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RESEARCH ARTICLE

A threat to a virtual hand elicits motor cortex activation

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Abstract We report an experiment where participants observed an attack on their virtual body as experienced in an immersive virtual reality (IVR) system. Participants sat by a table with their right hand resting upon it. In IVR, they saw a virtual table that was registered with the real one, and they had a virtual body that substituted their real body seen from a first person perspective. The virtual right hand was collocated with their real right hand. Event-related brain potentials were recorded in two conditions, one where the participant's virtual hand was attacked with a knife and a control condition where the knife only struck the virtual table. Significantly greater P450 potentials were obtained in the attack condition confirming our expectations that participants had a strong illusion of the virtual hand being their own, which was also strongly supported by questionnaire responses. Higher levels of subjective virtual hand ownership correlated with larger P450 amplitudes. Mu-rhythm event-related desynchronization in the motor cortex and readiness potential (C3–C4) negativity were clearly observed when the virtual hand was threatened—as would be expected, if the real hand was threatened and the participant tried to avoid harm. Our results support the idea that event-related potentials may provide a promising non-subjective measure of virtual embodiment. They also support previous experiments on pain observation and are placed into context of similar experiments and studies of body perception and body ownership within cognitive neuroscience.

Keywords Body ownership · Rubber hand illusion · Virtual reality · Motor cortex · Pain · ERPs

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Introduction

When someone anticipates that a knife might stab their hand that is resting on a table, they would be likely to attempt to move the threatened hand out of the way. They would expect to feel considerable pain should the knife stab it. In this paper, we consider what happens when a person's real body is visually substituted by a life-sized virtual body, and they see a threat or attack to a hand of this virtual body. Our experiment investigates brain activity in response to events that would cause pain to the observer were these 'pain observation' events to occur in reality. Our contribution lies in introducing a new technique for the study of such pain observation, by using immersive virtual reality (IVR) for the scenario and stimulation, while recording brain activity with EEG. Our work contributes to the growing field of body representation, how the brain represents the body, as well as presenting results on pain observation.

Several brain imaging techniques have used pain observation experiments to understand the associated mental processes. Methods that employ magnetic resonance imaging (fMRI) have found that the anterior cingulate cortex and the right insula brain regions are associated with such pain observation (Jackson et al. 2005; Gu and Han 2007). Studies that have examined the event-related potential (ERP) temporal dynamics involved in empathy, measured as the response to observation of pain in others, especially prominent in the motor cortex area, have found greater P450 responses for painful images compared to neutral images (Fan and Han 2008; Li and Han 2010; Meng et al. 2012, 2013). These effects were modulated by the realism of the presentation and were stronger with greater realism (Fan and Han 2008). Similarly, in studies using transcranial magnetic stimulation (TMS), participants have shown a reduction in motor evoked potentials (MEPs) resulting from watching a hand undergoing a painful situation (Avenanti et al. 2005). Experiments combining both pain observation and electrical stimulation have shown modulations in the somatosensory evoked potentials (SEP), particularly prominent in the centroparietal locations, with larger amplitudes for the P450 component when observing a painful situation (Bufalari et al. 2007).

This automatic empathy response is elicited involuntarily (Preston and de Waal 2002) through a bottom-up process. However, it can also be modulated consciously (top–down), for example under instructions of subjective pain estimation, generating stronger P450 responses (Fan and Han 2008).

Interestingly, pain observation studies that have focused on frequency power spectra (FPS) decomposition have shown a depression in the mu-rhythm during painful conditions, using magnetoencephalography (MEG) and EEG (Cheng et al. 2008; Yang et al. 2009). This abolition or suppression of the mu-rhythm when observing painful situations has been interpreted to be in agreement with previous observations about the involvement of this oscillatory activity in the execution of voluntary movements (Neuper et al. 2005). The mu-ERD is described as a circumscribed desynchronization in the upper alpha frequency band (in the range of about 9–12 Hz) when a participant performs a motor action (Pfurtscheller and Lopes da Silva 1999) or motor action observation (Muthukumaraswamy and Johnson 2004). Moreover, previous studies have found that mu-ERD can also be triggered as an unconscious mechanism to avoid painful events (Babiloni et al. 2008). When a sound alerted participants 2.5 s prior to an electrical painful stimulation at the left index finger, a suppression of the mu-rhythm was elicited, as if the participant had tried to move the hand to avoid harm. This effect was not elicited during non-painful stimulation.

We present a pain observation experiment in the context of a whole body ownership illusion in immersive virtual reality (IVR). The IVR was delivered through a wide field-of-view head-tracked stereo head-mounted display (HMD). This set-up substitutes a person's own body by a virtual body seen from a first person perspective (1PP), such that when participants look down towards their body they would see a virtual body replacing their own (Fig. 1). In the

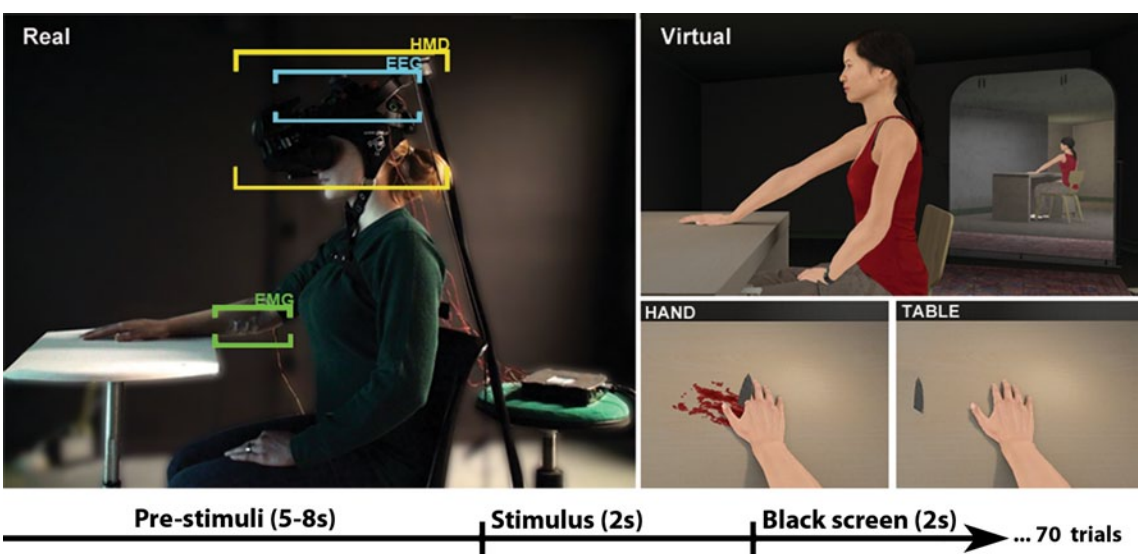

Fig. 1 Real: the participant wearing the HMD and EEG cap. Virtual: the IVR with the gender-matched collocated virtual avatar. HAND: the virtual hand stabbed by the knife. TABLE: the virtual table stabbed by the knife (control condition)—the two experimental conditions seen by the participant when looking towards his hand from the first person perspective

