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**Body representation shapes the responses to  
threatening stimuli**

Tutor: Professor ANGELO MARAVITA

Doctoral Thesis by:

DANIELE ROMANO

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## **- General Introduction -**

The body, the object we know the best (de Vignemont, 2011), holds a very complex representation in the brain because it is our unique referent for sensory-motor experience (Berlucchi & Aglioti, 1997, 2010). The sensory experience, in particular, is deeply rooted on our body surface so that we can map stimuli at different levels of complexity, from the most elementary signal to higher level representations referred either to the body itself or to the external space (Medina & Coslett, 2010; Medina et al., 2013). Furthermore, incoming stimuli approaching the body without touching it, can be interpreted as potential threats and are constantly monitored by a sophisticated neural network (Ploghaus, 1999).

A critical function, which allows a complete representation of the body, is the feeling that a given body part belongs to one's own body (de Vignemont, 2011), however bodily self-consciousness is not considered anymore a unitary inviolable concept. Recent experimental evidences suggest that it is rather a result of multisensory bodily signal integration in the brain (Blanke, 2012).

Since the first experimental induction of changes in limb-ownership and location in the rubber-hand illusion (Botvinick & Cohen, 1998), the bodily self-consciousness and in particular the sense of ownership for one's own body has been extensively investigated by means of several techniques and experimental paradigms in healthy participants, such as the rubber hand illusion (RHI) (Botvinick & Cohen, 1998; Tsakiris, 2010), the mirror box (Romano, et al., 2013a), the Full Body Illusion (Ehrsson, 2007; Ionta et al., 2011; Lenggenhager, et al., 2007), and Virtual Reality environment (Perez-Marcos, et al., 2009). Multisensory body representation has been proposed to be crucial for bodily self-identification and for other aspects of bodily self-consciousness (Botvinick & Cohen, 1998; Ehrsson, et al., 2005; Lenggenhager et al., 2007; Salomon, et al., 2012; Tsakiris, et al., 2007; Tsakiris, 2010), however it has also been shown to be critical for any sensory perception, including pain.

## **Body Representation**

The body representation is generally defined as the representations of our body mapped in the brain (Berlucchi & Aglioti, 1997, 2010).

This very general definition characterizes the brain as containing several body representations, some of them are responsible for the processing of primary sensory input or for sending motor output (Zeharia, et al., 2012). Beyond this first level of representations, a growing body of literature addressed the existence of several supplementary representations of the body in the brain of an higher cognitive order, not depending on single sensory modalities, which are involved in complex behaviours (de Vignemont, 2011; Haggard, et al., 2013; Longo, et al., 2010). From now on, we will refer to this high cognitive order representations with the term “body representation”.

An agreement about a cognitive model of the body representation is lacking and several proposals can be found in literature (de Vignemont, 2010; Head & Holmes, 1911; Longo et al., 2010; Makin, et al., 2008; Schwoebel & Coslett, 2005; Tsakiris, 2010). A first distinction for different body representations has been proposed by Head and Holmes at the beginning of the XX century. In their seminal work centered on the acquired sensory disturbances following brain damage (Head & Holmes, 1911), they proposed a dyadic taxonomy to distinguish the body schema from the body image. The authors proposed the body schema as a dynamic representation of the body, necessary to program actions and interact with objects in space. The main features of the body schema, as described by Head and Holmes, is still shared by most of the authors working on body representations and is now commonly defined as the dynamic representation of the body part location oriented to action execution (de Vignemont, 2010; Head & Holmes, 1911; Kammers, et al., 2006; Longo, et al., 2009). The body schema is typically considered more automatic than its counterpart (i.e. the body image in the dyadic taxonomy), more dynamic and less accessible to consciousness.

Conversely the body image received less agreement and seems to hold opposite features, thus it is supposed to be accessible by consciousness and less dependent upon sensory

input and feedbacks. The body image was supposed to include all the other information about the body not included in the body schema (de Vignemont, 2010; Longo, et al., 2009). A further differentiation was proposed by Schwoebel and Cosslett (Schwoebel & Cosslett, 2005) who proposed a triadic taxonomy, instead of the previous dyadic one, where the body image is split in two components: the body semantics – i.e. the lexical knowledge about the body –, and the body structural description, which should be a representation in between the body schema and the body semantics, that holds knowledge about the body metrics, the relationship between the different body segments and the body surface.

Both taxonomies are interesting, and can answer and predict a lot of behavior and neuropsychological conditions, however it nowadays seem that both of them are unable to account for all the features characterising our knowledge and use of the body (Sedda, 2011). For example, none of the present models can fully explain the sense of ownership of a body part nor how such a feeling builds up and is maintained in the brain (de Vignemont, 2010).

One of the most important issues with these taxonomies is that they seem unable to answer the questions about bodily self-consciousness and how we construct it.

Bodily self-consciousness has been proposed to encompass self-identification – the *sense of ownership* - (the experience that ‘I’ identify with a body), self-location (the experience of where ‘I’ am located), and a first-person perspective (from where ‘I’ experience the world), but does also relates to the sense of agency (the experience that ‘I’ am the agent causing ‘my’ actions) (Blanke & Metzinger, 2009; Blanke, 2012; Ferri, et al., 2012).

Recent experimental evidences suggest that bodily self-consciousness is the result of multisensory bodily signal integration in the brain (Blanke, 2012). This has been investigated by inducing bodily illusion in healthy people, and by exploring neuropsychological and psychiatric symptoms that exhibit disorders of body representation (de Vignemont, 2010).

The first experimental induction of changes in limb-ownership and location in healthy people was demonstrated by Botvinick & Cohen in 1998 with the paradigm of the Rubber Hand Illusion (RHI) (Botvinick & Cohen, 1998; Tsakiris, 2010). The RHI paradigm consists of the stimulation of a fake arm, which is seen by the participant, and the simultaneous somatosensory stimulation of the participants biological arm, which is hidden from his/her view. This induces feelings of surprise in the participant and alters the subjective localization of tactile stimuli, which are referred to the fake hand. A modified feeling of ownership for the fake hand, usually assessed through subjective scales (Bekrater-Bodmann, et al., 2012; Botvinick & Cohen, 1998; Ijsselstein, et al., 2006; Longo, et al., 2008; Petkova & Ehrsson, 2009), is observed in the RHI.

The sense of body ownership modulates the perception and localization of sensory stimuli, as well as the reaction to incoming threatening stimuli. Typically the sense of ownership correlates with the level of emotional activation for sudden threats directed to the fake hand (Armell & Ramachandran, 2003; Ehrsson, et al., 2007; Guterstam, et al., 2011). Interestingly, such an increased sense of ownership for the rubber hand seems to be accompanied by a relative decrease in the sense of ownership for the participant's biological hand (Barnsley, et al., 2011; Moseley, et al., 2008), although a complete agreement about this point is still lacking (de Vignemont, 2011).

