Title

A preliminary investigation into psychophysiological effects of threatening a perceptually embodied rubber hand in healthy human participants

Authors

Mark I. Johnson [1,2]* [#a], Emily Smith [1] [#a], Sarah Yellow [1] [#a], Matthew R Mulvey [1,2] [#b]

1. Faculty of Health and Social Sciences, Leeds Beckett University, United Kingdom
2. Leeds Pallium Research Group, www.leeds.ac.uk/pallium, United Kingdom

[#a]Faculty of Health and Social Sciences
Leeds Beckett University
City Campus
Leeds LS1 3HE
United Kingdom
Telephone: (UK) 0113 2832600, Fax: 0113 2833124

[#b]Leeds Institute of Health Sciences
University of Leeds
Charles Thackrah Building
Leeds, UK
LS2 9LJ

*Corresponding author:
e-mail: m.johnson@LeedsBeckett.ac.uk

Running Header:
Visually threatening a perceptually embodied rubber hand

ABSTRACT

Background and aims

Threatening a perceptually embodied rubber hand with noxious stimuli has been shown to generate levels of anxiety similar to that experienced when a real hand is threatened. The aim of this study was to investigate skin conductance response, self-reported anxiety and the incidence, type and location of sensations when a perceptually embodied rubber was exposed to threatening and non-threatening stimuli.

Methods

A repeated measures cross-over design was used whereby 20 participants ($\geq$18 years, 14 females) received a threatening (syringe needle) and non-threatening (soft brush) stimulus to a perceptually embodied rubber hand. Perceptual embodiment was achieved using a soft brush to synchronously stroke the participant's real hand (out of view) and a rubber hand (in view). Then the investigator approached the rubber hand with a syringe needle (threat) or soft brush (non-threat).

Results

Repeated measures ANOVA found that approaching the perceptually embodied rubber hand with either stimulus produced statistically significant reductions in the rated intensity of response to the following questions ($p<0.01$): 'How strongly does it feel like the rubber hand is yours?'; 'How strongly does it feel like the rubber hand is part of your body?'; and 'How strongly does it feel you can move the rubber hand?'. However, there were no statistically significant differences in scores between needle and brush stimuli. Repeated measures ANOVA on skin conductance response found statistically significant effects for experimental Events (baseline; stroking; perceptual embodiment; stimuli approaching rubber hand; stimuli touching rubber hand; $p<0.001$) but not for Condition (needle versus brush $p=0.964$) or experimental Event x Condition interaction ($p=0.160$). Ten of the 20 participants (50%) reported that they experienced a sensation arising from the rubber hand when the rubber hand was approached and touched by either the needle and/or brush but these sensations lacked precision in location, timing, and nature.

Conclusion and Implications

Our preliminary findings suggest that the increase in arousal in response to stimuli entering the peripersonal space may not be selective for threat. There was tentative evidence that more intense sensations were experienced when a perceptually embodied rubber hand was approached by a threatening stimulus. Our findings provide initial insights and should serve as a catalyst for further research.

HIGHLIGHTS

We approached a perceptually embodied rubber hand with a needle and brush

An increase in anxiety was selective for needle but an increase in arousal was not

50% of participants reported somatic sensation when stimuli approached the rubber hand

'Tingling' was the most common somatic sensation reported

KEYWORDS
Perceptual embodiment, Pain, Rubber hand illusion, Skin conductance response

INTRODUCTION

The sense of self and body ownership is essential for the performance of complex movements and is driven by the integration of visual, tactile and proprioceptive inputs in cortical and sub cortical areas responsible for multisensory processing [1-4]. Embodiment refers to the subjective experience of having a sense of one's own body [5] and can be studied using techniques that elicit perceptual embodiment of inanimate objects. The 'rubber hand illusion' is a technique where a rubber hand is embodied so that the individual experiences a sense that the rubber hand is part of their own body [6]. Perceptual embodiment of a rubber hand is achieved by participants observing the rubber hand being brushed (in view) whilst their real hand is synchronously brushed out of view. After a short time the brush sensation feels as if it is arising from the rubber hand and the rubber hand feels as if it is part of the body (i.e. perceptually embodied)[7, 8]. It is possible to perceptually embody a rubber hand using painful-tactile stimuli (e.g. a sharp pin) in much the same way as using non-painful-tactile stimuli (e.g. a brush) [9].

The sense of self and body ownership may have a role in protection from injury. Lloyd et al. [10] provided evidence that regions of the contralateral posterior parietal cortex were involved in discrimination of painful and non-painful stimulation of a perceptually embodied rubber hand in the peripersonal hand space. Activity in the superior and inferior regions of the parietal cortex increased when individuals observed a sharp painful stimulus applied to a rubber hand that had been placed over their real hand, but only when the rubber hand was spatially congruent to the real hand. Threatening a perceptually embodied rubber hand with injury has been shown to evoke feelings that are similar to those experienced when threatening real limbs. Ehrsson et al. [11] found that threatening perceptually embodied objects generated levels of anxiety similar to that experienced when a real hand was threatened. The desire to withdraw the rubber hand from the threat was stronger when the intensity of perceptual embodiment was high. Armel et al. [12] found that strong skin conductance responses, which reflect levels of physiological arousal, occurred when a perceptually embodied rubber hand was threatened by an apparently injurious stimulus such as forceful bending of a finger of the rubber hand. Likewise, Hagni et al. [13] reported elevated skin conductance in participants playing a first-person perspective virtual reality game that involved two virtual arms interacting with virtual balls rolling towards the viewer. When the right virtual arm was apparently stabbed by a knife causing ‘bleeding’ larger increases in skin conductance were observed when participants imagined virtual arms to be their own compared with not imagining virtual arms to be their own. However, studies investigating the effect of threatening a perceptually embodied rubber hand are few and do not control for general arousal that may arise from non-threatening stimuli entering the peripersonal space.