experiment, the participant's stationary right virtual hand, which was collocated with the real right hand resting on a table, was repeatedly threatened by a virtual knife, thereby reproducing in IVR previously conducted pain observation experiments (Avenanti et al. 2006; Bufalari et al. 2007; Gu and Han 2007; Fan and Han 2008; Li and Han 2010; Meng et al. 2012, 2013). The painful stimulation was compared to a control where the same knife attacked only the virtual table that was spatially registered with the real table on which the hand was resting (Fig. 1). In short, we measured EEG responses, which resulted in similar ERPs compared to previous experiments, with greater P450 effects on CP3 for the painful condition compared to the control condition (Bufalari et al. 2007; Fan and Han 2008; Li and Han 2010; Meng et al. 2013).

We used IVR for the study of pain observation due to recent results which show that virtual reality can be used to induce an illusion of ownership over a virtual body. This work has its origin in the rubber hand illusion, where it has been shown that synchronous tactile stimulation of a visible rubber hand and the experimental subject's corresponding hidden real hand, results in an illusion of ownership over the rubber hand (Botvinick and Cohen 1998; Armel and Ramachandran 2003; Ehrsson et al. 2004; Tsakiris and Haggard 2005). Here the rubber hand is placed on a table in front of the subject in an anatomically plausible position, with the corresponding real hand out of sight behind a screen. When the real and rubber hand are synchronously brushed in the same location on each hand, then there is typically and quickly an illusion of ownership over the rubber hand. This result has been extended to a virtual hand in virtual reality (Slater et al. 2008) including, but less strongly, a table-top video projection of a hand (Ijsselsteijn et al. 2006), and the illusion is also reproduced when visuomotor synchrony is used rather than visuotactile (Sanchez-Vives et al. 2010; Kalckert and Ehrsson 2012).

Similar multisensory techniques have been used for whole body ownership illusions—both illusions of displacement (or out of the body illusions) (Ehrsson 2007; Lenggenhager et al. 2007) and illusions of body substitution (Petkova and Ehrsson 2008). Evidence suggests that the dominant factor in such whole body illusions may be first person perspective (Slater et al. 2010; Petkova et al. 2011; Maselli and Slater 2013), though it is likely that additional multisensory stimulation such as visuotactile and visuomotor synchrony would also play a role. For a review of the field see Blanke (2012).

Typically, however, pain observation experiments present a series of pictures with hands or other extremities undergoing painful situations, and they compare the brain response of the participants to the activation produced by pictures where the same extremities do not undergo painful situations (Avenanti et al. 2006; Bufalari et al. 2007; Fan and Han 2008; Li and Han 2010). Many of these experiments present scissors and needles perforating the extremities as painful stimuli. A potential advantage of IVR, however, is that there is greater ecological validity, going beyond the presentation of two-dimensional, static stimuli. With IVR there is a life-sized, three dimensional virtual body seen in stereo that visually substitutes the obscured real body of the participant, which can be virtually attacked. Hence, the level of realism can be greatly enhanced. In the present study, participants saw a knife attacking the hand of their virtual body, the virtual body therefore acting as a surrogate for the real body in the context of pain observation.

Materials and methods

Apparatus

Participants were fitted with a stereo NVIS nVisor SX111 head-mounted display (HMD). This has dual SXGA displays with 76°H × 64°V degrees field-of-view (FOV) per eye, totalling a wide field-of-view 111° horizontal and 60° vertical, with a resolution of 1,280 × 1,024 per eye displayed at 60 Hz. Head tracking was by a 6-DOF Intersense IS-900 device. The virtual environment was programmed in the XVR system (Tecchia et al. 2010) and the virtual character rendered using the HALCA library (Gillies and Spanlang 2010).

Procedures

Nineteen healthy volunteers—9 male, 10 female; aged 25 ± 4.0 (SD) years—all right-handed—participated in the experiment. The experimental protocol was approved by the Universitat de Barcelona Ethics Committee (Spain), and all the participants gave written informed consent and were paid 10€ for their participation.

Participants entered the virtual reality and saw a virtual body (avatar) from 1PP that was consistent with their gender and skin colour. The virtual scene consisted of the avatar seated on a chair with its virtual right hand placed on a desk. In the laboratory, the participant was seated with his/her real right hand collocated with the avatar's hand and resting on a table. The left hand was placed comfortably on the participant's lap. Participants were asked to relax and keep their arms and hand still throughout the experiment (Fig. 1).

Participants were encouraged to freely look around for 60 s to familiarize themselves with the environment while keeping their arms still and collocated with those of the virtual body. After the familiarization time, we told participants several times to fix their gaze on the virtual hand resting on the table and to keep their real hand still. We did not ask them to perform any other task at all, such as pain judgment, but only to fixate on the virtual hand.

Stimuli

Participants repeatedly experienced two conditions in a within-group design: condition HAND where the knife stabbed the virtual right hand, and condition TABLE where the knife stabbed the table, 15 cm away from the right hand (Fig. 1). The experiment consisted of 70 trials repeating the HAND and TABLE conditions (30 HAND and 40 TABLE). A trial consisted of three parts:

1. Pre-stimulus: the participant looked at the virtual hand (5–8 s).
2. Stimulus: a knife appeared in the HAND or TABLE (2 s).
3. Black screen: a black screen appeared (2 s).