The RHI opened the route of bodily self-consciousness investigation in healthy participants and now is characterized by several bodily illusion based on multisensory conflict and integration like, but not limited to, the mirror box illusion (Romano, et al., 2013a), the Full Body Illusion (Ehrsson, 2007; Lenggenhager, et al., 2007), and Virtual Reality environment (Perez-Marcos, et al., 2009).

Of particular interest for this thesis, in the full-body-illusion (FBI) the principles of the RHI are extended to the entire body. Similarly to the RHI, the FBI can be induced: thus congruent visuo-tactile stimulation at the trunk can induce self-identification and self-location changes with respect to a virtual or fake body presented in front of the participants (Aspell, et al., 2009; Lenggenhager et al., 2007).



Promising results from the investigation of bodily self-consciousness in healthy people induced researchers to produce several cognitive models that tried to explain how the mind creates the sense of body ownership (see the special issue “The sense of body” published in *Neuropsychologia* in 2010). Tsakiris, in reviewing the literature about RHI (Tsakiris, 2010), proposed that a body segment have to pass three check-points to be attributed to oneself: specifically, in the RHI, the fake hand must have the shape of a hand compatible with participant’s hand, holding a compatible posture with the real hand and receiving congruent visuo-tactile stimulations; if and only if all these three conditions are satisfied the rubber hand can be embodied (Tsakiris, 2010). The neural network involved in these serial evaluations it is supposed to include subsequently the following areas: right Temporo-Parietal Junction, S1 and S2, ventral Pre-Motor, Posterior Parietal Cortex and the ownership feeling should finally emerge following the integration of all the signals in the right posterior Insula (Tsakiris, 2010).

In a different model Makin and colleagues proposed that the self location bias induced by the RHI depends on the recalibration of a complex system that involves occipital, frontal and parietal cortices and includes areas like posterior Inferior-Parietal Sulcus, anterior Inferior-Parietal Sulcus, Pre-Motor Cortex, S1 and the visual cortex. Such changes are supposed to be driven by multisensory recalibration of the coordinates of reference mainly because of recalibration of multimodal neurons (Makin, et al., 2008).

Bodily self-consciousness has been investigated inducing bodily illusions in healthy people, but also studying patients presenting with disruption of the body representation. Interestingly the clinical picture of body representation disruptions seems to show stronger effects, and therefore is likely to be more informative, than those induced by experimental manipulations in healthy subjects (e.g. the aforementioned RHI) (de Vignemont, 2011).

Many symptoms of disrupted body representation have been described such as autotopagnosia - i.e. the inability to verbally report the spatial relations of body parts – (Schwoebel & Coslett, 2005), the Alien Hand Syndrome – i.e. the acquired presence of

involuntary and unwilling goal-directed movements of the contralesional hand - (Romano, et al., 2013b), anosognosia for hemiplegia – i.e. the lack of awareness for acquired motor deficit - (Jenkinson & Fotopoulou, 2010), or the out of the body experience – i.e. the transient experience of feeling oneself outside of one's own physical body - (Bünning & Blanke, 2005). One of the most intriguing and informative neuropsychological disorder of body representation is somatoparaphrenia, defined as the acquired delusions and confabulations about the contralesional side of the body (Vallar & Ronchi, 2009).

Somatoparaphrenia is a well known disorder of body representation often associated with right brain lesions (Bottini, et al., 2002; Invernizzi, et al., 2013; Vallar & Ronchi, 2009) and represents a challenging situation for studying the sense of body ownership. Patients affected by somatoparaphrenia typically deny the ownership of their contralesional limbs (usually the left), which they attribute to others, such as to the nurse, to the doctor or to relatives not even present (Bottini, et al., 2002), or in general to someone else who could not possibly be there (Pugnaghi, et al., 2011). A critical question unanswered by previous experimental studies is whether somatoparaphrenia corresponds only to verbal delusion or whether it is also accompanied by coherent behavioral and physiological correlates, thus reflecting a deeper disruption of body representation. Behavioral effects consistent with the somatoparaphrenic delusion have been shown. For example, one patient showed an increase in tactile sensitivity after she was told that the touch would have been delivered to the arm of the person to whom she was attributing the limb ownership (Bottini, et al., 2002). In a different study two patients recovered a normal ownership sensation when they looked at themselves from an allocentric perspective such as in a frontal mirror (Fotopoulou, et al., 2011), moreover it has been shown also that self-touch of impaired hand can increase the sense of ownership over it (van Stralen, et al., 2011). However, the physiological markers of such striking confabulations were not investigated at all.

More recently it has been described a new class of patients showing a peculiar and radical desire for one's own healthy limb amputation (First, 2005; Hilti et al., 2013; Sedda,

2011). This striking conditions, namely Body Integrity Identity Disorder (BIID) or Xenomelia, is associated with a foreign feeling for the limb that is so strong that leads to the desire for amputation. The mechanisms underlying the desire for amputation of a perfectly working limb are far from being completely understood, however there is a growing body of literature that is trying to differentiate neurological factors - i.e. the underrepresentation of that specific body segment of the body - (Berti, 2013; Hilti, et al., 2013; McGeoch, et al., 2011), from social and psychological factors (Brugger, et al., 2013; Sedda, 2011).

De Vignemont distinguished the judgment of ownership from the feeling of ownership (de Vignemont, 2011), hypothesizing that the feeling of ownership is a more primitive sensation than beliefs or explicit judgments and is the critical determinant of the ownership of one's biological body part (de Vignemont, 2007).

Conversely it is not clear whether the increased ownership for external objects, as in the RHI, typically measured with questionnaires (Botvinick & Cohen, 1998; Longo, et al., 2008), merely intercepts the judgment of ownership or penetrates the body representation more deeply, up to the level that a profound feeling of ownership for the fake body part is established. From this point of view BIID symptoms seem to affect the feeling of ownership, given that patients still judge themselves as the biological owners of that limbs.

It is worth nothing that a comprehensive model of body representation is not yet available (Blakemore, et al., 2002; Blanke & Metzinger, 2009; Head & Holmes, 1911; Makin, et al., 2008; Mancini, et al., 2011a; Medina & Coslett, 2010; Tsakiris, 2010) and all those proposed so far failed to fully account for the neuropsychological conditions described above, and in particular y somatoparaphrenia and BIID (Berti, 2013; de Vignemont, 2010; de Vignemont, 2011; Longo, et al., 2010; Sedda, 2011) for which a more comprehensive model is still needed.