Moreover, there has been little research on sensations evoked by stimuli that threaten a perceptually embodied rubber hand. Lewis and Lloyd [14] found that they were able to produce phantom-like experiences in non-amputees by inducing a sense of embodiment in a rubber hand that had a finger removed. Twenty eight out of 30 participants reported experiencing a sense of presence of the absent finger and seven out of 28 of these participants reported tingling or numbness in the missing phantom finger. Guterstam et al. demonstrated that sensations could be referred to an empty space creating a sense of having an invisible hand [15] and creating a sense of two right (or left) hands [16]. Neither of these studies systematically document stimuli–evoked sensations misattributed to a perceptually embodied rubber hand. The aim of this study was to investigate skin conductance response, self-reported anxiety and the incidence, type and location of sensations when a perceptually embodied rubber hand was exposed to threatening and non-threatening stimuli. The study was designed to evaluate whether arousal associated with approaching a perceptually embodied rubber hand was selective for threatening objects. We hypothesised that there would be a larger increase in arousal when threatening stimuli entered the peripersonal space compared with non-threatening stimuli.

METHODS

A repeated measure cross-over study was designed with each participant taking part in one experiment where they perceptually embodied a rubber hand which was exposed to a threatening (syringe needle) and non-threatening (soft brush) stimulus. The order of presentation of stimuli was randomised between experiments by a technician independent to the study using a computerised random number generator and sealed envelope method. Twelve participants received the threatening stimulus first. The study was approved by the Research Ethics Sub-Committee of Leeds Beckett University.

Participants, recruitment and selection

A convenience sample of unpaid healthy human volunteers (mean ± SD age = 21.0 ± 1.41 years, 14 females) was recruited by announcements in lectures throughout the university. This was a preliminary investigation and the sample size used was based on sample sizes used in similar studies [13, 17].

All participants were students of undergraduate or postgraduate taught course university courses. Interested individuals were briefed about the nature of the study and provided with a participant information pack that stated that the purpose of the study was to investigate whether it was possible to create the sense that a rubber hand could feel like it was part of the body and to take some physiological measurements during the process. Participants were also told that the rubber hand would be exposed to different stimuli. Volunteers were given 48 hours before being formally invited to take part in the study. During the study visit volunteers were screened for eligibility (≥18 years with no existing medical condition). Volunteers were excluded if they: had an ongoing medical condition (e.g. diabetes, osteoarthritis) or previous history of heart and circulatory disorders (e.g. vasculitis, thrombosis); were currently seeking medical care; were taking any medication or were likely to take any medication during the week preceding the study visit; were pregnant; were currently experiencing pain; had an upper limb injury within the previous six months; experienced disturbances in skin sensations of the forearm; regularly exposed their hands to extremes of cold. Participants were asked to refrain from engaging in vigorous exercise, consuming alcohol or caffeine products, or smoking (e.g. tobacco) 12 hours before the study visit. Participants signed written consent before the experiment and were reminded that they could withdraw at any time without any reason.

Procedure

Each experiment was conducted in a physiology laboratory by two female investigators of White British ethnic origin (ES and SY). Participants were seated throughout the experiment with both arms resting on a table with the left hand placed on a pillow. Sensors were attached to the middle and index finger of the left hand of the participant to monitor skin conductance response. The right hand of the participant was placed within a canvas box so that it was out of view and a rubber hand aligned parallel to the canvas box so that it looked like it could be part of the participants body (i.e. visually congruent to the real hand, Figure 1). The same rubber hand was used for each participant with no attempt to match the physical appearance of the hand to that of the real hand. The skin tone of the rubber hand was white, fair (skin type II - Fitzpatrick skin tone classification scale [18]), and was similar to the skin colour of all participants.

[Insert Figure 1 here]

A 5 minute pre-experiment silent period was used to relax and acclimatise the participant and to stabilise skin conductance after which the experimental timing began (Figure 2). A two minute baseline measurement was taken to ensure stabilisation of skin conductance whilst the participant stared at a point on the table with the rubber hand out of view. Real time skin conductance recording continued for the remainder of the experiment and data captured at various time points (described later). After the baseline recording participants were asked to stare at the rubber hand and the investigator simultaneously stroked the rubber hand (in view) and the right hand (out of view) with a soft brush (SENSELab brush-05, SOMEDIC, Hörby Sweden) for 4 minutes. Stroking of the hands was synchronous using brush strokes down the fingers and thumb, and occasional tapping of the knuckles. Participants were instructed to state 'Now' if they experienced a subjective tactile sensation of stroking that seemed to arise from the rubber hand. They were told to remain silent if they did not experience a subjective tactile sensation of stroking arising from the rubber hand. After 4 minutes of stroking participants verbally rated the intensity of

their subjective experience of aspects of perceptual embodiment on a 11 point numerical scale (1 = ‘Not at all strongly’ and 10 =‘The strongest imaginable’) to the following 4 questions based on questions previously used by adapted from Mussap and Salton [19]:
Q1. How strongly does it feel like the sensation of stroking is coming from the rubber hand?
Q2. How strongly does it feel like the rubber hand is yours?
Q3. How strongly does it feel that the rubber hand is part of your body?
Q4. How strongly does it feel that you could move the rubber hand?

[Insert Figure 2 here]

After the last question the investigator approached the rubber hand with either the threatening stimuli (needle attached to an empty syringe) or the non-threatening stimuli (soft brush). The order of presentation of stimuli was randomised to control for carry-over effects between the conditions. Threatening stimuli were delivered as 15 rapid lunges of the needle toward the rubber hand without touching the rubber hand itself (approximately 1 lunge per second, [11]). Then the needle was slowly inserted into posterior surface of the rubber hand as if to take a blood sample (~5s) and held in position for a further 10 seconds before being removed. Non-threatening stimuli were delivered as 15 rapid lunges of a soft brush (SENSELab brush-05, SOMEDIC, Hörby Sweden) toward the rubber hand without touching the rubber hand itself (approximately 1 lunge per second). Then the end of the brush hairs were pushed against the posterior surface of the rubber hand (~5s) and stroked up and down the middle finger for 10 seconds (7 strokes). Immediately after presentation of stimuli, participants rated the intensity of their subjective experience of aspects of perceptual embodiment using the 4 questions described previously. In addition, participants were asked the following questions designed by the investigators:
Q5. How anxious were you when I initially approached the rubber hand with the stimulus? (1 = ‘not at all anxious’ and 10 = ‘the most anxious imaginable’)
Q6. Did you feel any sensation in the rubber hand during the stimulus? (‘Yes’/’No’)
If ‘yes’, how strong were these sensations? (1 = ‘no sensation’ and 10 = ‘the strongest sensation imaginable’).