During the first 10 trials only the TABLE condition was presented to acclimatize participants to the trial evolution and the black screen. Then, there were 6 predefined blocks of 10 trials each, each block had 5 HAND and 5 TABLE, with the order randomized within each block. The order in which these blocks were presented to the participants was randomized for each participant. After the 70 trials, the screen went black and the experiment ended. This virtual reality exposure lasted for 15 min. (See Electronic Supplementary Material Video for an overview of the whole experiment). Participants then completed a questionnaire about their virtual experience, in which they answered the following questions:

1. *Ownership* I felt as if the hand I saw in the virtual world might be my hand.
2. *Harm Hand* I had the feeling that I might be harmed when I saw the knife inside the hand.
3. *Harm Table* I had the feeling that I might be harmed when I saw the knife outside the hand.
4. *No Ownership* The hand I saw was the hand of another person.
5. *Body Threat* I saw the knife as a threat to my body.

Responses to these statements were on a 5-point Likert scale where 1 was anchored to strong disagreement and 5 to strong agreement. Questions 1 and 4 were related to the sense of ownership of the hand, with question 1 expected to record high scores while question 4 expected to record low scores. These two questions are similar to those used in previous studies to measure ownership illusions (Banakou et al. 2013; Llobera et al. 2013), for example: 'How much did you feel that the virtual body was your body' for the ownership question and 'How much did you feel that the virtual body was another person?' as a control for the no ownership or 'How much did you feel that the virtual body you saw when you looked down at yourself was your own body' versus 'How much did you feel as if you had two bodies'. Moreover, question 1 is similar to that used by the original Botvinick and Cohen (1998) paper 'I felt as if the rubber hand was my hand'. Questions 2 and 3 were to examine whether there was any feeling of harm in response to the knife being in the condition HAND or TABLE. Question 5 was a consistency check to control questions 2 and 3; we expected similar responses to *Harm Hand*.

Electrophysiological recording

Both EEG and electromyography (EMG) were recorded using an gUSBamp[1] amplifier with a resolution of 30nV; the electrodes were set to cover the motor cortex area and surrounding: FC3, FC4, C3, C4, CP3 and CP4 located according to the 10/20 standard EEG recording; the reference was set with an ear clip on the left ear lobe; the ground was positioned on the forehead; electrodes in the face measured ocular activity (EOG). Three EMG electrodes were placed in the flexor carpi ulnaris muscle of the right arm to measure whether participants moved their hand. All the electrodes were kept to impedances below 10 kΩ. The data were recorded using Matlab with a sampling frequency of 512 Hz. Trials that were contaminated, i.e. exceeding amplitudes of ± 100 μV by any electrode or by the EOG were rejected off-line; 3.8 % of the trials were excluded due to artefacts (2.26 ± 2.42 trials per participant).

EEG data analysis

In order to study the effects of the stimuli on the pain sensitivity, event-related potential (ERP) components were analysed as in Fan and Han (2008), Li and Han (2010) and Meng et al. (2012). The stimulus-locked ERP helped us to determine the pain-related levels of the participants with respect to the HAND condition, where a higher P450 activity was expected in case of a pain response.

The ERPs in both conditions, HAND and TABLE, were averaged separately for each subject. The ERPs were also used to better study the lateralization part of the readiness potential (RP) in order to detect which hemisphere was more active. The RP has been previously related to movement preparation, and it is generally calculated as the double subtraction of C3–C4 (Eimer 1998), considering right and left hand movements. As in our case we only used right hand manipulations we report only one side C3–C4

[1] The EEG equipment was supplied by Guger Technologies, www.gtec.at.

subtraction. An increase in negativity is expected when a movement is prepared with the contralateral hemisphere. Thus, negative amplitude might reflect a right-hand movement preparation.

Apart from the ERPs, frequency bands were also evaluated. To account for variations, we used short-time power spectra as described in Pfurtscheller and Lopes da Silva (1999). Power spectral density (PSD) was calculated as the superimposed 1-s power spectra calculated over the event-related EEG for the HAND and TABLE conditions for both the reference and activity periods.

EMG data analysis

EMG data were filtered with a band pass of 20–250 Hz selected according to the recommendations of Fridlund and Cacioppo (1986) and keeping the frequency range where the primary energy in the surface EMG signal is located. As is common practice, the root mean squared (RMS) processing technique was used (Fridlund and Cacioppo 1986). The RMS of the signal was computed with a sliding window of 500 ms in order to detect whether right arm muscles were activated at any moment. For the purpose of this experiment, subjects were asked not to move their hand under any circumstance, and the plan was that trials showing EMG activation would be discarded.

Results

Hand movements

A critical question for this experiment was whether participants did actually move their threatened hand or not. Real hand movement had to be negligible, otherwise it would increase activation in the motor cortex. This was assessed using the EMG data. The RMS was calculated for the pre-stimuli reference period (−1 to 0 s) and the post-stimuli activation period (0.7–1.7 s), and these periods correspond to the time when the motor cortex was found to be activated. Using a repeated measures ANOVA, comparing HAND-BASELINE vs. TABLE-BASELINE no significant difference nor effects were found in the RMS, $F(1,18) = 2.685$, $P = 0.119$. Other timings also did not show any activation, and the same ANOVA analysis was later used to analyse the mu-ERD. These results suggest that the participants did not move their real hand during the experimental period (see ‘Discussion’ where this issue is revisited).

Questionnaire

Here we consider whether the set-up did induce an illusion of ownership over the virtual hand, and whether the stabbing knife was subjectively experienced as a threat. Figure 2 shows the box plot ($n = 19$) for the questionnaire responses that were designed to assess this. It is clear that the illusion of ownership was high (the median level of ownership is 5), and the no ownership score was comparatively low (the median is 2). The Wilcoxon matched pairs sign-rank test (two-sided) comparing *Ownership* with *No Ownership* shows that this difference is significant ($z = 3.89$, $P < 0.0001$). The illusion of harm to the hand (*Harm Hand*) has median 3, and *Harm Table* has median 1. The paired sign-rank test again shows these to be significantly different ($z = 3.74$, $P < 0.0002$). The threat to the body as a whole (*Body Threat*) also has median 3 and is significantly different from *Harm Table* ($z = 3.59$, $P < 0.0003$). Although the medians of *Harm Hand* and *Body Threat* are the same, the greater range of the former leads to it being significantly greater overall ($z = 2.36$, $P < 0.018$).