## **Pain Processing**

Pain is an extremely common daily-life sensory experience. The International Association for Study of Pain (IASP) defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Recommended by the IASP Subcommittee on Taxonomy, 1979). Acute pain is a complex sensation, usually generated by nociceptive input (Treede, 2006) even if it is possible to feel pain in absence of nociception (Craig, et al., 1996; Craig, 2002; Ehrsson, et al., 2007; Lloyd, et al., 2006). As a complex sensation, pain experience seems to emerge from the co-activation of a complex network in the brain, originally called neuromatrix (Melzack, 1989) and currently referred to as pain matrix (Ploghaus, 1999; Tracey & Mantyh, 2007), which comprises a large neural network brain areas related to primary discriminative-somatosensory analysis, namely S1 and S2 as well as associative multimodal areas including the posterior parietal cortex, anterior insula and anterior cingulate cortex (ACC) (Iannetti & Mouraux, 2010; Price, 2000), however an agreement on the role of such a network is still debated, and it has been proposed that this matrix would be a salience detection system and would not be limited to the processing of painful stimulations (Iannetti & Mouraux, 2010; Legrain, et al., 2011).

Moreover a primary, pure nociceptive cortex has not been identified up to now (Bushnell, et al., 1999; Garcia-Larrea, 2003), therefore some authors consider inappropriate talking about pain as a pure specific sensation (Auvray, et al., 2010) like touch or vision. Congruently the nociceptive system shows a great specificity on the periphery having specific sensory pathways, but there is a progressive integration with other modalities at higher stages that starts early in S1 (Haggard, et al., 2013). Such a complex brain substrate is justified by the multicomponential nature of pain experience that includes both cognitive and sensory aspects. This double nature of pain experience is nicely shown by the experimental modulation of pain experience through a large range of experimental manipulations including crossmodal signals (Longo, et al., 2009; Torta, et al., 2013), emotions or meditation induced states (Brown & Jones, 2010; Rhudy, et al.,

2010; Rhudy, et al., 2008; Williams & Rhudy, 2009; Zeidan, et al., 2011), attention and expectations (Babiloni, et al., 2008; Brown, et al., 2008a; Clark, et al., 2008; Porro, et al., 2002), social factors (Avenanti, et al., 2010; Forgiarini, et al., 2011).

An important distinction must be pointed out between acute pain and chronic pain. As said, acute pain is a complex sensation, usually generated by nociceptive input that leads unpleasant and uncomfortable sensation and a potential danger for the body tissue safety (Recommended by the IASP Subcommittee on Taxonomy, 1979), while chronic pain is defined as “pain or discomfort, that persisted continuously or intermittently for longer than 3 months” (Elliott, et al. 1999).

Different neural mechanisms are likely to underpin chronic and acute pain (Moseley, et al., 2005); indeed chronic pain is a condition of long lasting, impossible to ignore, pain without a constant noxious stimulus that usually dominates the patient's mental life. This condition of overexposure to painful sensation plastically induces long lasting modification of the body representation (Moseley, et al., 2005; Moseley, 2005) that could, per se, change the patterns of response induced by external stimuli. Furthermore, in behavioral terms, it has been show that the same cognitive modulation induced either in healthy subjects affected by acute pain or in chronic pain patients, can lead to opposite results (Mancini, et al., 2011b; Moseley, et al., 2008; Ramachandran, et al., 2009). Moreover it may be difficult to assess the causal associations between chronic pain and changes in the body representation (Haggard, et al., 2013).

For the above reasons, in order to study the link between body representation and pain processing, in the present thesis we studied the experimental condition of acute pain, where changes of pain perception can be more easily related to contingent manipulations of body representation, avoiding any long-lasting effects of chronic pain conditions, that would more difficult to control experimentally or interpret.

The multicomponential nature of the pain experience have been classically divided in two main aspects: the nociception and the cognitive aspects of pain (Brown & Jones, 2008; Ploghaus, 1999; Rainville, et al., 1999).

Nociception is defined as the afferent neural activity transmitting sensory information about noxious stimuli (Iannetti & Mouraux, 2010; Treede, 2006), giving information about the location of the stimulus, the intensity and the quality of the stimulation (pressure, thermal, electrical).

The cognitive component of pain is a more heterogeneous category, including very different contributions that go from attentional (Brown, et al., 2008a; Sprenger, et al., 2012) to perceptual (Gallace, et al., 2011; Longo, et al., 2009), to social (Forgiarini, et al., 2011; Valeriani, et al., 2008) interactions. Cognitive aspects of pain are well captured by measuring the anticipatory responses to pain, that precede the stimulus onset (Ploghaus, 1999).

Thus, although, nociceptive stimuli are processed through specific sensory pathways (Lenz, et al., 2010), similarly to non painful stimuli, pain can be critically modulated by cognitive manipulations in a top down fashion (Haggard, et al., 2013).

Pain is evidently entangled with the state of one's own body and is quite impossible to describe pain without referencing to the body (Haggard et al., 2013). It has been proposed that pain is deeply linked to the spatial structure of the body and thus the representation of the body and the surrounding peripersonal space are critical not only for preparing motor responses to pain, but also for the functional sensory organization of pain itself (Haggard et al., 2013).

As we said cognitive aspects of pain include very different cognitive abilities, critical to our work also perceptual manipulations of the body interact with the processing of noxious stimuli, for example, Gallace and colleagues showed that pain experience for noxious stimuli delivered to the hands is significantly reduced by simply crossing the arm (Gallace, et al., 2011). Also, information contained in the visual target have been shown to be effective for modulating pain: previous experimental works have shown that looking at one's own body, but not to an object or at another person's body, while receiving a painful stimulus, produces analgesic effects (Longo, et al., 2012; Longo, et al., 2009).

In a different study Mancini and colleagues demonstrated that, by changing the visual size of one hand, is possible to impact the experience of a painful stimulation (Mancini, et al., 2011b) in a similar way of non-noxious stimuli processing which is influenced by the perceived size of the hand (Bernardi, et al., 2013; Pavani & Zampini, 2007).

The relationship between the body and pain processing is further justified by the most important function of the nociceptive system, namely the safety of tissues, protecting the body from actual or potential damage (Haggard et al., 2013) due to potentially noxious stimuli that are within, and/or rapidly moving toward the space surrounding our body (Graziano & Cooke, 2006; Graziano, et al., 2002). Furthermore defensive actions are precise and coordinated (Cooke, et al., 2003) in such a way that motor response to pain imply a representation of the peripersonal space around the body to anticipate the sensory consequence of the incoming stimulus. This sector of space, namely the peripersonal space (Rizzolatti, et al., 1981a, 1981b), holds peculiar features due to its richness of multisensory interactions, especially between spatially near visual stimuli and tactile stimuli on the body (Farnè, et al., 2005; Macaluso & Maravita, 2010).

Therefore pain anticipation is a crucial ability of the human being. It allows us to understand potentially dangerous situations, in order to increase alertness and carry out appropriate defensive behavior. This is reminiscent of the notion of “defensive flight zone” in animals. In the 1950s Heini Hediger described the urge to protect the zone near the body as the primary goal of any creature, more important than food or sex. He defined this zone as the “flight distance”, and later as “flight zone”. Graziano and colleagues further characterized Hediger’ claims in non-human primates, by reporting avoidance behaviors in response to stimuli rapidly approaching the body or air puffs directed to single bodily regions (Cooke & Graziano, 2003; Graziano, et al., 2002).

