefore, the main effect of condition was not considered statistically significant [$F(3,120) = 2.78$, $p = 0.044$, $\eta_p^2 = 0.065$, power = 0.69]. The condition x order interaction was not statistically significant [$F(15,120) = 1.29$, $p = 0.216$, $\eta_p^2 = 0.139$, power = 0.76].

Heat pain tolerance

The main effect of condition was not statistically significant [$F(3,120) = 0.149$, $p = 0.930$, $\eta_p^2 = 0.004$, power = 0.77]. The condition x order interaction was not statistically significant [$F(15,120) = 0.412$, $p = 0.793$, $\eta_p^2 = 0.049$, power = 0.24].

[Please insert Figure 3 here Line graph thermal tests]

Proprioceptive drift

The main effect of condition was not statistically significant [$F(3,120) = 1.98$, $p = 0.120$, $\eta_p^2 = 0.047$, power = 0.50], indicating that the magnitude of proprioceptive drift of the hidden hand experienced by the participants did not differ among the four conditions. The main effect of time point was not statistically significant [$F(1,40) = 0.44$, $p = 0.510$, $\eta_p^2 = 0.011$, power = 0.10], indicating that the magnitude of proprioceptive drift of the hidden hand experienced by the participants did not differ between pre-thermal tests and post-thermal tests (Figure S1). The condition x order interaction was not statistically significant [$F(15,120) = 0.38$, $p = 0.98$, $\eta_p^2 = 0.046$, power = 0.23]. The condition x time point interaction was not statistically significant [$F(2.40,95.99) = 0.137$, $p = 0.938$, $\eta_p^2 = 0.003$, power = 0.075]. The condition x time point x order interaction was not statistically significant [$F(15,120) = 0.60$, $p = 0.86$, $\eta_p^2 = 0.071$, power = 0.37].

Embodiment questionnaire

Results from Friedman's ANOVA for each statement of the questionnaire are summarised in Table 1. Participants rated their level of agreement with the statements 'It feels like I am looking directly at my right hand rather than at a reflection of the hand' and 'It feels as if the reflection of the hand is my real hand' at three time points: baseline, pre-thermal tests, and post-thermal tests. Results indicated that the adaptive phase enhanced the embodiment experience when participants observed the magnified and minified reflections. The painful stimuli did not weaken participants' embodiment experience (Figure 4).

[Please insert Table 1 here]

[Please insert Figure 4 here - Box-plots of embodiment questionnaire]

Participants rated their level of agreement with the other six statements of the questionnaire at two time points: pre-thermal tests and post-thermal tests. For the statement 'It feels as if I could move the reflection of the hand without having to move my left hand' the Friedman's ANOVA rendered a significant result (Table 1). Pairwise comparisons indicated that perceptual embodiment of the reflection of the hand when observing the magnified and minified reflection was stronger after the painful stimuli (Figure 5).

[Please insert Figure 5 here Box-plots of embodiment questionnaire]

For the statements 'It feels as if the reflection of the hand is part of my body', 'It feels as if my right hand is in the same location as the reflection of the hand' and 'It feels as if I move my right hand the reflection of the hand will move too' the Friedman's ANOVA rendered significant results (Table 1). However, pairwise comparisons using the Wilcoxon signed ranks test showed no significant difference between comparisons (Figure 5).

DISCUSSION

Our findings show that observing a reflection of a hand in front of a mirror does not affect pain threshold nor tolerance to contact-heat stimuli applied to a hand hidden behind a mirror when compared with a no-reflection condition. Altering the size of the reflection of the hand does not affect pain threshold nor tolerance. Participants were more sensitive to changes in warmth when observing a magnified reflection of the hand compared with a no-reflection condition.

We found that the perceptual experience that the reflection of the hand was the participant's real hand was stronger after the adaptive phase, in which participants watched a mirror reflection of a hand in front of a mirror performing finger movements synchronised with movements of the hand hidden behind a mirror. Altering the size of the reflection of the hand did not affect the strength of the perceptual experience. Observing a normal-sized, magnified and minified reflection of the hand in front of the mirror and a no-reflection condition did not influence estimates of proprioceptive drift. Painful contact-heat stimuli did not influence estimates of proprioceptive drift and embodiment.