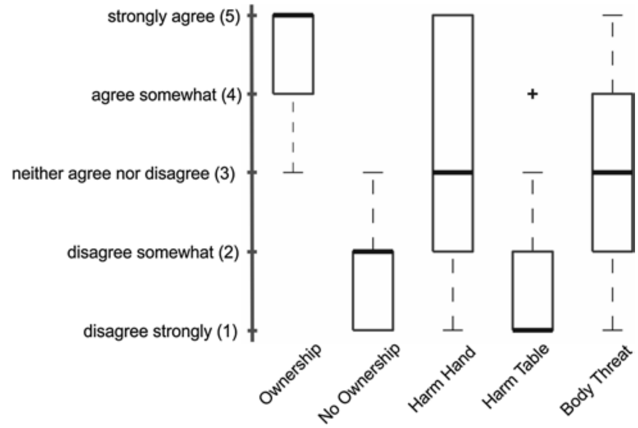

Fig. 2 Box plots showing the responses to the questionnaire. The *thick lines* are the medians, and the *boxes* are the interquartile ranges (IQR). The whiskers follow the standard convention of extending to 1.5 times the IQR or the maximal/minimal data point

Table 1 shows that *Ownership* is positively correlated with *Harm Hand* which is positively correlated with *Body Threat*. *Body Threat* is also positively correlated with *Ownership*. There are no other significant correlations. This is important since illusory ownership of the hand should go along with the feeling of threat to that hand or to the body, since without illusory ownership there is no actual threat. These results are consistent with the original hypothesis that the stronger the illusion of ownership the greater the tendency of participants to give higher ratings to the Harm questions. We consider these relationships in greater depth in “Relationship between Questionnaire Scores, P450 and mu”.

ERP stimulus-locked activity

The pain sensitivity levels of the participants for the HAND and TABLE conditions were assessed using

Table 1 Spearman correlation coefficients between the questionnaire scores

	Ownership	Harm Hand	Harm Table	No Ownership	Body Threat
Ownership	1.000				
Harm Hand	0.726 (0.000)	1.000			
Harm Table	0.162 (0.508)	0.302 (0.209)	1.000		
No Ownership	−0.048 (0.844)	0.079 (0.749)	−0.125 (0.611)	1.000	
Body Threat	0.481 (0.037)	0.774 (0.000)	0.418 (0.075)	−0.179 (0.463)	1.000

P values for test of 0 correlation (shown in brackets). $P = 0.000$ means $P < 0.0005$, $n = 19$

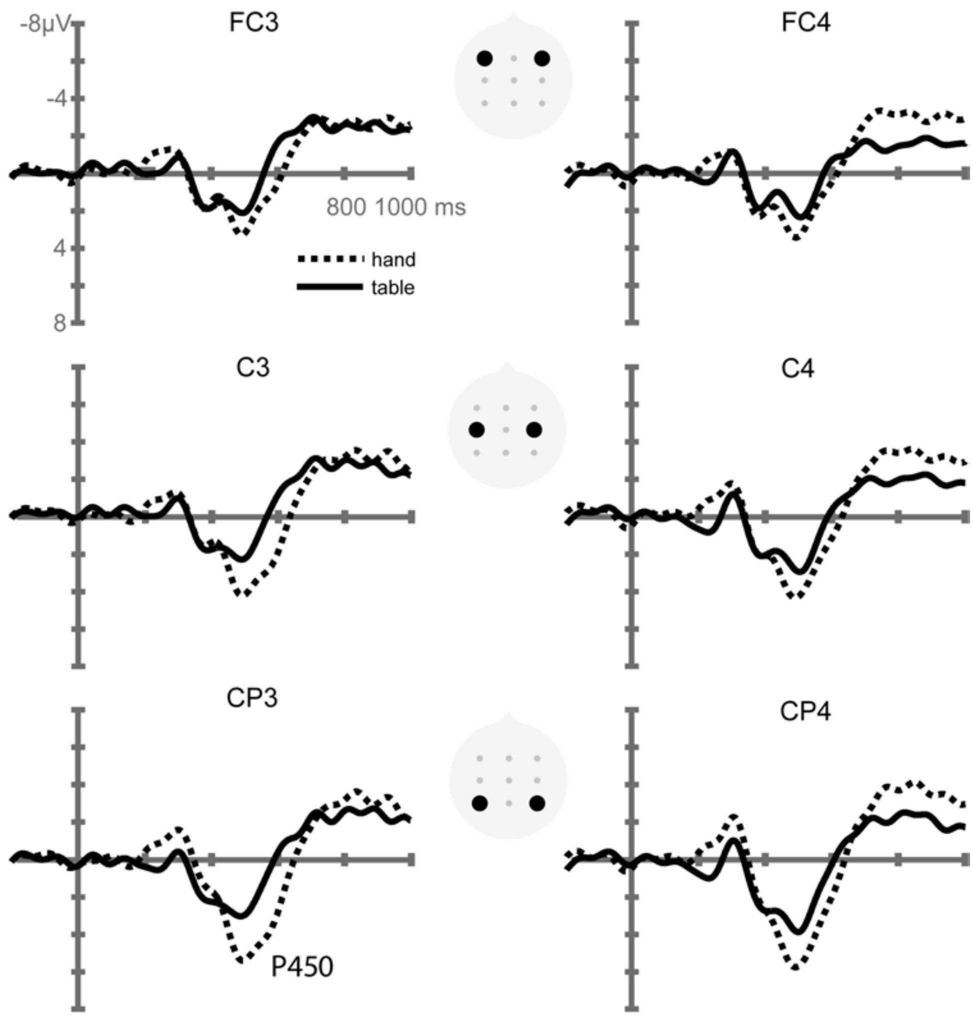

Fig. 3 Grand averaged stimulus-locked ERPs for six representative front, central and parietal electrode locations. A significant increase in the amplitude of the P450 is observed in the HAND condition mainly at C3 and CP3 locations. Baseline from −200 to 0 ms, time 0 indicates the stimuli onset; a low pass filter 12 Hz half-amplitude cut-off was applied

stimulus-locked ERPs depicted in Fig. 3. A repeated measures ANOVA P450 [condition (HAND/TABLE) × electrode (frontal/central/centroparietal) × hemisphere (left, right)] in the time window 420–620 ms on the original real voltage data showed a significant main within subjects effect for condition [$F(2,18) = 6.977$, $P = 0.017$] and for electrode position [$F(2,36) = 21.401$, $P < 0.001$]. A centroparietal distribution was observed for the P450 component as reflected by the significant interaction between condition and electrode [$F(2, 36) = 7.640$, $P = 0.002$] (the peak value was observed at CP3, see Fig. 3). We conducted further post hoc pairwise comparisons between both conditions (hand and table) at parietal and central electrodes; the paired samples *t* test were significant for the P450 at