A recent study shows the existence of a similar hand-centered coding system of the visual space in humans, where approaching objects can rapidly modulate corticospinal excitability in hand centered coordinates (Makin, et al., 2009). This finding is compatible with the existence of a mechanism that anticipates the impact with approaching objects

and suggests that the human peripersonal space has an adaptive role as a protective safety barrier to incoming threats (Cardinali, et al., 2009b). The features of peripersonal space are reminiscent of some characteristics of the body schema in such a way that it has been proposed that the two definitions might underlie the same concept or at least being part of a continuum of interface between body and space (Cardinali et al., 2009a).

The meaning of such interactions between body, space and pain is that the processing of painful stimuli is impacted by cognitive and perceptual states relative to the body and the surrounding space, in a top-down fashion. This assumption is fundamental for our working hypothesis as we tried to interact with the processing of painful stimuli by changing the representation of one's own body.

## **General Aim**

Bodily self consciousness is a blooming field of research where a lot of questions are still unsolved. From a psychological point of view de Vignemont proposed an agenda of main issues (de Vignemont, 2011) wondering what is the fundamental role of body ownership, what grounds the sense of ownership, how is the sense of ownership related to bodily sensations, action and emotion, and whether it is possible to feel a sense of ownership for any extracorporeal object.

I cannot say to have answered any of these questions, however the contribution of this thesis should be interpreted in the framework of trying to figure out some of the relations between emotions and sensations, on one side, and the feeling of ownership, on the other. To this aim we conducted a series of studies investigating either patients presenting with body representation disorders and healthy people experiencing bodily misperceptions.

In the present thesis I am going to present a progression of seven studies where I tried to characterize how body representation interacts with pain processing. Experiments were run on both healthy participants and patients showing body representation disruptions, by means of recording responses from the autonomous nervous system and ratings of pain experience under different conditions of body representation distortion.



In Study 1 we designed and validated a novel experimental paradigm that was able to dissociate the cognitive from the nociceptive components of pain processing. The paradigm generally employed the measure of the Skin Conductance Response (SCR), which is an index of the electrical conductance of the skin due to sweating and represents a reliable, direct measure of sympathetic nervous system activation following psychological or physiological arousal (Deltombe, et al., 1998; Lykken & Venables, 1971). Previous evidence has shown that SCR increases in response to threatening stimuli (Armeli & Ramachandran, 2003a; Forgiarini, et al., 2011), pain perception (Rhudy, et al., 2009; Williams & Rhudy, 2009) and cognitive conflict (Kobayashi, et al., 2007). There are a lot of ways to capture and analyze SCR, such as counting of natural fluctuations (Storm, et al., 2005); recording of maximum amplitude with log transformation (Armeli & Ramachandran, 2003a); averaging (Bradley, et al., 2008; Forgiarini, et al., 2011; Kobayashi, et al., 2007; Lang, et al., 1993); integral calculation (Dubé, et al., 2009); percentage of increase (Hägni, et al., 2008). However, it seems that the most widely used and reliable measure for event related SCR paradigms, especially those related to pain, are the peak-to-base and the peak-to-peak indexes (Bellodi, et al., 2013; Breimhorst et al., 2011; Ehrsson, 2007; Rhudy, et al., 2007; Williams & Rhudy, 2009). This measure, in particular, seemed more sensitive to capture responses that are locked to a brief stimulation, than methods like fluctuations counting or averaging, which are more suitable for long lasting stimulus exposure (usually time windows longer than 5sec).

In the first experiment of Study 1 (Experiment. 1.1) we aimed at evaluating whether the SCR to incoming painful stimuli was related to experience of pain generated by that stimulus. In the second experiment (Experiment 1.2) we measured the SCR to noxious and neutral stimuli, that actually touched the skin or approached the body without contacting it. The latter condition - namely simulated contact – is of particular interest as it is sensitive to pain anticipation, which is considered a good proxy of cognitive aspects of pain (Colloca, et al., 2006; Hsieh, et al., 1999; Ploghaus, 1999), and can capture pain responses independently from noxious stimulations. It is worth noting that the paradigm

used in this experiment, with the specific modifications for the peculiarity of each study, is the one used in most of the studies presented in this manuscript. Finally in the third experiment (Experiment 1.3) we measured whether pain anticipation response was generally due to the vision of a salient stimulus or specifically increased when stimuli actually approach the body surface, thus threatening it.

In Study 2 we used the pain anticipation paradigm (Experiment. 1.2) in patients showing the striking neuropsychological condition of somatoparaphrenia – i.e. the acquired delusion that one's own limb belongs to someone's else -. In this study we hypothesized that if the confabulations of the patients represent the deranged status of their body representation, we should find physiological evidence of a different processing for stimuli directed to the impaired hand than those directed to the spared hand. We compared responses from somatoparaphreic patients with other two categories of right brain damaged patient: patients with anosognosia for hemianaesthesia – i.e. the lack of awareness for acquired somatosensory deficit -, but preserved sense of ownership; and a group of hemiplegic patients without deficit of awareness and ownership. We supposed that the deep representation of one's own body, and not merely its vision, is determinant to anticipate incoming sensory stimulation properly, thus we expected that pain anticipation responses in somatoparaphreic patients would be lacking selectively when stimuli were directed to the left, contralesional hand, whose ownership is defective.

In Study 3, I had the opportunity for studying a very peculiar population of patients presenting with Body Integrity Identity Disorder (BIID). These are individuals without any history of neurological or psychiatric illness, but with a persistent and deep desire to receive the amputation of a given limb. What these patients typically refer is a sense of disownership for that body part, which is felt as not belonging to their body, despite their full acknowledgment that the body part is, in fact, theirs, that their desire is indeed unusual sensation, and that an eventual amputation would have serious consequences. The discussion around these patients is currently quite hot for several reasons that goes from ethical problems that they carry out, to the more scientific understanding of the disease

and its diagnostic criteria (Brugger, et al., 2013; First, 2005; McGeoch, et al., 2011; Müller, 2009; Sedda, 2011). Nonetheless, this condition represent a challenging and interesting situation to study body representation disorders. Following the idea explored in somatoparaphrenia that unrepresented body part should show reduced anticipatory response to pain, we tested pain anticipation with eight BIID patients, expecting a reduced SCR to painful stimuli approaching the limb they desired to amputee.

In the first three studies we assessed whether body representation is necessary to properly evaluate incoming painful stimuli. In Study 4 (Experiment 4.1 and 4.2) we tried to modulate pain processing by manipulating the body representation. We changed the visual size of one of the hands, target of the painful stimulation, either by increasing and decreasing the visual size. In this conditions we measured the autonomic responses to incoming and contacting painful stimuli and the referred experience of unpleasantness and stimulus intensity. There is a growing body of research about how to modulate pain experience by changing the visual size feedback coming from the body (Mancini, et al., 2011b; Moseley, et al., 2008; Ramachandran, et al., 2009) and here we sought for evidence about the physiological underpinnings of such visual modulations of pain responses.