Q7. What did it feel like when the stimulus approached but did not touch the rubber hand? (open question)
Q8. What did it feel like when the stimulus touched the rubber hand? (open question)

Participants then indicated the location, intensity and quality of any sensations experienced from the perceptually embodied rubber during the stimulus by shading and annotating a diagram of the arm (i.e. pain chart). This completed the first stage of the experiment.

There was a 15 minute rest period where participants were disconnected from skin conductance recording equipment and allowed to sit quietly. The second stage of the experiment commenced after this rest period and consisted of a repeat of the procedure used in stage 1 except that the other stimulus was applied. At the end of the experiment the participant was given an opportunity to express their thoughts about any aspect of the experiment.

Skin conductance response

Skin conductance was used as an indicator of physiological arousal and recorded in real time throughout stage 1 and stage 2 of the experiment. Skin conductance was measured at index and middle fingers using finger electrodes and a skin conductance response amplifier (low voltage, 75 Hz AC excitation, 1000Hz sample rate, automatic zeroing) connected to a PowerLab data acquisition device (ADInstruments Ltd, Oxford, U.K. [20, 21]). It is common for changes in skin conductance response to occur within 2 to 5s of stimulus onset [22] so it was decided to use a 10 second interval as the region of interest. Data was captured for 10 seconds for each experimental event as follows: at the end of baseline; at the start of stroking (stroking); "Now" (perceptual embodiment); stimulus approaching rubber hand (approach); and stimulus touching rubber hand (touch). Mean skin conductance response was calculated for each 10 second region of interest and used as an index of skin conductance response.

Data analysis

Baseline measures of time to subjective report of perceptual embodiment were checked for parity using a paired t-test for continuous data between brush and needle stimuli. Scores for questions using the numerical rating scales were reported as mean ± standard deviation (SD). A repeated measures ANOVA was used to determine the effect of Stimulus (two levels: pre-stimulus; post-stimulus) and Condition (two levels: brush and needle) for questions two, three and four. Skin conductance data was analysed using a repeated measures ANOVA to determine the effect of experimental Event (five levels: baseline, stroking; perceptual embodiment; approach; touch) and Condition (two levels: brush and needle). If Mauchly's test of Sphericity was not assumed then a Greenhouse-Geisser correction was used for the data set. Alpha was set at 0.05 and adjustment made for multiple comparisons using the Bonferroni correction.

RESULTS

Twenty participants expressed interest in the study and all started and completed the experimental session (mean ± SD age = 21.0 ± 1.41 years, 14 females). During stage one of the experiment one participant did not state 'Now' to indicate they had experienced a subjective tactile sensation of stroking arising from the rubber hand within 4 minutes of brush stroking. This participant rated the intensity of sensation of stroking as coming from the rubber hand as 2 out of 10 (Q1) but the intensity of their subjective experience of perceptual embodiment as strong (Q2 = 8/10; Q3 = 7/10; Q4 = 9/10). In stage 2 of the experiment this participant stated 'Now' within 74 seconds. All other participants stated 'Now' for stage 1 and stage 2 of the experiment. There was no statistical significant difference between mean ± SD time for participants to report that they were experiencing a subjective tactile sensation of stroking arising from the rubber hand before the stimulus (pairwise difference = 0.42 ± 28.23s, n=19, paired t-test, t (18) = 0.065, p = 0.949).

Repeated measures ANOVA on Q2 'How strongly does it feel like the rubber hand is yours?' found effects for Stimulus (F(1,19)=12.99, p=0.002) but not for Condition (F(1,19)=1.41, p=0.249) or Stimulus x Condition interaction (F(1,19)=0.073, p=0.789). Repeated measures ANOVA on Q3 'How strongly does it feel like the rubber hand is part of your body?' found effects for Stimulus (F(1,19)=14.67, p=0.001) but not for Condition (F(1,19)=4.51, p=0.133) or Stimulus x Condition interaction (F(1,19)=0.459, p=0.506). Repeated measures ANOVA on Q4 'How strongly does it feel you can move the rubber hand?' found effects for Stimulus (F(1,19)=16.65, p=0.001) but not for Condition (F(1,19)=0.859, p=0.366) or Stimulus x Condition interaction (F(1,19)=0.178, p=0.677). Thus, approaching the perceptually embodied rubber hand with either stimulus produced a reduction in subjective reports of aspects of perceptual embodiment but there were no differences in the magnitude of change between needle and brush (Table 1, Figure 3). Participants recorded higher scores to Q5 'How anxious were you when I initially approached the rubber hand with the stimulus?' when the rubber hand was approached by the needle compared with the brush (pairwise difference brush - needle mean±SD = -3.55±2.66, t (19)=-5.957, p<0.001, paired t-test, Table 1).

[Insert Table 1 here]

[Insert Figure 3 here]

Ten of the 20 participants (50%) reported experiencing a sensation arising from the rubber hand when the rubber hand was approached and touched by a stimulus. Six out of ten of these participants reported experiencing a sensation arising from the rubber hand by both the needle brush and the brush; 2/10 participants reported experiencing a sensation arising from the rubber hand only by the brush; and two participants reported experiencing a sensation arising from the rubber hand by the needle. The majority of sensations were experienced arising from the back of the rubber hand at the site of stimuli (Figure 4). Scores to Q7 'How strongly did these sensations associated with the stimulus feel like they were coming from the rubber hand?' were higher when the rubber hand was approached and touched by the needle than by the brush, although this analysis was based on data from only 6 participants ($p=0.041$, Table 1). Seven of the eight participants that reported a sensation when approached by the brush described the sensation as "tingling" and one participant reported "numbness with pins and needles". Five of the eight participants that experienced a sensation when approached by the needle reported "tingling", one reported "numbness", one "pressure" and one a "rubber sensation".