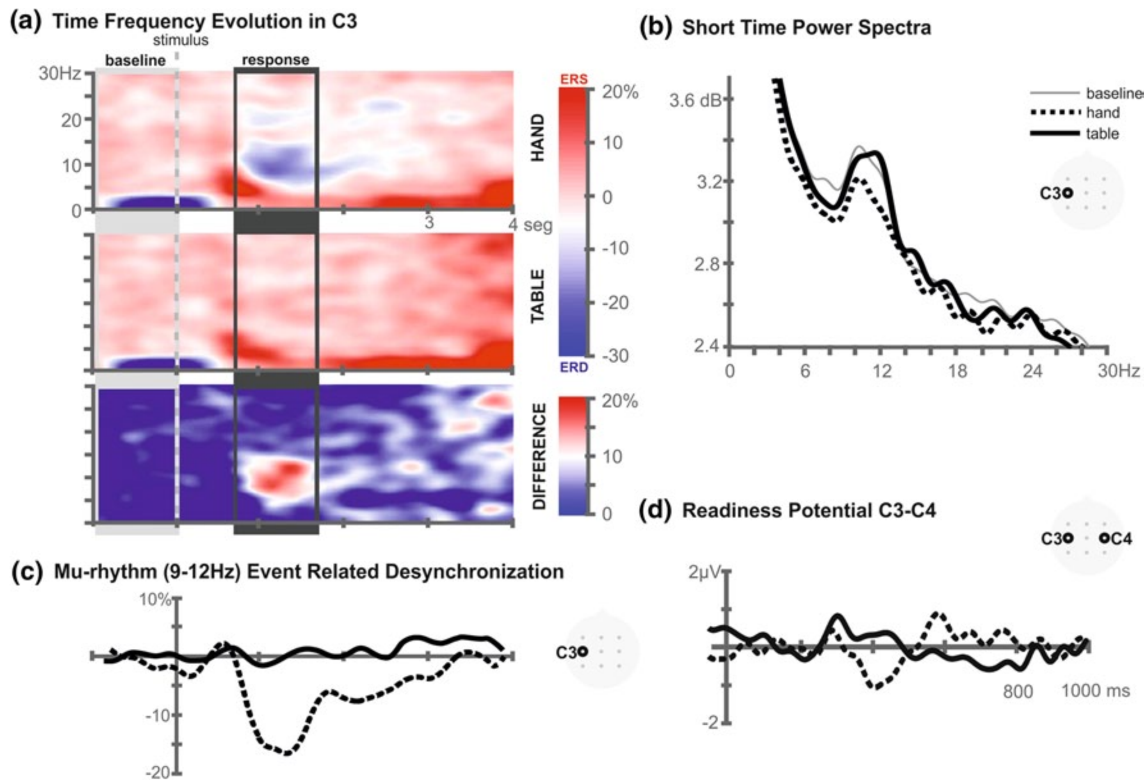

Fig. 4 **a** Time frequency evolution of the two conditions and the difference in the spectral activity. **b** Grand averaged 1-s short-time power spectra calculated from EEG data (electrode C3) recorded. The baseline corresponds to the range −1 to 0 s before the stimuli and the activity period corresponds to the range 0.7–1.7 s after the stimuli. Both the baseline and TABLE frequency spectra show a peak in the mu-rhythm that is attenuated in the HAND condition. **c** Grand averaged mu-rhythm (9–12 Hz) event-related desynchronization for the C3 electrode. **d** Grand averaged RP (C3–C4) subtraction between the brain activity in the two hemispheres shows movement preparation effects. Low pass filter 8 Hz, half-amplitude cut-off

C3 and CP3 electrodes [$t(18) = 3.438$, $P = 0.003$ and $t(18) = 3.637$, $P = 0.002$, respectively]. These results are consistent with the P450 effects induced when a pain estimation task was performed in previous studies (Fan and Han 2008; Li and Han 2010; Meng et al. 2012).

Frequency power spectral density

To determine whether participants showed a different frequency response to the attack (HAND) versus the control stimulus (TABLE), we performed a 1-s power spectra analysis (see Fig. 4a–c). In Fig. 4a, the time frequency evolution of the two conditions and the difference in the spectral activity can be observed; further representation of the mu-rhythm evolution can be found in Fig. 4c; and the 1-s power spectral differences between the reaction (0.7–1.7 s) and the baseline (−1 to 0 s) can be found in Fig. 4b. The three visualizations show a clear attenuation in the mu-rhythm during the HAND condition.

The 1-s power spectrum of the mu-rhythm (9–12 Hz) in both conditions (hand-baseline vs. table-baseline) was used for the statistical analysis. A repeated measures ANOVA with three factors [condition (HAND/TABLE) × electrode (frontal/central/centroparietal) × hemisphere (left, right)] was run to analyse the desynchronization. We found a significant main within-subject effect for the condition [$F(1,18) = 12.235$, $P = 0.003$]. The distribution of this component was dependent on the electrode position as reflected by the significant interaction [condition × electrode $F(2, 36) = 8.751$, $P = 0.001$]. Further post hoc tests comparing the conditions in the parietal and central electrodes showed most prominent desynchronization during the HAND condition in C3 [$t(18) = -3.482$, $P = 0.003$] and CP3 [$t(18) = -3.670$, $P = 0.002$]. These results are similar to the mu-ERD effects induced when an imaginary hand movement is performed (Pfurtscheller and Lopes da Silva 1999; Neuper et al. 2005).