Up to the fourth study we aimed at investigating the relevance of body representation for the processing of painful stimuli. However it is worth noting that one's biological body part was always visually available and directly involved in these studies. In the last three studies proposed, we tried to figure out whether the body representation interact with pain processing also when the relevant body is, in fact, an external object undergoing a process of embodiment.

In Study 5 we implemented, in a controlled way, the full body illusion (FBI) combining virtual reality techniques and robotics. Similarly to the RHI (Botvinick & Cohen, 1998) in the FBI congruent visuo-tactile stroking of one's own back and the back of an avatar, seen in front of the participant at 2 meter distance, induces an increased sensation of ownership for the virtual body and a mislocalization of oneself towards the avatar

(Lenggenhager, et al., 2007; Pfeiffer, et al., 2013). In two experiments we manipulated the degree of ownership felt for different avatars by changing the congruency of visuo-tactile stroking. Moreover we proposed, as virtual items to embody, either the pictures of a normal body seen from the back, or a neutral object (white rectangle) or a scrambled human figure (see Fig. 5.1). We hypothesized that under illusory conditions of increased ownership for the normal virtual body, painful stimuli should be processed as during the observation of one's own body showing analgesic effects (Longo, et al., 2012; Longo, et al., 2009), conversely when the avatar was an object, a non-anatomical body, or else it was a human figure, but stroked in an incongruent fashion with the participant's own body, the ownership illusion should decrease or be absent and thus the response to painful stimuli should not be reduced.

In the following Study 6 we used again virtual reality to induce a FBI on the participant's legs, using a first person visual perspective (Ehrsson, 2007). Then we presented a visual feedback showing either anatomically congruent or incongruent image of the legs being threatened by a noxious stimulus. Critically, also the size of the legs was manipulated, in order to be either larger or smaller than the participant's legs. Crucially, we expected, as in Study 4, that the size of the virtual legs would interact with the processing of painful stimuli, but only when the virtual body was provided in a posture that was anatomically compatible with that held by the participant.

In the final Study 7 we studied the bodily mapping of painful stimuli, beyond the boundaries of the body itself. In a series of three experiments (Experiment 7.1, 7.2 and 7.3) we evaluated whether the embodiment of an external, non-bodily object – i.e. a 40 cm long stick consisting of a hand-held wooden stick with a needle at the end – can induce plastic changes in the anticipatory response to painful stimuli. In these experiments we evaluated whether a motor training with a tool changes the anticipatory response to threatening stimuli in the far and peripersonal space, according to the notion of the incorporation of hand wielded tools in the body representation (Cardinali, et al. 2011; Maravita & Iriki 2004; Maravita, et al., 2001).

Taken together the seven studies outlined in the present work are aimed at figure out how pain experience and its anticipation is processed under different conditions of body representation . Specifically, we sought to show that the vision of one's own body is not enough to modulate pain processing without the mental representation of the body itself. Also we have shown that when body representation is extended, through experimental manipulation, to a virtual body or an hand held tool, the processing of threatening stimuli directed to those extracorporeal objects can be modulated to a similar extent as when they are directed to the body itself.

The present set of studies provide novel experimental evidence showing the critical influence of body representation for the mapping of sensory experience, in particular pain processing, and how the sense of ownership critically governs this interaction.



## **- Study 1 -**

# **Dissociating pain anticipation from nociception: validation of a novel Skin Conductance Response (SCR) paradigm.**

### **Introduction**

Pain is a sensory experience that plays the crucial role of protecting our body from potentially dangerous stimuli. A primary, pure nociceptive cortex has not been identified up to now (Bushnell et al., 1999; Garcia-Larrea, 2003), so acute pain is generally considered a complex sensation, usually generated by nociceptive input (Treede, 2006) that emerges from the co-activation of a complex network in the brain, called pain matrix (Ploghaus, 1999), which comprises areas related to primary discriminative-somatosensory analysis, namely S1 and S2 as well as associative multimodal areas including the posterior parietal cortex, anterior insula and anterior cingulate cortex (ACC) (Iannetti & Mouraux, 2010; Price, 2000).

Such a complex brain substrate is justified by the multicomponential nature of pain experience that includes both cognitive and sensory aspects (Clark et al., 2008; Ploghaus, 1999).

Nociception is responsible for the sensory analysis of the physical characteristics of the noxious stimulation, giving information about the location of the stimulus, the intensity and the quality of the stimulus (pressure, mechanical, thermal, electrical), while that comprising the cognitive aspects of pain is a more heterogeneous category, including a large number of components ranging from attentional (Brown et al., 2008a; Sprenger et al., 2012) to perceptual (Gallace et al., 2011; Longo, et al., 2009), to emotional (Rhudy et al., 2007), and even social (Forgiarini et al., 2011; Valeriani et al., 2008) factors.

Pain is a subjective experience and, for this reason, the way of measuring pain is a critical and challenging issue. The easiest and most used way to measure pain is by asking the receiver to rate it via visual analogue scales (Moseley & Wiech, 2009; Preißler et al., 2012), numerical scales (Brown et al., 2008a; Longo et al., 2012), and questionnaires

(Cleeland & Ryan, 1994; Lewis et al., 2010). Although subjective ratings are fundamental to understand the subjective experience of pain, they clearly suffer a lack of objectivity, might be not sensitive enough to capture subtle changes and cannot dissociate between cognitive and sensory aspects of pain. A more objective way to measure pain is the measurement of pain thresholds (Hänsel et al., 2011; Mancini et al., 2011b) that assesses the limit at which a sensory stimulation becomes painful; any variation of this pain threshold is considered to be a more reliable index of a modulation of pain processing. However, even if it is less influenced by external factors than ratings, pain thresholds cannot yet differentiate nociception from cognitive aspects of pain perception.

One way to capture the cognitive aspects of pain is that of measuring the anticipatory responses to incoming threats that shortly precede stimulus onset and that have been differentiated from nociception also at neural level (Hsieh et al., 1999; Jensen et al., 2003; Ploghaus, 1999; Porro et al., 2002). For this reason we designed a new experimental paradigm (Experiment 1.2), that should be able to dissociate the cognitive aspects of pain from the global (cognitive plus nociceptive) experience of it. In order to do so we measured the Skin Conductance Response (SCR), which is an index of the electrical conductance of the skin due to sweating and represents a reliable, direct measure of sympathetic nervous system activation following psychological or physiological arousal (Mordkoff et al., 1967; Deltombe et al., 1998). Previous evidences have shown that SCR increases in response to threatening stimuli (Armel & Ramachandran 2003; Forgiarini et al. 2011), pain perception (Rhudy et al. 2009; Williams & Rhudy 2009) and cognitive conflict (Kobayashi et al., 2007). In a series of three experiments here we evaluated whether the SCR is predictive of the pain ratings in absence of any experimental manipulation. Then we measured SCR to incoming painful and neutral stimuli to show its sensitivity to the painful stimulus. Critically, in this experiment stimuli were administered in two contact conditions: real and simulated. In the former, stimuli really contacted participants' hand assessing for the global sensory experience; in the latter stimuli approached the body without eventually contacting the skin, thus assessing only the



anticipatory response. In the third experiment we further validated the paradigm by measuring SCR to painful stimuli provided at different distances from the body, in order to prove that any eventual modulation of SCR was due to the evaluation of the consequence for the incoming threat for the body.