We did not detect differences in heat pain threshold nor tolerance when participants were observing a normal-sized, magnified and minified reflection of the hand and a no-reflection condition, and these findings are consistent with previous studies (Johnson and Gohil 2016; Torta et al., 2015). Johnson and Gohil (2016) measured pain threshold, intensity, and tolerance of the hand hidden behind a mirror using cold-pressor pain. The authors found that observing a normal-sized, magnified and minified reflection of the hand and a direct view of the hand being stimulated did not alter pain in healthy participants. It was hypothesized that as visual feedback techniques are used to change perceptual dysfunctions of limbs in some chronic painful states the technique may have less influence on experimentally-induced pain in pain-free healthy participants.

A visually-mediated reduction on experimentally induced pain in healthy participants has been reported (Longo et al., 2009; Longo et al., 2012; Mancini et al., 2011). Longo et al., (2009) found a reduction in pain intensity when participants observed a normal-sized reflection of the hand compared with a reflection of an object. Likewise, Mancini et al., (2011) found that observing a normal-sized reflection of the hand increased heat pain threshold when compared with the reflection of an object. The authors also found that magnifying the reflection of the hand increased heat pain threshold and minifying the reflection of the hand decreased it (Mancini et al., 2011). The use of different feedback to facilitate embodiment during the adaptive phase and different stimuli to evoke pain may explain differences in findings. During the rubber hand illusion, a visuo-tactile feedback is used to facilitate embodiment, in which participants observe a rubber hand being stroked whilst their real hand is stroked in synchrony but hidden from view (Botvinick and Cohen 1998). Using visuo-tactile feedback to facilitate embodiment of a reflection of a hand can be challenging, and authors have used different modalities. Longo et al., (2009) and Mancini et al., (2011) used a visual feedback, and during the adaptive phase participants observed the reflection of the hand at rest. We used visuo-motor feedback, because this technique has been used in the rehabilitation of painful hands and is known to enhance feelings of ownership and agency (Wittkopf et al., 2017). Medina et al., (2015) found that watching the reflection of the hand in front of a mirror moving in synchrony with the hand hidden behind the mirror increased the intensity of embodiment of the reflection of the hand when compared to a condition where participants observed the reflection of the hand resting on the table. Findings of studies that have used visuo-motor feedback to facilitate embodiment suggest that the perceptual experience is stronger when there is greater spatial temporal congruence between the real and the viewed hand (Asai 2016; Sanchez-Vives et al., 2010).

Results from warm detection threshold measures indicate that the sensitivity to warmth increased when participants observed the magnified reflection of the hand. This is consistent with previous reports of increased sensitivity to a sensory stimulus when observing a magnified view of the stimulated limb. Kennett et al., (2001) reported an increase in tactile discrimination threshold when participants observed a magnified arm being stimulated compared with direct view and no view of the arm. It was concluded that tactile acuity improves when greater visual detail of the body surface is seen. Romano and Maravita (2014) studied the skin conductance response to a noxious stimulus approaching and touching the finger of healthy participants while observing the hand through normal and magnifying lenses. When participants observed the magnified hand, there was an increase in skin conductance response while the noxious stimulus was approaching the hand, and a decrease when it touched the hand. It was suggested that observing a noxious stimulus approaching the hand through a magnifying lens might enhance the processing of incoming sensory information of the viewed image during pain anticipation. In our study, participants were aware that heat pain threshold would be measured after warm detection threshold and the increased sensitivity to warmth could represent an anticipatory response to the subsequent painful stimuli.

Participants reported stronger feelings of ownership towards the reflection of the hand after the adaptive phase, and the exposure to painful stimuli did not weaken the perceptual experience and did not alter measures of proprioceptive drift, which is consistent with previous studies (Kammers et al., 2011; Medina et al., 2015; Siedlecka et al., 2014; Wittkopf et al., 2017). Although the Friedman's ANOVA rendered significant results for three questionnaire statements, we failed to detect the differences in pairwise comparisons. A possible explanation is a limitation of the scale in which the strength of agreement with the statements was measured. Embodiment is a subjective phenomenon and it is quantified using questionnaires that capture aspects of subjective experience, i.e., perceived location, ownership, and agency (Lewis and Lloyd 2010; Longo et al., 2008). Investigators have also measured proprioceptive drift (Tsakiris and Haggard 2005). During the rubber hand illusion participants report proprioceptive drift of their real hand towards the rubber hand, and the amount of drift is positively correlated with aspects of the subjective embodiment experience (Botvinick and Cohen 1998), although sometimes the two measures can be dissociated (Lloyd et al., 2013). The phenomenon of embodying a reflection of a hand has been quantified using instruments adapted from those used to quantify the embodiment of a rubber hand, and it can be problematic (Asai 2016). Investigations into aspects influencing the embodiment of a reflection of a limb are needed to improve outcome measures.