Readiness potential

To detect which hemisphere was more activated, and thus if there was any movement preparation (Eimer 1998), we calculated the RP as C3–C4. An increase in negativity is expected if a movement is prepared with the contralateral hemisphere.

Table 2 Spearman's correlation coefficients between the questionnaire scores and EEG variables

	Ownership	Harm Hand	Harm Table	No Ownership	Body Threat	P450	mu
p450	0.287	0.389	0.113	−0.021	0.289	1.0000	
	(0.0000)	(0.0000)	(0.089)	(0.751)	(0.0000)		
mu	0.266	0.093	−0.035	−0.169	0.040	0.029	1.0000
	(0.0000)	(0.160)	(0.601)	(0.011)	(0.545)	(0.658)	

$n = 228$. Overall $R^2 = 0.26$, $F(5,222) = 15.59$, $n = 228$

Shapiro–Wilk test for normality of residuals: $P = 0.10$

$P = 0.0000$ means $P < 0.00005$

Figure 4d depicts the response-locked RP (C3–C4) activity that was analysed via a paired samples t test for time window 300–500 ms. A significant difference between conditions was found [$t(19) = -2.237$, $P = 0.038$]. This result shows negativity in the contralateral hemisphere (left, C3 electrode) during the HAND condition (mean = −0.455, SD = 1.183), which indicates right-hand pre-movement activity versus a more positive response during the TABLE condition (mean = 0.419, SD = 1.221).

Relationship between questionnaire scores, P450 and mu

Here we examine the relationship between the EEG response variables (P450, mu), the *condition* (TABLE, HAND), and the subjective responses from the questionnaire. Table 2 shows strong positive correlations between *Ownership* and each of P450 and mu, and a positive correlation between *Harm Hand* and P450. There is a negative correlation between *No Ownership* and mu.

Correlations do not imply causality, but the fact that there are very strong correlations between variables obtained in totally different ways (questionnaire and electrical recordings from the scalp) suggests that there is something to be explained. It would be surprising indeed if these were just coincidental, especially given the underlying supposition of this paper that the level of ownership would be reflected in brain activity in just the way that these correlations suggest. In particular, given the set-up and based on previous results showing that body ownership is likely to be induced as a result of 1PP (Slater et al. 2010), we would expect that the level of ownership would be positively associated with the feelings of threat to the hand and the body, which in turn would influence the P450 and mu values. These would also be influenced by the manipulated condition (i.e. whether the knife penetrated the hand or was close to it but did not penetrate).

Conventional approaches would have to treat these different relationships in separate linear models (for example, using regression) that cannot assess multiple simultaneous effects. To address this, we turned to path analysis—for example (Kaplan 2009)—which can model multiple simultaneous stochastic equations. Although not conventional in this domain of research, it is a powerful method that we have used before in the context of body ownership studies (Kilteni et al. 2013; Llobera et al. 2013; Maselli and Slater 2013; Steptoe et al. 2013).

Path analysis is particularly appropriate in the case when there are several strong correlations between variables, and a hypothesized model specifying potential causal relationships among them. The model is expressed as a set of stochastic equations (not necessarily linear) with the dependent variables on the left-hand sides and the functional specifications of the model relationships on the right (plus random error). Path analysis estimates the resulting covariance matrix of this model (and the parameters involved in the equations) typically using maximum-likelihood estimation. It can unravel spurious correlations, for example, when x and y are apparently highly correlated but where actually they are each influenced separately by another variable z. A model that consisted of $z \rightarrow x$, $z \rightarrow y$, $x \rightarrow y$, where '$u \rightarrow w$' represents a potential causal relationship from u to w (e.g. an equation of the form $w = \alpha + \beta u + \varepsilon$) would find that the path $x \rightarrow y$ was not significant (in spite of a high correlation between x and y). We used path analysis to try to isolate potential causal paths from correlations. For the path analyses, we used maximum-likelihood estimation, with robust standard errors, available in Stata 13 (www.stata.com), and the questionnaire responses were modelled as ordinal logistic variables.

We fitted the model allowing *Ownership* to influence *Harm Hand*, *Harm Table* and *Body Threat*. In turn, these could influence P450 and mu, which were also influenced by condition. We fitted the path model and deleted paths with significance levels less than 0.05. The resulting path model is shown in Fig. 5 and Table 3. It can be seen that *Ownership* is very strongly positively associated with the three harm variables. *Harm Hand* is very strongly positively associated with P450 and weakly with mu. *Harm Table* is weakly negatively associated with P450. *Condition* is strongly positively associated with P450 and negatively with mu. The overall fit of the model is good as shown by the last column of Table 3 which presents

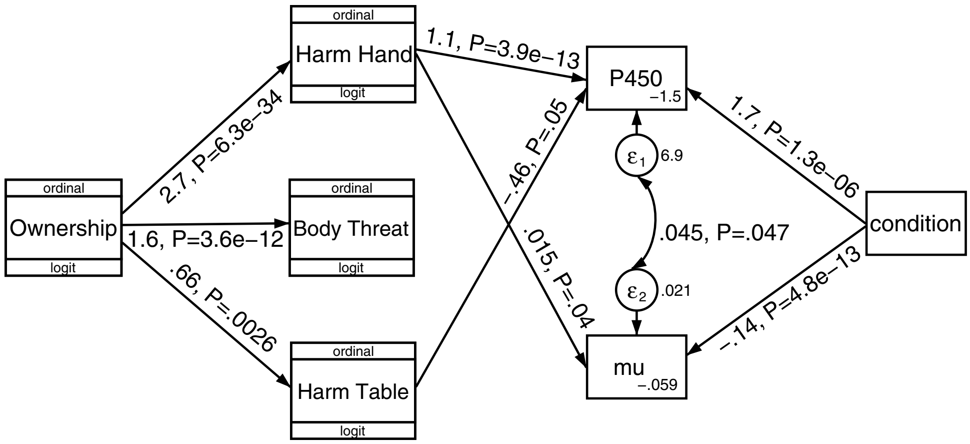

Fig. 5 Path analysis for P450 and mu and in relation to questionnaire variables *Harm Hand*, *Harm Table*, *Body Threat*, *Ownership* and condition (TABLE = 0, HAND = 1). The values on the paths are the path coefficients and the corresponding significance levels. The epsilon terms represent the random error term. The diagram can be interpreted as a set of simultaneous linear prediction equations. For example from Table 3, we can see that P450 = −1.50 + 1.69*condition + 1.08*(*Harm Hand*) − 0.46*(*Harm Table*) + epsilon. The *circles* are the random error terms and values by the *epsilon circles* are their variances. The *curved path* represents a covariance