### **Exp. 1.1 – SCR to noxious stimuli predicts pain ratings**

#### **Materials and Methods**

##### *Subjects:*

21 (13 females, mean age=24.45 s.d.=1.92) healthy participants, attending the Università degli Studi di Milano-Bicocca took part in the Experiment 1.1 after giving their informed consent. The experimental protocol was explained in detail, but the participants were blind to the specific purpose of the experiment. The experiment was conducted according to the principles of the Declaration of Helsinki (“Declaration of Helsinki,” 1996).

##### *SCR Hardware and Software*

SCR was collected through the Biopac biosignal amplifier MP150 and the specific module for galvanic skin responses GSR100C (Biopac System Inc., Goleta, California) connected to a dedicated PC through an optical link. The gain parameter was set at 5  $\mu\text{mho/V}$ ; the signal was sampled at 50 Hz. The signal was acquired by means of two silver electrodes (TSD203 electrodermal response transducer set) placed on the third phalanx of the index and middle fingers of the hand which was not stimulated. A saline conductive paste (GEL 101) was applied to the electrodes to improve signal-to-noise ratio. Data were digitalized with an A/D resolution of 16 Bits and then analyzed with the software AcqKnowledge 3.7 designed to work with the Biopac system.

##### *Experimental Procedure:*

Participants sat comfortably at a table with the experimenter sitting in front of them. They put both hands on the table with the palm facing up. One hand was kept under an opaque screen and was the target of subsequent stimulations, the other one was aside and was connected to the SCR recorder.

On each trial participants have to look in the direction of the hand hidden under the opaque screen. Noxious stimuli were manually delivered, by means of a needle (Cheng et al., 2007; Forgiarini et al., 2011; Höfle, et al., 2012), to the pad of the middle finger of the hand hidden under the opaque screen, by a trained experimenter. The stimuli and the target hand were never visible throughout. After 10 seconds of each stimulation participants were asked to judge the intensity of the stimulus and separately the unpleasantness of the stimulation by rating them on separate verbal scales that went from 1 (not unpleasantness at all / minimum intensity of stimulation) to 10 (the worst unpleasantness imaginable / the most intense stimulus). A total of 20 stimuli were administered to the participants in a single session. Eleven participants were stimulated on the right hand and the other ten on the left. The order of the questions was counterbalanced, eleven volunteers rated the intensity first and then the unpleasantness, and the other ten vice versa. The entire session took around 30 minutes.

#### *Data pre-processing:*

The Skin Conductance Level was recorded at DC level. An off-line digital high pass filter set at 0.05 Hz was applied to obtain phasic Skin Conductance Responses (Andreassi, 2000).

The SCR peak-to-peak measure (Bellodi et al., 2013; Benedek & Kaernbach, 2010; Tronstad, et al., 2013) was then computed for each trial as the difference between the maximum and the minimum value detected in a 6-seconds post-stimulus.

Manual markers identifying each stimulus were added to the SCR trace by the computer keyboard, at the moment that the stimulus was administered.

#### *Data analysis*

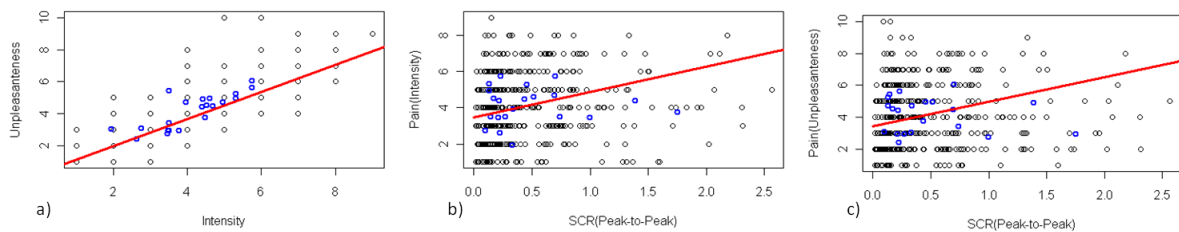
Data were analyzed with statistical package software R 2.13 (<http://www.r-project.org/>).

We run a standard correlation analysis (Pearson's  $r$ ) for the two ratings expecting a strong correlation but not a complete overlap of information ( $.75 < r < .90$ ). Then we tested in two separate models whether the SCR can predict the rating expressed for a specific stimulus. In order to do so we used an ANOVA with random effect (mixed model)

(Baayen, et al., 2008), that controls the effect of intersubject variability by analyzing all single data point acquired and grouping the subjects as a vector. The two models were settled to predict the ratings (unpleasantness, or intensity) from the SCR signal as independent variables (fixed effects), and including participants (N= 21) and trials (from 1 to 20) as random effect variables.  $R^2$  was calculated to estimate the effect size.

## Results

The correlation between the ratings of unpleasantness and intensity of the stimuli is significant and strong ( $r = .807$ ,  $p < .001$ ).



**Fig.1.1** Experiment 1.1 results. In a) the correlation analysis of intensity and unpleasantness ratings; b) shows the effect of SCR to predict pain intensity rating and, while c) shows the effect of SCR for predicting unpleasantness rating. Black dots indicate for each data point, blue dots are the mean of each subject, the red lines express the strength of the effect.

The ANOVAs with random effect shows that the SCR predicts both ratings.

Intensity rating - Fixed effects: estimate Intercept= 3.465; SCR= 1.417,  $t$ -value= 4.591,  $R^2 = .347$ . Random effects: Trial (Intercept)= .126; Subjects (Intercept)= 1.275; Residual= 2.358. Number of observations: 408, groups: Trial: 20; Subjects: 21.

Unpleasantness rating - Fixed effects: estimate Intercept= 3.464; SCR= 1.529,  $t$ -value= 4.506,  $R^2 = .342$ . Random effects: Trial (Intercept)= .19; Subjects (Intercept)= 1.688; Residual= 2.766. Number of observations: 408, groups: Trial: 20; Subjects: 21.

## Discussion

The aim of the experiment was to assess the relationship between SCR to noxious stimuli, and the unpleasantness and intensity ratings expressed by the participants for the same stimuli (Breimhorst et al., 2011). We have two basic findings in this experiment.

First we observed that skin conductance responses to painful stimuli is predictive of the subsequent judgment for the same stimulation. That basically means that, with the present paradigm, without any experimental manipulation, the autonomic responses to painful stimuli are strongly related to the explicit experience of pain.