[Insert Figure 4 here]

Skin conductance response

Repeated measures ANOVA identified statistically significant effects for experimental Event ($F(1.4,26.5)=27.609$, $p<0.001$). Pairwise comparisons identified statistically significant increases in skin conductance response for each experimental event compared with other experimental events, except for Baseline compared with 'Now' (Table 2). There were no statistically significant effects for Condition ($F(1,19)=0.002$, $p=0.964$) or experimental Event x Condition interaction ($F(1.6,30.9)=1.992$, $p=0.160$). There were no significant differences between needle and brush conditions at any experimental event point (Figure 5).

[Insert Table 2 here]

[Insert Figure 5 here]

DISCUSSION

This study was designed to evaluate whether arousal associated with approaching a perceptually embodied rubber hand was selective for threatening objects. It was found that approaching and touching a perceptually embodied rubber hand with needle or brush stimuli reduced the subjective experience of perceptual embodiment but there were no differences in the magnitude of response between the stimuli. Participants reported being more anxious when the perceptual embodied hand was approached by the needle but there were no differences in skin conductance response between needle and brush. This suggests that the increase in arousal in response to stimuli entering the peripersonal space was not selective for threat. Half of the participants reported that they experienced a sensation arising from the rubber hand when the rubber hand was approached and touched by a stimulus and there was tentative evidence that more intense sensations were experienced when approached by the needle compared with the brush.

Skin conductance response reflects autonomic nervous system arousal that is not voluntarily controlled and was used to control for the possibility that participants were responding to task demands to please the investigators. Skin conductance response increased in the phases leading up to perceptual embodiment, consistent with other investigations and reflecting general physiological arousal [12, 23, 24]. The phases leading up to perceptual embodiment have also been associated with various physiological correlates including proprioceptive drift [25], local skin cooling [26, 27], local histamine reactivity [28] and alterations of neural activity in the brain [29], although confounding variables reduce the reliability of these correlates as indicators of the subjective experience of the sense of self and body ownership [30, 31].

The skin conductance response associated with stimuli threatening the perceptually embodied rubber hand is likely to reflect increased general physiological arousal associated with increased anxiety. Armel et al. [12] found that skin conductance response increased when the real finger (out of view) was lifted into a non-painful position and the fake finger of the rubber hand (in view) was bent into a position that would likely cause pain. Ehrsson et al. [11] reported increased levels of anxiety when participants observed a needle approaching a perceptually embodied rubber hand and this was associated with activity in the insula and anterior cingulate cortex involved with anticipation and experience of pain. They also reported increased activity in medial motor areas including the supplementary motor area and pre-supplementary motor area associated with anticipatory responses such as the sense of agency and the urge to withdraw the perceptually embodied rubber hand. Hagni et al. [13] found that skin conductance responses but not heart rate increased when participants observing virtual arms imagined to be their own were presented with an unexpected threat (i.e. a knife stabbing the virtual arm). They suggested that the low realism and short duration of the virtual reality task may have contributed to the lack of measurable effect on heart rate. Ma and Hommel [17] investigated whether perceptual embodiment and skin conductance (affective resonance) were affected by a ball hitting (non-injurious impact) or a knife cutting a virtual hand. They found that embodiment and affective response to ball hitting or knife cutting could be dissociated reflecting different underlying mechanisms. They suggested that affective reactions to stimuli that are perceived to threaten a body part are generated by bottom-up processes irrespective of body ownership whereas stimuli that are perceived to be non-threatening are generated by top-down processes.

Our study extends these previous findings by including a non-threatening stimulus to determine whether this response was due to an aversive response specifically related to the threatening stimulus. There were no differences in skin conductance response between non-threatening and threatening stimuli, despite higher levels of reported anxiety when the perceptual embodied hand was approached by the needle (threat). This suggests that there is an increase in general arousal in response to a stimulus entering the peripersonal space rather than a specific aversive response [32]. Possible confounds were the small sample size and the small number of trials for each condition with only one trial per condition for each participant (20 repeats per condition).

Interestingly, 50% (n=10) of participants reported a sensation arising from the perceptually embodied rubber hand evoked by the needle and/or brush approaching the rubber hand with 80% (n=8) of these participants reporting sensations within each subcategory of stimuli. It is tempting to infer from these findings that some individuals are susceptible to experience stimulus-evoked sensations in the perceptually embodied rubber hand irrespective of the type of stimuli presented. However, follow-up studies with larger sample sizes and control questions are needed to ensure that participants were not responding randomly to please the investigator. Armel et al. [12] found that only two out of 120 participants (1.6%) experienced pain when they observed a finger of a perceptually embodied rubber hand bent backwards into a potentially painful position whilst their real finger (out of view) was also bent back into a non-painful position. Some participants reported that the embodied rubber hand felt anaesthetised, a phenomenon also observed in our study. Lewis and Lloyd [14] removed a finger from a rubber hand and simultaneously stroked the space previously occupied by the rubber finger and the real finger (out of view) in a synchronous fashion. They found that 28 out of 30 non-amputees experienced a sense that a real finger was present in the space previously occupied by the rubber finger. Sixteen of these 28 participants (57%) reported phantom sensations including alteration in the perceived size of the finger (14/28, 50%) and/or tingling or numbness (7/28, 25%). The proportion of participants that reported a sense of a phantom finger was similar to the proportion of participants that reported experiencing a stimulus evoked sensation from the perceptually embodied rubber hand in our study although the incidence of tingling and numbness was slightly lower and the context under which these sensations were generated was different.