Study limitations

Changes in the size of the viewed body part are proportional to changes in depth perception. In our study, a minified hand was seen further away from participant's body midline, whereas a magnified hand was seen closer to it. It is known that it is more difficult to embody a rubber and virtual hand when it is placed further away from the participant's body midline (Lloyd 2007; Nierula et al., 2017). Furthermore, our paradigm investigates the immediate visual illusory effects on a transient stimulus that activates a non-sensitised nociceptive system and it cannot be ruled out a general distraction effect. Attentional elements related to the use of visual feedback techniques should be addressed in future studies.

Implications for pain management

Recent systematic reviews indicate that mirror visual feedback is effective for pain reduction when used as a prolonged treatment (Boesch et al., 2016) and in patients with complex regional pain syndrome (Thieme et al., 2016). Furthermore, visual distortions of the size of body parts have been used to modulate pain perception of patients with disrupted mental representation of the affected body part (Boesch et al., 2016). Studies investigating the use of mirror visual feedback for pain management have given little attention to the embodiment of the reflection of the limb. Even though there were no differences in responses to experimentally-induced pain in pain-free participants in the present study, we found that participants reported stronger feelings of embodiment after a 3-min period of watching a reflection of a hand performing synchronised finger movements with the real hand. The inclusion of an adaptive phase with visuo-motor feedback could improve the effectiveness of the use of visual feedback techniques in clinical settings; as it is known that the embodiment of the viewed body part is important for pain reduction when using such techniques (Foell et al., 2014; Martini et al., 2014; McCabe 2011).

In conclusion, observing a reflection of the hand in front of a mirror did not alter heat pain threshold nor tolerance when compared with a no-reflection condition. Magnifying and minifying the reflection of the hand did not alter heat pain threshold nor tolerance. Participants were more sensitive to changes in heat when observing the magnified reflection compared with the no-reflection condition. The perceptual embodiment of the reflection of the hand was stronger after a 3-min period of watching a mirror reflection of a hand performing finger movements synchronised with movements of the hand hidden behind the mirror, and this experience was not weakened by the painful stimulation. Researchers and clinicians using visual feedback techniques may consider including an adaptive phase with visuo-motor feedback to facilitate embodiment of the viewed body part.

Author Contributions

All authors contributed to conception, design, analysis and interpretation of data. The first author collected the data and drafted the manuscript. All authors critically revised the article for important intellectual content. All authors gave final approval for publication.