A second important result can be found in the correlation analysis of the subjective scales. The ratings showed a strong correlation that confirm that the two questions are exploring different aspects of the same general experience of pain (Longo, et al., 2009) but they do not overlap. Hypothetically the intensity rating reflects more the judgment of sensory features of the stimulus, while the unpleasantness should reflect more the subjective feelings associated with that stimulation. The present experiment shows that not only the SCR is a good measure for nociceptive stimuli (Breimhorst et al., 2011), but it is also a good automatic measure for the pain experience of unpredictable stimulations.

### **Exp. 1.2 – Dissociating the contribution of cognitive aspects of pain from nociception: a novel SCR paradigm.**

#### **Materials and Methods**

##### *Subjects*

12 new, right handed, healthy participants (6 females, mean age= 24.32 s.d.= 2.1), recruited among the students attending the Università degli Studi di Milano-Bicocca took part in the Experiment 1.2 after giving their informed consent. A further sample of 24 right handed, healthy elder volunteers (12 females, mean age= 68.2 s.d.= 5.6) took part in the experiment with the specific purpose to check the reliability of this paradigm with people of comparable age to atypical population of stroke patients (see Study 2).

The experimental protocol was explained in detail, but the participants were blind to the purpose of the experiment. The experiment was conducted according to the principles of the Declaration of Helsinki ("Declaration of Helsinki," 1996).

### *Somatosensory Stimuli*

Two different kinds of stimuli were administered: noxious (delivered through the same needle as in Exp. 1.1) and neutral (delivered through a cotton swab) (Cheng et al., 2007; Forgiarini et al., 2011; Höfle, et al., 2012). The stimuli could be administered in two contact conditions (Factor: contact): real or simulated. In the real condition the needle and the cotton swab touched the skin of the back of the hand (between the thumb and the index finger) for about 0.5 seconds in an area of approximately 1 cm<sup>2</sup>. In the simulated condition the stimulus approached the same area of the skin, but stopped at a distance of approximately 0.5 cm from the skin where the stimulus stayed still for about 0.5 seconds and then retracted.

Non-painful tactile stimuli were delivered in order to compute the anticipatory response to pain and to reduce the adaptation of SCR which is known to be very quick in presence of repetitive stimulations (Levinson & Edelberg, 1985). Stimuli were delivered either to the right and left hand, thus eight different conditions were available in the experimental paradigm: Painful Real Right, Painful Real Left, Painful Simulated Right, Painful Simulated Left, Neutral Real Right, Neutral Real Left, Neutral Simulated Right, Neutral Simulated Left.

### *SCR Hardware and Software*

SCR was collected through a SC-2701 biosignal amplifier (Bioderm, UFI, Morro Bay, California) connected to a dedicated PC through a serial port. The gain parameter was set at 10  $\mu\text{mho/V}$ ; the signal was sampled at 10 Hz. The signal was acquired by means of two silver electrodes (1081 FG Skin Conductance Electrode) placed on the first phalanx of the index and ring fingers of the right hand for six participants and vice versa for the other six. A saline conductive paste was applied to the electrodes to improve signal-to-noise ratio. Data were digitalized at 12 bit resolution using the SC-2701 dedicated software.

### *Experimental Procedure:*

Participants sat comfortably at a table with the experimenter sitting in front of them. They were asked to put both hands on the table with the palm facing down. Each trial started

with participants gazing at the fixation point placed at the center of a 40-cm tall vertical opaque board placed at 50 cm distance in front of them. A trained experimenter delivered one of two somatosensory stimuli (Factor: stimulus) to one of the two hands (Factor: hand), by approaching it with a smooth, continuous movement. Neutral or painful stimuli emerged unpredictably, in random sequence, from behind the opaque board and participants were instructed to gaze at them along their entire trajectory.

A total of 64 tactile and noxious stimuli were administered to the participants in a single session, while the Skin Conductance Response (SCR) was recorded. The 64 stimuli were divided into 8 independent blocks of 8 stimuli each (1 per condition); the stimuli were randomized within each block. A pause was introduced after 4 blocks, or at the end of any block if the volunteer asked for a rest. The entire session took around 30 minutes.

#### *Data pre-processing:*

The SCR peak-to-base measure (Breimhorst et al., 2011; Lykken & Venables, 1971; Rhudy et al., 2010) was computed for each trial as the difference between the maximum value detected in a 6-seconds post-stimulus time window and the baseline calculated as the average value of a 300-millisecond pre-stimulus time window.

Manual markers identifying each stimulus type were added to the SCR trace by the computer keyboard, at the moment that the stimulus became visible to the participant.

The peak-to-base measures were then normalized within-subject and converted in Z-scores (Rhudy et al., 2007; Rhudy et al., 2008; Williams & Rhudy, 2009; Rhudy et al., 2010), given the well-known large inter-subject variability of SCR (Lykken & Venables, 1971; Fowles et al., 1981) however either the raw SCR and the standardized SCR were analyzed to check the reliability of the two methods with healthy participants in normal conditions.

#### *Data analysis*

Either the raw SCR and the standardized data were analyzed with SPSS 21 (IBM® SPSS® Chicago, Illinois), STATISTICA 6.0 (StatSoft, Italy, <http://www.statsoft.it>) and G\*Power 3.1 (<http://www.pscho.uni-duesseldorf.de/abteilungen/aap/gpower3/>)

separately for the two samples. A General Linear Model was used on SCR data, factoring: Stimulus (painful/neutral), Contact (real/simulated) and Hand (left/right), as within subject factors. This resulted in two 2\*2\*2 repeated-measure ANOVAs design.

Achieved power and effect size, measured with the partial eta squared ( $\eta^2$ ) evaluating the degree of association between an effect and the dependent variable, namely the proportion of the total variance that is attributable to a main factor or to an interaction (Cohen 1973), are also provided.

Significant level was set at  $< .05$ , Fisher post-hoc tests were used when appropriate.

## Results

### *Young participants*

The ANOVA on raw SCR showed a main effect of stimulus ( $F(1,11)= 14.426$   $p < .01$ ,  $\eta^2 = .567$ , power = .932; painful = .17 (average)  $\mu S \pm (St.Err).04$ , neutral = .02  $\mu S \pm .02$ ) and a main effect of contact ( $F(1,11)= 15.411$   $p < .01$ ,  $\eta^2 = .584$ , power = .946; real = .12  $\mu S \pm .03$ , simulated = .07  $\mu S \pm .03$ ) moreover the interaction between stimulus and contact was significant ( $F(1,11)= 8.61$   $p \leq .01$ ,  $\eta^2 = .439$ , power = .97; painful real = .22  $\mu S \pm .06$ , painful simulated = .12  $\mu S \pm .04$ , neutral real = .02  $\mu S \pm .01$ , neutral simulated = .02  $\mu S \pm .02$ ). The main effect hand ( $F(1,11)= .116$   $p = .74$ ,  $\eta^2 = .010$ , power = .061) and the other interactions were not significant.