Table 3 Path analysis for P450 and mu

	Coefficient	SE	z	P	r, P
P450					0.53, $P = 0.0000$
Condition	1.69	0.35	4.84	0.000	
Harm Hand	1.08	0.15	7.26	0.000	
Harm Table	−0.46	0.23	−1.96	0.050	
Const.	−1.50	0.57	−2.63	0.009	
mu					0.45, $P = 0.0000$
Condition	−0.14	0.02	−7.23	0.000	
Harm Hand	0.01	0.01	2.05	0.040	
Const.	−0.06	0.02	−2.42	0.015	
Harm Hand					
Ownership	2.71	0.22	12.14	0.000	
Harm Table					
Ownership	0.66	0.22	3.02	0.003	
Body Threat					
Ownership	1.56	0.22	6.95	0.000	

Condition = 0 (TABLE), 1 (HAND). r, P are the Pearson's correlations and significance levels between fitted and observed values of the response variables. $P = 0.00{*}0$ means $P < 0.00{*}05$

the correlations between values fitted by the model and the observed values of the response variables P450 and mu.

Now turning attention to the RP, this is based on a different set of data ($n = 38$), since RP is a bipolar difference in the activity between the C3 and C4 electrodes in the motor cortex so cannot be considered at the same time as P450 and mu. Applying path analysis to these data, only condition and *Harm Table* are significantly related to RP. Hence, an ordinary regression can be used (although still we allow robust standard errors). The result is shown in Table 4, where condition (HAND) is negatively associated with RP but positively associated with *Harm Table*. This is consistent with a lateralization between hemispheres occurring during the preparation of a motor action, the RP (C3–C4) is more negative when there is preparation to move the right hand (Eimer 1998), which in the current experiment is the attacked hand. Therefore, a reduction in RP for higher scores in *Harm Hand* question indicates stronger preparation of movement.

Table 4 Regression for RP

	Coefficient	SE	t	$P > t$
Condition	−0.87	0.38	−2.28	0.029
Harm Table	0.34	0.16	2.18	0.036
Const.	−0.10	0.41	−0.25	0.802

Condition = 0 (TABLE), 1 (HAND) $F(2,35) = 8.48$, $R^2 = 0.17$, $P = 0.001$, $n = 38$, Shapiro–Wilk (test for normality of residual errors) $P = 0.24$

Discussion

Our results suggest that participants instinctively avoided a virtual knife stab to their virtual body, thus activating the motor cortex and generating a mu-ERD, and a RP, as would be expected if their real hand were threatened. Our study reproduced the results of Fan and Han (2008), Li and Han (2010), and Meng et al. (2012, 2013) in terms of ERP correlates, showing significant evidence that pain effects were found, with the mean P450 showing greater amplitudes at the CP3 electrode location in the HAND compared to the TABLE condition.

Importantly, participants had been instructed not to move their hand during the whole experiment—and this was verified by the EMG analysis. However, it is important to note that measurements of the flexor carpi alone could not have detected very subtle movements, a reason for caution. Nevertheless, when doing the ERP study, we found motor cortex activation in the HAND condition with a significantly greater negative RP (C3–C4), associated with the intention of moving the right hand, and this RP was probably an instinctive reaction to the harm that could not be controlled consciously by the participants.

Additionally, we found that when the virtual hand was attacked with the virtual knife, it elicited significant motor cortex activation. A significant mu-ERD was found when the knife attacked the hand—especially prominent in the C3 electrode—as if the participants were trying to avoid harm. This suppression of the mu-rhythm in the HAND condition could be interpreted as being in agreement with previous observations about the involvement of this oscillatory desynchronization when a participant performs a hand motor action (Pfurtscheller and Lopes da Silva 1999). Besides, this effect reproduces the results of Yang et al. (2009), Perry et al. (2010) and Whitmarsh et al. (2011), although we believe that the illusory feeling of ownership over the virtual body was likely much greater than in any previous pain observation experiment. Furthermore, this illusory ownership provoked more prominent responses with greater similarity to those described by Babiloni et al. (2008) in preparation for an electrical painful stimulation of the left index finger.

A recent paper (Evans and Blanke 2013) showed that synchronous visual-tactile feedback during the hand ownership illusion generates mu-ERD in the sensorimotor cortex similar to the one produced during motor imagery BCI. Although in our experiment no tactile feedback was provided, we postulate that their results are compatible with our findings and suggest that the correlations found in the current experiment between the mu-ERD and P450 with the ownership illusion question may be related by a similar mechanism to the one they describe. Future research could assess whether tactile feedback would enhance the experience in the current scenario and inhibit any existing sensory mismatch. In our study, tactile feedback was avoided to prevent overlaying activities in the sensorimotor cortex between the interpretation of tactile sensory information and the efferent motor reactions (Yetkin et al. 1995). It would have been very difficult to dissociate the effects of the tactile stimulation from the subconscious motor reaction to the harm. However, regarding the sensory mismatch, some participants reported a strange feeling in their finger at the end of the experiment which would indicate that they were having illusions of tactile stimulation through a top–down mechanism.

According to Pfurtscheller and Lopes da Silva (1999), an ERS in the beta-rhythm would typically be found in hand motor imagery when the movement finishes. Nevertheless, in the current experiment we could not find significant beta rebound.

We have shown that automatic neural mechanisms, such as pain responses, that occur in reality occurred in this case in response to events in the virtual reality scenario of this study. This is in line with previous findings that people do tend to have similar responses in IVR as they would to similar situations in reality (Sanchez-Vives and Slater 2005), and this study seems to confirm this at the level of brain activity as measured by EEG.