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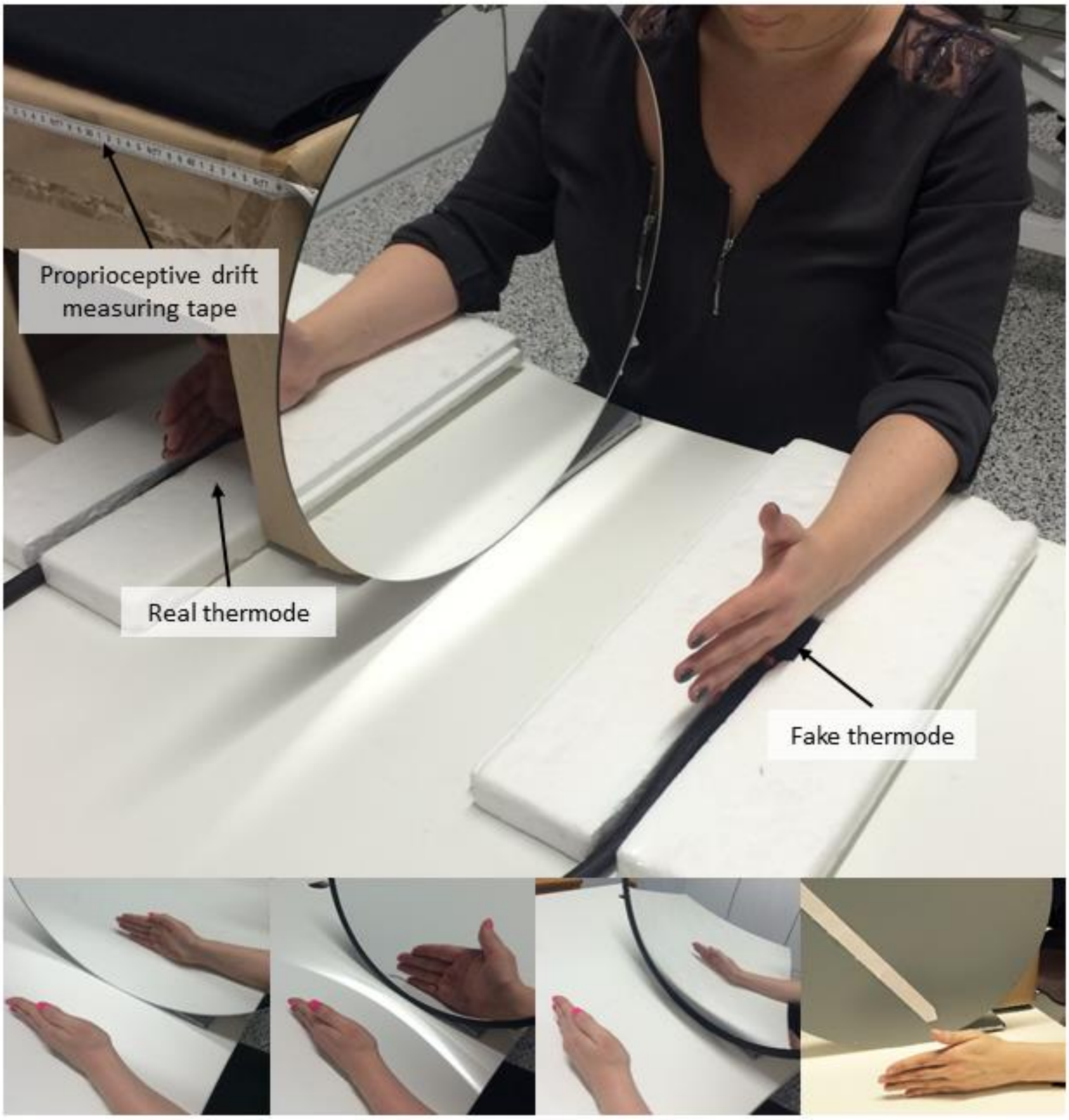

Figure 1. Experimental set up (top). Three experimental conditions, normal-sized reflection, magnified reflection, minified reflection, and no-reflection condition in order (bottom).

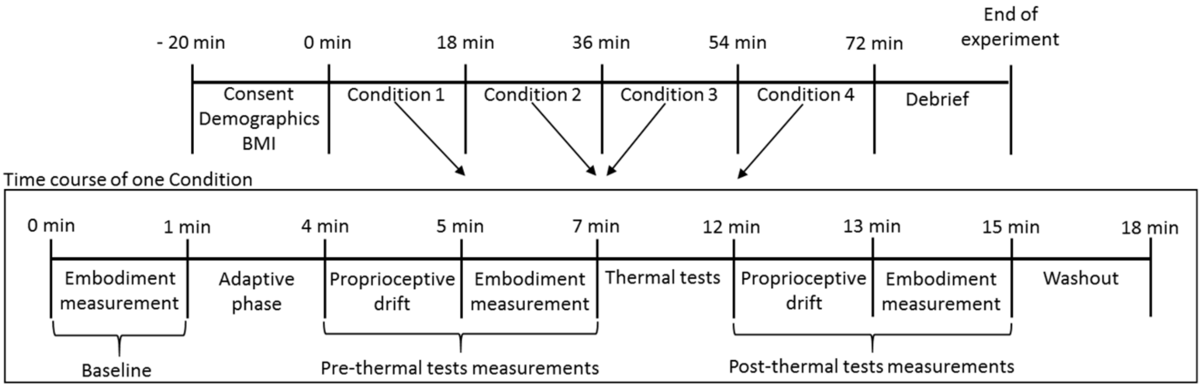

Figure 2. Time-course of the overall experiment and of one condition.