In our study participants reported ‘tingling’ and ‘numbness’ arising from the perceptually embodied rubber hand evoked by both needle and brush stimuli and there were no differences in the nature of sensations between the stimuli. Sensations were not elicited on each presentation of the stimuli and there was an approximation of localisation of sensations to the posterior region of the rubber hand. This lack of precision in the location, timing, and nature of these sensations may be due to the absence of peripheral input from somatosensory receptors (e.g. mechanoreceptors and nociceptors). The process of perceptual embodiment of a rubber hand involves integration of somatotopic and visual frames of reference, i.e. somatosensory input from the skin of the real hand (out of view) and visual input from the eyes watching the rubber hand being stroked. The cerebrum appears to attribute stronger reliance on the visual frame of reference to generate a final perceptual experience that involves distortion of position sense (proprioceptive drift) of the real hand which is relocated to the space occupied by the rubber hand [33] [9, 34].

Recently, research has focussed on the contribution of perceptual embodiment and visual expectation to pain perception. Studies have found that perceived body ownership may increase pain threshold and decrease pain intensity to experimentally induced pain [34, 35], although Mohan et al. [33] found that pain threshold was not affected during the rubber hand illusion (in view) when the real hand (out of view) received noxious thermal stimuli. Chang et al. [36] found that the sympathetic response to acupuncture needle stimulation was influenced by visual expectation rather than by modifications of body ownership producing greater sympathetic responses measured by skin conductance. Suggested mechanisms for visually induced analgesia include disruption of nociceptive processing caused through conflict in the reference frames needed by the brain to localize somatosensory input, and also by a decrease in homeostatic control as the hand is disowned [37, 38].

Shortcomings in our study need to be recognised. Our outcome measures relied on the subjective report of perceptual embodiment gleaned from responses to questions that implied that participants should feel 'something arising' from the rubber hand. The absence of a condition that exposed a non-embodied rubber hand to stimuli meant that it was not possible to isolate with certainty that outcomes were affected by observing the actions of stimuli on a rubber hand per se. We plan to include a non-embodied rubber hand as a control in follow-up studies. Moreover, the inclusion of synchronous and asynchronous stroking patterns on real and rubber hands coupled with questions that capture subjective experience when the illusion is not expected to arise could be used to control for expectancy associated with the illusion. We included mean skin conductance response as an objective correlate of the presence of embodiment, although follow-up studies could also include other correlates such as proprioceptive drift to improve confidence in self-reports [39]. Skin conductance measurements of peak to peak or incremental slopes, rather than mean skin conductance response may be more reliable when measuring arousal responses in short time windows. Concern associated with asking questions related to perceptual embodiment of the rubber hand before delivery of stimuli may have interfered with the embodiment experience was not borne out by inspection of raw and summary data for questions 1 to 3 over the course of the experiment. There were minor differences in the salience of stimuli with the needle inserted into the back of the rubber hand and the brush slowly stroked across the rubber hand rather than pressed against the back of the rubber hand. Participants would have observed differences in the type and amount of movements of the stimuli and this could cause attentional differences and confound the comparison. Moreover, we did not measure baseline anxiety (using for example using the State-Trait Anxiety Inventory) so it was not possible to determine relationships between baseline anxiety and stimulus-evoked anxiety.

Two participants made slight withdrawal movements of their real hand when the needle contacted the rubber hand. One other participant closed their eyes at this point. This would have disrupted agency and visual input confounding embodiment measures. These three participants were included in the statistical analysis of 10 participants that experienced stimulus evoked sensations. Excluding these participants from the analysis of Q7 ‘How strongly did these sensations associated with the stimulus feel like they were coming from the rubber hand?’ would have meant that there would not have been a sufficiently sized sample to perform the statistical analysis. Larger sample sizes and multiple repetitions of stimuli may overcome this problem in follow-up studies. Consideration also needs to be given to how to reduce movement artifact.

In conclusion, our preliminary findings suggest that the increase in arousal in response to stimuli entering the peripersonal space may not be selective for threat. Half of the participants reported that they experienced a sensation arising from a perceptually embodied rubber hand when the rubber hand was approached and touched by a stimulus. There was tentative evidence that more intense sensations were experienced when approached by the needle compared with the brush. The confounders mean that our findings should be used to provide initial insights of phenomena and serve as a catalyst for further research.

ACKNOWLEDGEMENT
The authors wish to express thanks to Dr Osama Tashani for technical support and the anonymous reviewers for their valuable advice.

AUTHORS CONTRIBUTIONS
Conceived and designed the experiments: MIJ, ES, SY, MRM
Performed the experiments: ES, SY
Analysed the data: MIJ, ES, SY
Wrote the paper: MIJ, ES, SY, MRM

DISCLOSURE STATEMENT
There are no potential or actual conflicts of interest for any of the authors

REFERENCES

[1] Gentile G, Petkova VI, Ehrsson HH. Integration of visual and tactile signals from the hand in the human brain: an FMRI study. J Neurophysiol.105:910-22.

[2] Ehrsson HH, Spence C, Passingham RE. That's my hand! Activity in premotor cortex reflects feeling of ownership of a limb. Science. 2004;305:875-7.

[3] Ehrsson HH, Fagergren A, Ehrsson GO, Forssberg H. Holding an object: neural activity associated with fingertip force adjustments to external perturbations. J Neurophysiol. 2007;97:1342-52.

[4] Ehrsson HH, Holmes NP, Passingham RE. Touching a rubber hand: feeling of body ownership is associated with activity in multisensory brain areas. J Neurosci. 2005;25:10564-73.

[5] Longo MR, Schuur F, Kammers MP, Tsakiris M, Haggard P. What is embodiment? A psychometric approach. Cognition. 2008;107:978-98.

[6] Botvinick M, Cohen J. Rubber hands 'feel' touch that eyes see. Nature. 1998;391:756.

[7] Valenzuela Moguillansky C, O'Regan JK, Petitmengin C. Exploring the subjective experience of the "rubber hand" illusion. Front Hum Neurosci. 2013;7:659.

[8] Lewis E, Lloyd DM. Embodied experience: A first-person investigation of the rubber hand illusion. Phenomenol Cogn Sci. 2010;9:317-39.

[9] Capelari ED, Uribe C, Brasil-Neto JP. Feeling pain in the rubber hand: integration of visual, proprioceptive, and painful stimuli. Perception. 2009;38:92-9.