Additionally, the results are useful for understanding the neural and cognitive mechanisms of body perception. We have shown that neural responses (P450, mu and RP) are correlated with the subjective level of the ownership illusion and the subjective illusions of harm and threat to the body. It seems quite remarkable that these variables being in principle totally unrelated to one another (electrical brain signals measured from the scalp compared with scores in a questionnaire) are nevertheless apparently strongly related. This correlation provides a cross validation that both the questionnaire responses and the electrical signals relate to the same underlying brain processes. However, correlations should not be confused with causation, and the path analysis proved useful for investigating a causal model between the observed variables. For example, although there is a positive correlation between *Body Threat* and P450 (Table 2), which might simplistically be interpreted as a direct causal relation, in the context of the path model this can be seen as spurious. The model provides an alternative interpretation that *Ownership* is positively associated with *Harm Hand*, which in turn is positively associated with P450, but also *Ownership* is positively associated with *Body Threat*. Overall, the path analysis was able to unravel possible relationships that would otherwise not be apparent and provide a quantitative assessment of a model.

From the path model, P450 is higher in the HAND compared to the TABLE condition, and it is also higher the stronger the subjective feeling that the hand might be harmed. But whose hand? A possible caveat in the interpretation of the results is that we cannot easily dissociate some of the intrinsic factors that may be modulating the pattern of ERP responses observed, for example, between empathy and body ownership. Previous empathy related studies (Fan and Han 2008; Li and Han 2010; Meng et al. 2012, 2013) suggest that the P450 component is mostly associated with empathy processing. Here, however, it appears to be related to ownership, given that *Harm Hand* specifically refers to harm to the participant ('… *I might be harmed* …') a statement that is not compatible with an interpretation that only favours empathy.

Empathy refers to the capacity to respond and understand experiences of another person (Decety and Jackson 2004). Brain activity associated with empathic responses occurs, for example, in the context of pain observation of the (even violet coloured) hand of a stranger (Avenanti et al. 2010). However, the same study shows that it is not generated when the hand belongs to racial out-group members (specifically white individuals observing black hands). However, recent evidence suggests that ownership by white individuals over a black rubber arm reduces implicit racial bias (Maister et al. 2013) as does ownership of a dark skinned virtual body (Peck et al. 2013). Since Avenanti et al. (2010) found that the degree of implicit racial bias and empathy responses were negatively correlated, we could conclude that embodiment in the body of another might be likely to increase empathy towards that person or the stereotype or group that the person represents (other things being equal). So although empathy and body ownership are not the same, they are related—for body ownership may be used to manipulate the degree of empathy.

Perhaps, the common factor between empathy and body ownership is perspective taking (Lamm et al. 2007). Perspective taking denotes the ability to see the world from the eyes of another, and metaphorically to put yourself in the shoes of another. It has been shown, for example, that perspective taking can improve attitudes towards others such as racial or ethnic out-groups (Swart et al. 2010). However, virtual embodiment provides a technological method for actually realizing perspective taking—when embodied in a virtual body it is literally the case that you see through the eyes of a (virtual) other, so it is not especially surprising that virtual embodiment can lead to a change in empathy, since it well realizes perspective taking. However, we would argue that in the present study empathy plays less of a role—except in the tautological sense that you might have 'maximal' empathy towards yourself, your own body. We suggest that this 'maximal' level of empathy may be a reason why stronger reactions were found in the motor cortex in our experiment in comparison with previous pain observation studies.

In our study, we observed new effects (mu-ERD and lateralization) that have not been reported before in previous empathy studies. We believe that these strong effects were observed due to the strong embodiment illusion. Previous research has shown that embodiment can be modulated by different combinations of self-location and body ownership (Longo et al. 2008; Kilteni et al. 2012). In our set-up, the control condition TABLE in which the knife did not appear where the hand was located, but 15 cm away, did not trigger the brain activation, indicating therefore that the possibility of harm to the own body played an important role. Our results show that the exploitation of virtual body ownership illusions could be useful for further understanding the underlying neural mechanisms involved in cognitive processes of perception. Besides, the measurements of cognitive processes provide a promising tool to measure virtual embodiment.

This may also have implications not only for the measurement of virtual body ownership but also to discriminate the strength of this illusion, so that people reacting with a stronger EEG activation—greater P450 amplitude the virtual hand is attacked—may have a stronger illusion than people with a weaker P450 amplitude. This is indicated in the path diagram (Fig. 5) where the subjective level of ownership is seen to be indirectly associated with both P450 and mu.

The questionnaire responses indicated generally a very strong illusion of ownership over the virtual body. This could explain why the brain responses observed—P450, RP and mu-ERD—were larger in comparison with previous experiments reported in the literature as observed above. A future experiment could explicitly test this by reducing the level of ownership through a third person perspective rather than a first person perspective condition (Slater et al. 2010; Petkova et al. 2011; Peck et al. 2013). For example, this would involve observing the reactions to seeing somebody else being attacked in an immersive virtual environment. These results could also be further studied by focusing on the effects of self-location with respect to the threat stimuli. In general, the neural responses by themselves may provide a non-subjective measure of embodiment, however, the current findings are based on correlations of both objective and subjective measures. Further studies may explore the extent in which ERPs may be exploited as an objective measure of embodiment.

Conclusions

The present study suggests that when a person is in an immersive virtual reality and has a body ownership illusion towards a virtual body that apparently substitutes their own body, there are autonomic responses that correspond to what would be observed were the events to take place in reality. Overall automatic brain mechanisms—P450—were found in this variation of the classical pain observation experiment, which is consistent with what Bufalari et al. (2007), Fan and Han (2008), Li and Han (2010) and Meng et al. (2012, 2013) previously reported. However, our set-up was not one concerned with participants experiencing empathy towards another person but rather experienced direct attacks to their own body, since both subjective and objective data point in that direction. The results support our initial hypothesis that a threat to a virtual hand, towards which the participant has an illusion of ownership, would significantly produce a harm prevention effect (measures using the RP (C3–C4) and oscillatory movement-related components, the mu-ERD) such as trying to move it away from the source of the harm. The questionnaire also confirmed high levels of ownership over the virtual body (see Fig. 2). In addition, the correlation between the automatic brain mechanisms—P450—and the subjective illusion of ownership opens the door for a new promising measure of virtual embodiment.

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