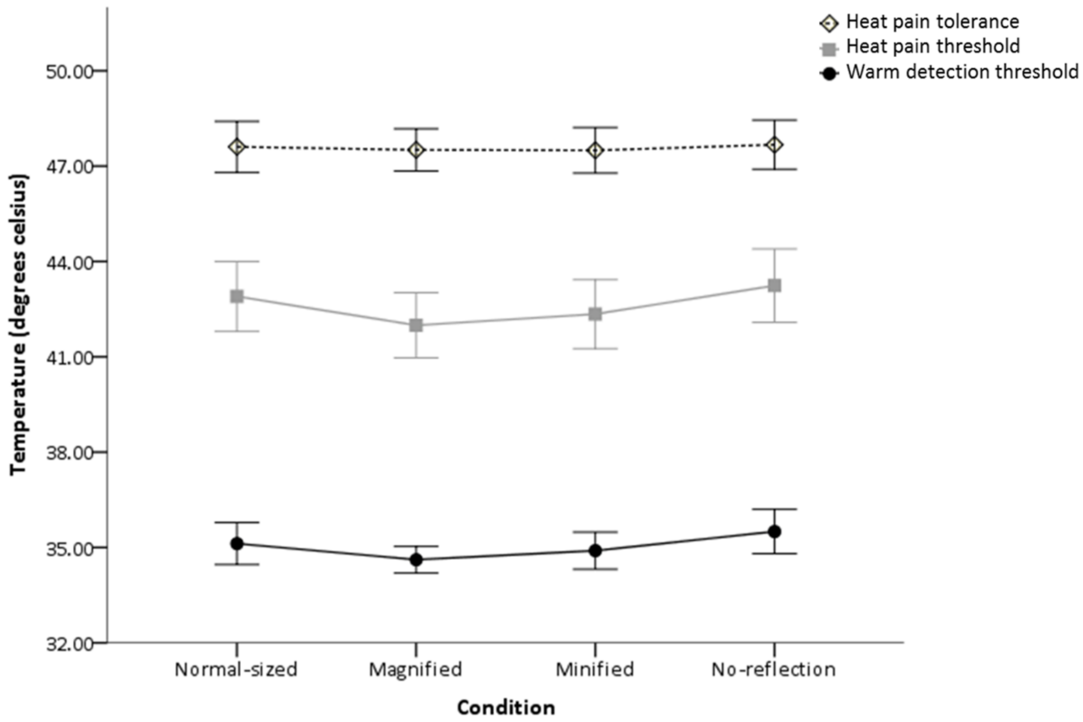

Figure 3. Mean temperature thermal tests. Error bars indicate standard error of the mean.