[10] Lloyd D, Morrison I, Roberts N. Role for human posterior parietal cortex in visual processing of aversive objects in peripersonal space. J Neurophysiol. 2006;95:205-14.

[11] Ehrsson HH, Wiech K, Weiskopf N, Dolan RJ, Passingham RE. Threatening a rubber hand that you feel is yours elicits a cortical anxiety response. Proc Natl Acad Sci U S A. 2007;104:9828-33.

[12] Armel KC, Ramachandran VS. Projecting sensations to external objects: evidence from skin conductance response. Proc Biol Sci. 2003;270:1499-506.

[13] Hagni K, Eng K, Hepp-Reymond MC, Holper L, Keisker B, Siekierka E, Kiper DC. Observing virtual arms that you imagine are yours increases the galvanic skin response to an unexpected threat. PLoS One. 2008;3:e3082.

[14] Lewis E, Lloyd DM, Farrell MJ. The role of the environment in eliciting phantom-like sensations in non-amputees. Front Psychol. 2012;3:600.

[15] Guterstam A, Gentile G, Ehrsson HH. The invisible hand illusion: multisensory integration leads to the embodiment of a discrete volume of empty space. J Cogn Neurosci. 2013;25:1078-99.

[16] Guterstam A, Petkova VI, Ehrsson HH. The illusion of owning a third arm. PLoS One. 2011;6:e17208.

[17] Ma K, Hommel B. The virtual-hand illusion: effects of impact and threat on perceived ownership and affective resonance. Front Psychol. 2013;4:604.

[18] Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol. 1988;124:869-71.

[19] Mussap AJ, Salton N. A 'rubber-hand' illusion reveals a relationship between perceptual body image and unhealthy body change. J Health Psychol. 2006;11:627-39.

[20] Farmer H, Tajadura-Jimenez A, Tsakiris M. Beyond the colour of my skin: how skin colour affects the sense of body-ownership. Conscious Cogn. 2012;21:1242-56.

[21] Dawson M, Schell A, Filion D. The electrodermal system. In: Cacioppo J, Tassinary L, Berntson G, editors. Handbook of Psychophysiology 3ed. New York: Cambridge University Press; 2007. p. 159–81.

[22] Edelberg R. Electrical properties of the skin. In: Brown CC, editor. Methods in psychophysiology. Baltimore: Williams and Wilkins; 1967. p. 1–53.

[23] Braithwaite JJ, Broglia E, Watson DG. Autonomic Emotional Responses to the Induction of the Rubber-Hand Illusion in Those That Report Anomalous Bodily Experiences: Evidence for Specific Psychophysiological Components Associated With Illusory Body Representations. J Exp Psychol Hum Percept Perform. 2014.

[24] Ocklenburg S, Ruther N, Peterburs J, Pinnow M, Gunturkun O. Laterality in the rubber hand illusion. Laterality. 2011;16:174-87.

[25] Tsakiris M, Haggard P. The rubber hand illusion revisited: visuotactile integration and self-attribution. J Exp Psychol Hum Percept Perform. 2005;31:80-91.

[26] Moseley GL, Olthof N, Venema A, Don S, Wijers M, Gallace A, Spence C. Psychologically induced cooling of a specific body part caused by the illusory ownership of an artificial counterpart. Proc Natl Acad Sci U S A. 2008;105:13169-73.

[27] Llobera J, Sanchez-Vives MV, Slater M. The relationship between virtual body ownership and temperature sensitivity. J R Soc Interface. 2013;10:20130300.

[28] Barnsley N, McAuley JH, Mohan R, Dey A, Thomas P, Moseley GL. The rubber hand illusion increases histamine reactivity in the real arm. Curr Biol. 2011;21:R945-6.

[29] Tsakiris M, Hesse MD, Boy C, Haggard P, Fink GR. Neural signatures of body ownership: a sensory network for bodily self-consciousness. Cereb Cortex. 2007;17:2235-44.

[30] Rohde M, Di Luca M, Ernst MO. The Rubber Hand Illusion: feeling of ownership and proprioceptive drift do not go hand in hand. PLoS One. 2011;6:e21659.

[31] Rohde M, Wold A, Karnath HO, Ernst MO. The human touch: skin temperature during the rubber hand illusion in manual and automated stroking procedures. PLoS One. 2013;8:e80688.

[32] Makin TR, Holmes NP, Ehrsson HH. On the other hand: dummy hands and peripersonal space. Behavioural Brain Research. 2008;191:1-10.

[33] Mohan R, Jensen KB, Petkova VI, Dey A, Barnsley N, Ingvar M, McAuley JH, Moseley GL, Ehrsson HH. No pain relief with the rubber hand illusion. PLoS One. 2012;7:e52400.

[34] Hegedus G, Darnai G, Szolcsanyi T, Feldmann A, Janszky J, Kallai J. The rubber hand illusion increases heat pain threshold. Eur J Pain. 2014;18:1173-81.

[35] Hansel A, Lenggenhager B, von Kanel R, Curatolo M, Blanke O. Seeing and identifying with a virtual body decreases pain perception. Eur J Pain. 2011;15:874-9.

[36] Chang DS, Kim YJ, Lee SH, Lee H, Lee IS, Park HJ, Wallraven C, Chae Y. Modifying Bodily Self-Awareness during Acupuncture Needle Stimulation Using the Rubber Hand Illusion. Evid Based Complement Alternat Med. 2013; 849602.

[37] Moseley GL, Gallace A, Spence C. Bodily illusions in health and disease: physiological and clinical perspectives and the concept of a cortical 'body matrix'. Neurosci Biobehav Rev. 2012;36:34-46.

[38] Moseley GL, Flor H. Targeting cortical representations in the treatment of chronic pain: a review. Neurorehabil Neural Repair. 2012;26:646-52.