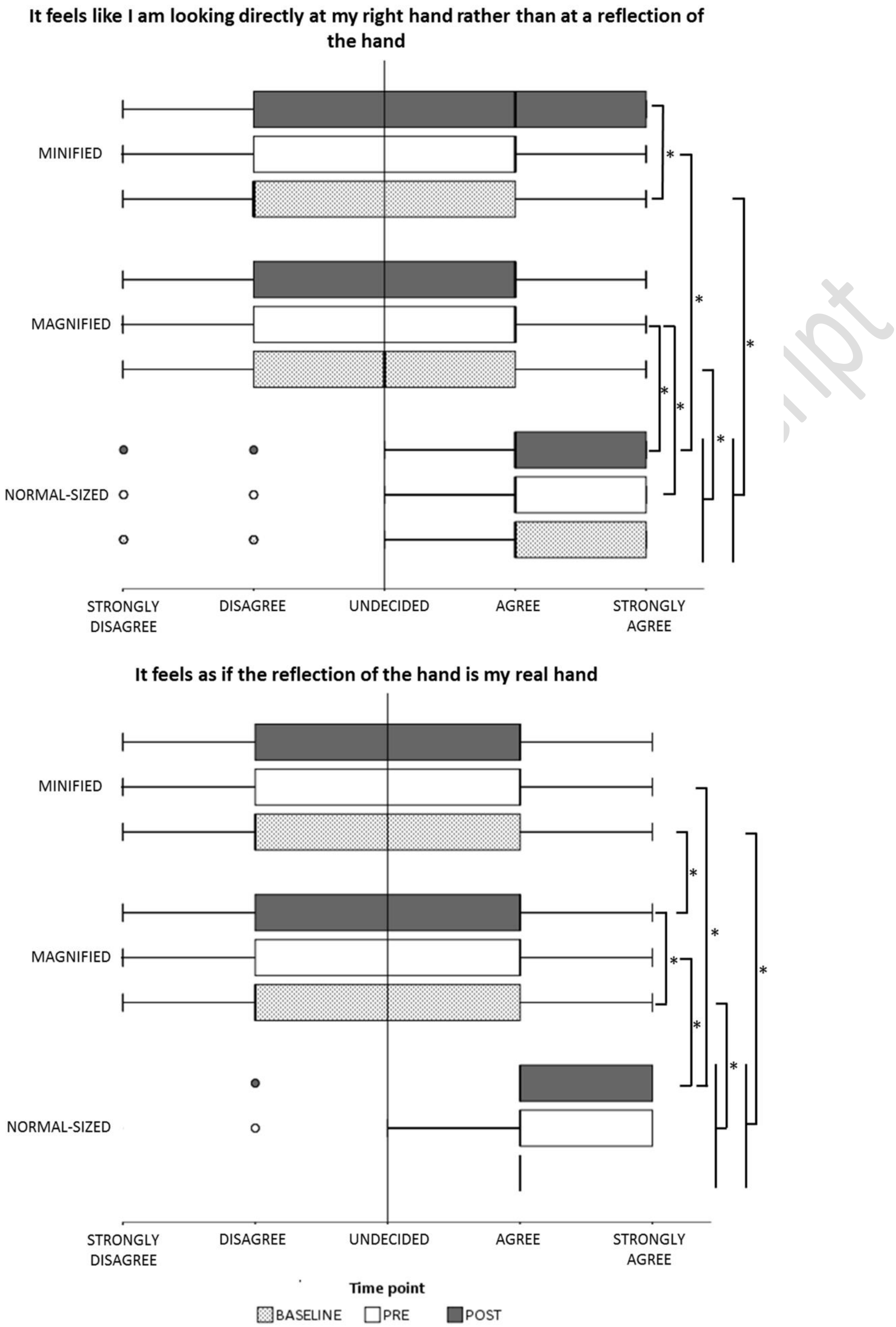

Figure 4. Box-plots of the embodiment questionnaire statement scores for each time point. Asteristks indicate statistical significance $p \leq 0.05$.