[39] Christ O, Reiner M. Perspectives and possible applications of the rubber hand and virtual hand illusion in non-invasive rehabilitation: Technological improvements and their consequences. Neurosci Biobehav Rev. 2014; 44, 33-44

FIGURE LEGENDS

Figure 1

a) Experimental set up showing the location of the right hand (out of view) in a canvas box, the left hand with electrodes for skin conductance response on fingers and thumb, and the rubber hand (in view) between the real hands; b) Technique used to perceptually embody the rubber hand; c) Soft brush used for the non-threatening stimulus; d) Syringe needle used for the threatening stimulus. The individuals in this figure are the authors and they have given written informed consent to publish these photographs.

Figure 2

Time line of experimental events. SCR = Skin Conductance Response

Figure 3

Mean $\pm$ Standard Error scores for questions 2 to 5. Repeated measures ANOVA found effects for Stimulus (pre and post stimulus, * = $p<0.01$) but not for Condition (brush and needle) or Stimulus x Condition interaction for self-reported items associated with perceptual embodiment (Q2-Q4). A paired t-test found that anxiety was lower for brush than needle (Q5, ** = $p<0.001$, n=20).

Figure 4

Location and nature of phantom sensations. The location of each sensation is represented as the outermost boundary created from the whole sample by overlaying each participant's pain chart on top of each other. No attempt was made to represent the intensity or reporting frequency of sensations in the diagram. N values represent the number of participants reporting the presence of a sensation.

Figure 5

Mean $\pm$ Standard Error skin conductance response (microsiemens) across experimental events (n=20).

TABLES

Table 1
Summary data for perceptual embodiment questions (Mean±SD, n=20). Responses to each question was rated on an 11 item numerical scale where 1 = ‘Not at all strongly’ and 10 = ‘Strongest imaginable’. * = statistical significance at p<0.05 between brush and needle, ** = statistical significance at p<0.001 between brush and needle. For Q2-Q4 repeated measures ANOVA found effects for Stimulus but not Condition, or Stimulus x Condition interaction. N/A – Statistical test Not Applicable.

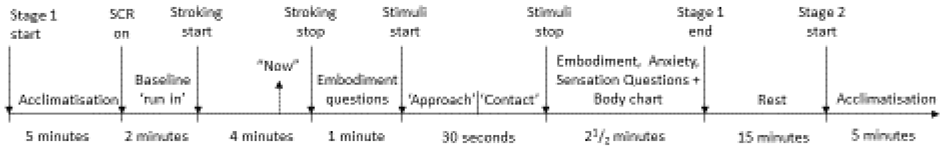
	Time to report tactile sensations arising from the rubber hand	Q1. How strongly does it feel like the sensation of stroking is coming from the rubber hand?	Q2. How strongly does it feel like the rubber hand is yours?	Q3. How strongly does it feel like the rubber hand is part of your body?	Q4. How strongly does it feel you can move the rubber hand?	Q5. How anxious were you when I initially approached the rubber hand with the stimulus?	Q6. Did you feel any sensations associated with the stimulus coming from the rubber hand?	Q7. How strongly did these sensations associated with the stimulus feel like they were coming from the rubber hand?
Before Stimulus								
Brush (non-threat)	38.65±29.71s	8.35±1.18	8.00±1.49	7.90±1.41	7.90±1.65	N/A	N/A	N/A
Needle (threat)	36.37±21.82s	8.00±2.03	8.25±1.74	8.05±1.76	8.20±1.40	N/A	N/A	N/A
After Stimulus								
Brush (non-threat)	N/A	N/A	5.90±2.55	5.65±2.64	5.70±2.62	3.35±2.50	“Yes” (n=8)	5.37±2.67(n=8)
Needle (threat)	N/A	N/A	6.25±2.88	5.95±2.60	5.80±2.80	6.90±2.17**	“Yes” (n=8)	7.50±1.41(n=8)*
Difference in change before and after brush and needle	N/A	N/A	0.10±1.65	0.15±2.03	-0.2±2.12			

Table 2
Mean differences (95% confidence intervals, n=20) for skin conductance response (microsiemens) across experimental Events. Values for p were adjusted for multiple comparisons using Bonferroni and statistical significance represented by *.

Condition	Baseline	Stroking	"Now" (Perceptual embodiment)	Stimulus 'Approach'
Stroking	1.162 (-0.35, 2.68) p=0.250			
"Now" (Perceptual embodiment)	2.70 (0.75, 4.65) p=0.003*	1.53 (0.81, 2.26) p<0.001*		
Stimulus 'Approach'	4.68 (1.69, 7.65) p=0.001*	3.51 (1.60, 5.43) p<0.001*	1.98 (0.36, 3.60) p=0.01*	
Stimulus 'Touch'	5.09 (7.85, 2.33) p<0.001*	3.93 (2.14, 5.71) p<0.001*	2.39 (0.83, 3.96) p<0.001*	0.42 ($8.31x10^{-5}$, 0.83) p=0.05*


Fig 1
a
b
c
d

Fig 2



Stage 1 start
SCR on
Stroking start
Stroking stop
Stimuli start
Stimuli stop
Stage 1 end
Stage 2 start
Acclimatisation
Baseline 'run in'
"Now"
Embodiment questions
'Approach'
'Contact'
Embodiment, Anxiety, Sensation Questions + Body chart
Rest
Acclimatisation
5 minutes
2 minutes
4 minutes
1 minute
30 seconds
2 1/2 minutes
15 minutes
5 minutes