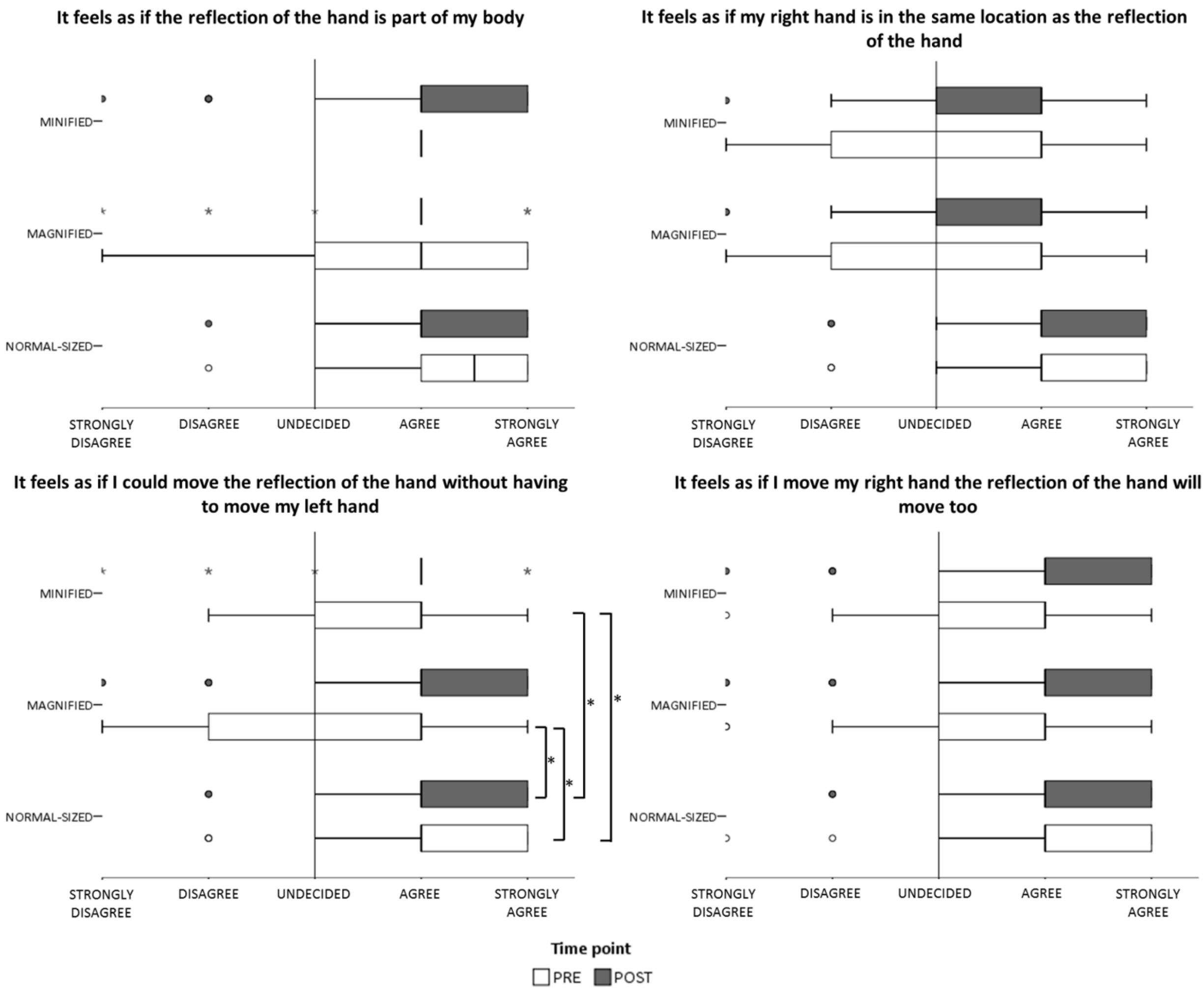

Figure 5. Box-plots of the embodiment questionnaire statement scores for each time point. Asteristks indicate statistical significance $p \leq 0.05$.

Table 1. Friedman's ANOVA results for each statement of the embodiment questionnaire.

Statement	χ^2[a]	*df*[b]	p[c]
It feels like I am looking directly at my right hand rather than at a reflection of the hand	80.83	8	0.001
Significant pairwise comparisons	Z[d]		p
Normal-sized baseline vs minified baseline	-2.043		0.012
Normal-sized pre vs minified baseline	-2.63		0.001
Normal-sized post vs minified baseline	-3.00		0.001
Normal-sized post vs minified pre	-2.076		0.010
Normal-sized baseline vs magnified baseline	-2.304		0.002
Normal-sized pre vs magnified baseline	-2.891		0.001
Normal-sized post vs magnified baseline	-3.261		0.001
Normal-sized pre vs magnified pre	-1.913		0.029
Normal-sized post vs magnified pre	-2.283		0.002
Magnified baseline vs magnified post	-1.913		0.029
Statement	χ^2	*df*	p
It feels as if the reflection of the hand is my real hand	99.91	8	0.001
Significant pairwise comparisons	Z		p
Normal-sized baseline vs minified baseline	-2.467		0.001
Normal-sized pre vs minified baseline	-3.391		0.001
Normal-sized post vs minified baseline	-3.500		0.001
Normal-sized post vs minified pre	-2.011		0.015
Normal-sized baseline vs magnified baseline	-2.500		0.001
Normal-sized pre vs magnified baseline	-3.424		0.001
Normal-sized post vs magnified baseline	-3.533		0.001
Normal-sized post vs magnified pre	-1.826		0.033
Magnified baseline vs magnified post	-1978		0.019
Minified baseline vs magnified post	-1946		0.024
Statement	χ^2	*df*	p
It feels as if I could move the reflection of the hand without having to move my left hand	49.79	5	0.001
Significant pairwise comparisons	Z		p
Normal-sized pre vs magnified pre	-1.630		0.001
Normal-sized post vs magnified pre	-1.554		0.001
Normal-sized pre vs minified pre	-1.261		0.018
Normal-sized post vs minified pre	-1.185		0.036
Statement	χ^2	*df*	p
It feels as if the reflection of the hand is part of my body	37.18	5	0.001
It feels as if my right hand is in the same location as the reflection of the hand	32.74	5	0.001
It feels as if I move my right hand the reflection of the hand will move too	26.29	5	0.001
It feels like I cannot tell where my right hand is	2.12	5	0.832
My right hand feels unusual	4.96	5	0.420

Abbreviations: χ^2 chi-square; *df* degrees of freedom, p significance value.