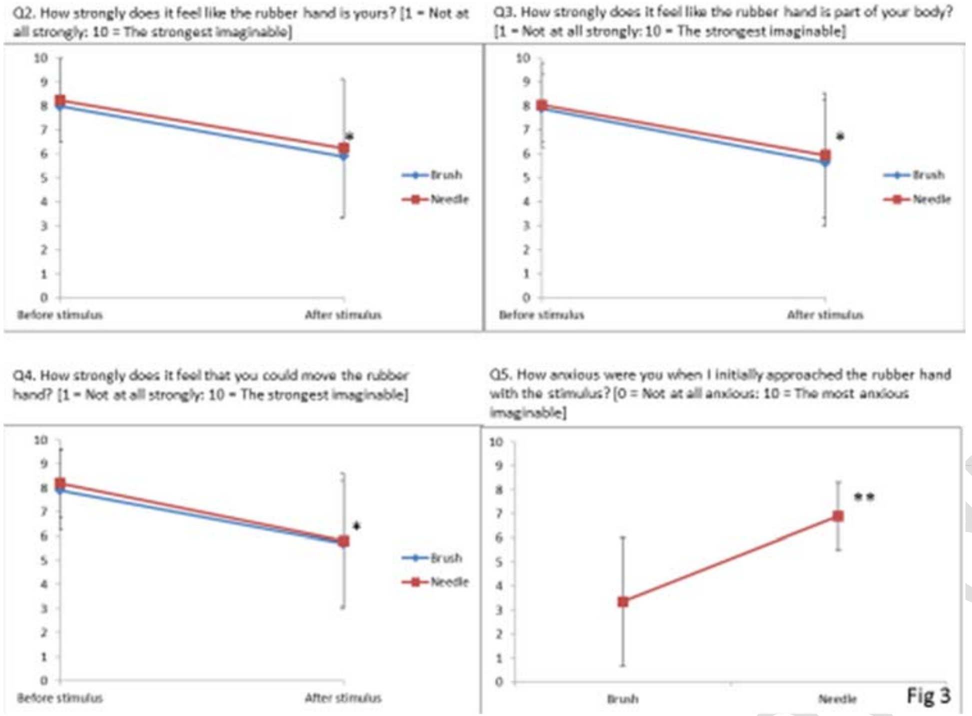

Q2. How strongly does it feel like the rubber hand is yours? [1 = Not at all strongly: 10 = The strongest imaginable]
Q3. How strongly does it feel like the rubber hand is part of your body? [1 = Not at all strongly: 10 = The strongest imaginable]
Q4. How strongly does it feel that you could move the rubber hand? [1 = Not at all strongly: 10 = The strongest imaginable]
Q5. How anxious were you when I initially approached the rubber hand with the stimulus? [0 = Not at all anxious: 10 = The most anxious imaginable]
Brush
Needle
Before stimulus
After stimulus
*
**

Fig 3

Fig 4

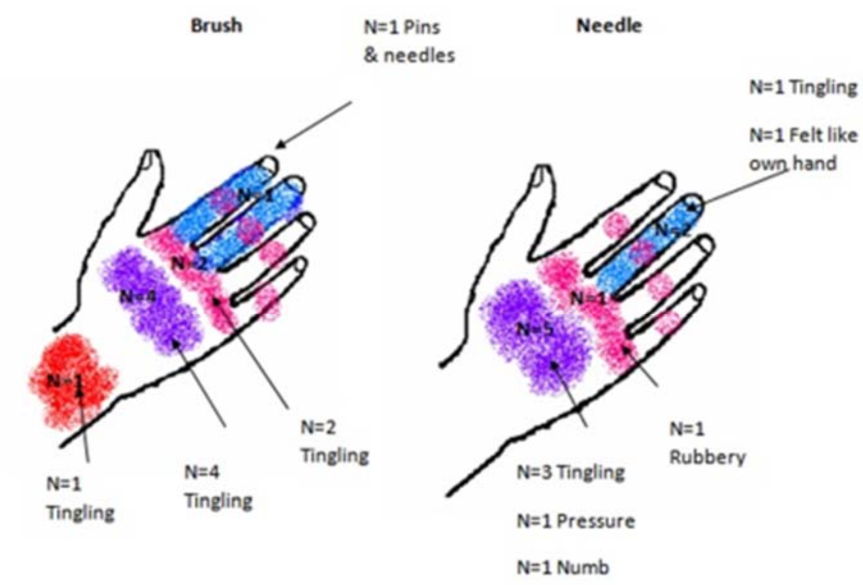
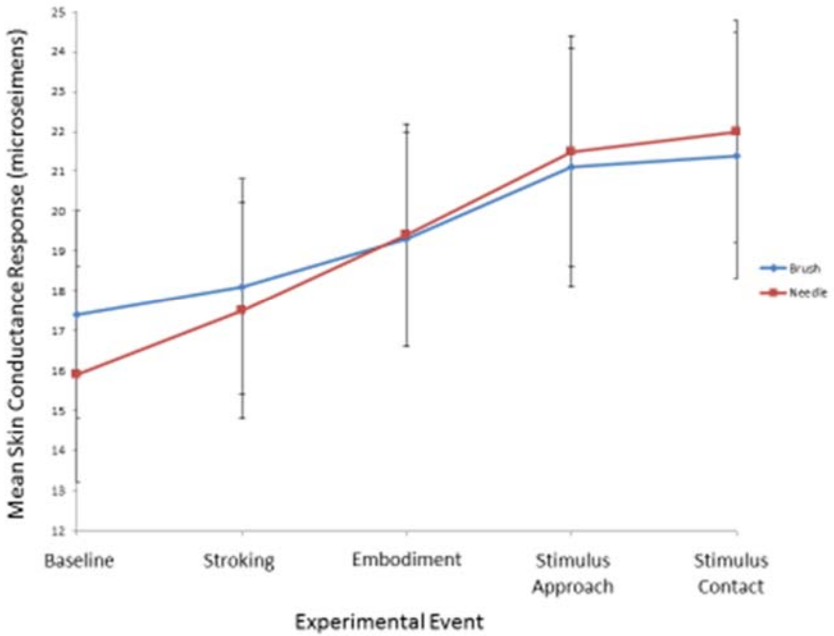

Brush
N=1 Pins & needles
N=1
N=2
N=4
N=1
N=1 Tingling
N=4 Tingling
N=2 Tingling
Needle
N=1 Tingling
N=1 Felt like own hand
N=2
N=1
N=5
N=3 Tingling
N=1 Pressure
N=1 Numb
N=1 Rubbery

Fig 5


Mean Skin Conductance Response (microseimens)
25
24
23
22
21
20
19
18
17
16
15
14
13
12
Baseline
Stroking
Embodiment
Stimulus Approach
Stimulus Contact
Experimental Event
Brush
Needle