Post-hoc analysis showed that painful real stimulations induced stronger SCR than all other conditions (all  $p < .01$ ), but also that painful simulated stimuli induced larger SCR than neutral stimuli (all  $p < .01$ ), finally neutral real and neutral simulated stimuli were equals ( $p = .93$ ).

The ANOVA on standardized SCR showed a significant main effect of stimulus ( $F(1,11)= 27.397$   $p < .001$ ,  $\eta^2 = .714$ , power = .997; painful = .32  $\pm .08$ , neutral = -.39  $\pm .06$ ), a main effect of contact ( $F(1,11)= 17.116$   $p < .01$ ,  $\eta^2 = .609$ , power = .964; real = .09  $\pm .04$ , simulated = -.16  $\pm .04$ ), and the interaction between stimulus and contact ( $F(1,11)= 11.617$

$p < .01$ ,  $\eta^2 = .514$ , power = .997; ; painful real =  $.56 \pm .12$ , painful simulated =  $.07 \pm .07$ , neutral real =  $-.38 \pm .06$ , neutral simulated =  $-.39 \pm .07$ ). The main effect hand ( $F(1,11) = .055$   $p = .819$ ,  $\eta^2 = .005$ , power = .055) and the other interactions were not significant.

Post-hoc analysis on significant interaction showed the same pattern of responses observed in raw SCR analysis (all  $p < .001$ , except neutral real Vs neutral simulated where  $p = .86$ ).

#### *Elder participants*

The ANOVA on raw SCR highlighted a main effect of stimulus ( $F(1,23) = 16.529$   $p < .001$ ,  $\eta^2 = .418$ , power = .973; painful =  $.39 \mu S \pm .08$ , neutral =  $.26 \mu S \pm .07$ ), a main effect of contact ( $F(1,23) = 15.364$   $p \leq .001$   $\eta^2 = .400$  power = .963; real =  $.36 \mu S \pm .08$ , simulated =  $.29 \mu S \pm .07$ ), and the interaction between these two main factors ( $F(1,23) = 8.113$   $p < .01$ ,  $\eta^2 = .261$ , power = .988; painful real =  $.45 \mu S \pm .09$ , painful simulated =  $.33 \mu S \pm .08$ , neutral real =  $.28 \mu S \pm .07$ , neutral simulated =  $.25 \mu S \pm .07$ ). The main effect hand ( $F(1,23) = .007$   $p = .934$ ,  $\eta^2 < .001$ , power = .051) as well as all the other interactions were not significant.

The ANOVA on standardized SCR showed again a significant main effect of stimulus ( $F(1,23) = 42.972$   $p < .001$ ,  $\eta^2 = .651$ , power > .999; painful =  $.25 \pm .04$ , neutral =  $-.3 \pm .04$ ), a main effect of contact ( $F(1,23) = 28.331$   $p < .001$   $\eta^2 = .552$  power = .999; real =  $.13 \pm .03$ , simulated =  $-.18 \pm .03$ ), and the interaction between stimulus and contact ( $F(1,23) = 6.666$   $p < .05$ ,  $\eta^2 = .225$ , power = .967; painful real =  $.5 \pm .08$ , painful simulated =  $.002 \pm .05$ , neutral real =  $-.23 \pm .06$ , neutral simulated =  $-.37 \pm .04$ ). The main effect hand ( $F(1,23) = .741$   $p = .398$ ,  $\eta^2 = .031$ , power = .131) and the other interactions were not significant.

Also in elder people post-hoc analysis for significant interaction showed that painful real stimulations induced stronger SCR than all other conditions (all  $p < .001$ ), but also that painful simulated stimuli induced larger SCR than neutral stimuli (all  $p < .05$ ), and finally neutral real and neutral simulated stimuli were equal again either with raw and standardized data analysis (raw SCR  $p = .31$ , standardized SCR  $p = .17$ )



## **Discussion**

We aimed at investigating whether noxious stimuli induced a skin conductance response stronger than neutral stimuli. Moreover we sought for dissociating the cognitive component of pain experience from nociception. We found that painful stimuli induced stronger autonomic responses than neutrals, critically painful stimuli in simulated contact condition induced bigger SCR than neutral stimuli as well as real contact painful stimuli.

As expected the strongest SCR was recorded when the noxious stimulus actually touched the hand adding to the cognitive aspects of pain – i.e. simulated stimulation – the contribution of nociception and the physical characteristics of the current stimulus. Interestingly both hands responded equally in each condition.

### **Exp. 1.3 – Increase SCR to incoming painful stimuli in peripersonal space.**

#### **Materials and methods**

##### *Participants*

14 right-handed participants took part in this experiment (4 males, mean age  $26 \pm 11$ ). All participants gave written informed consent; they were naïve to the experimental procedure and to the purpose of the study and none of them reported neurological, psychiatric, or other relevant medical problems. The protocol was carried out in accordance with the ethical standards of the Declaration of Helsinki (1996) and was approved by the ethical committee at the University of Milano-Bicocca.

##### *Experimental procedure*

Participants sat on a chair in a floodlit room with the experimenter sitting in front of them. During the experiment, participants were asked to relax, and carefully watch the approaching stimulus, namely a 4-cm long needle. On each trial, the experimenter manually moved the stimulus from behind the table (where it was invisible to the participant) towards the hand, in four spatial positions (Factor: distance): 1) touch: the needle eventually touched the right index fingertip; 2) near 1cm: the approaching needle stopped at 1cm from the fingertip; 3) near 5cm: the needle stopped at 5cm from the

finger; 4) far 40cm: the needle stopped at 40cm from the fingertip. The stimulus was moved towards the hand along two directions (Factor: axis): horizontal (H) and vertical (V). In the H condition, the experimenter raised the needle for 2cm from the table, and then approached the table at the given radial distance from the hand for each trial, or touched the hand itself; in the V condition the needle was raised at 50 cm above the hand, and then was manually lowered towards the hand, stopping at the given distance for each single trial, or touching the hand. Two rulers were fixed close to participants' hand, one for each axis, shielded from participants view by a cardboard box, the four spatial distances were marked upon them and were used as reference for the stimulations. The experimenter was trained to deliver manual stimuli at a speed as constant as possible. 16 blocks of trials were given, each comprising 8 trials, one for each distance on each axis for a total of 64 stimuli. The direction of stimulation along the two axes occurred in a counterbalanced fixed order (HVVH or VHHV) between subjects, while the four spatial distances were randomly stimulated. The total duration of the experimental session was about 30 min.

#### *SCR Hardware and Software*

Skin Conductance data were acquired with the same hardware, parameters set, and software of Experiment 1.2 following standard guidelines (Dawson et al. 2007). For each trial, we calculated the peak-to-base measure (Rhudy et al. 2010) as in Experiment 1.2.

#### *Data Analysis*

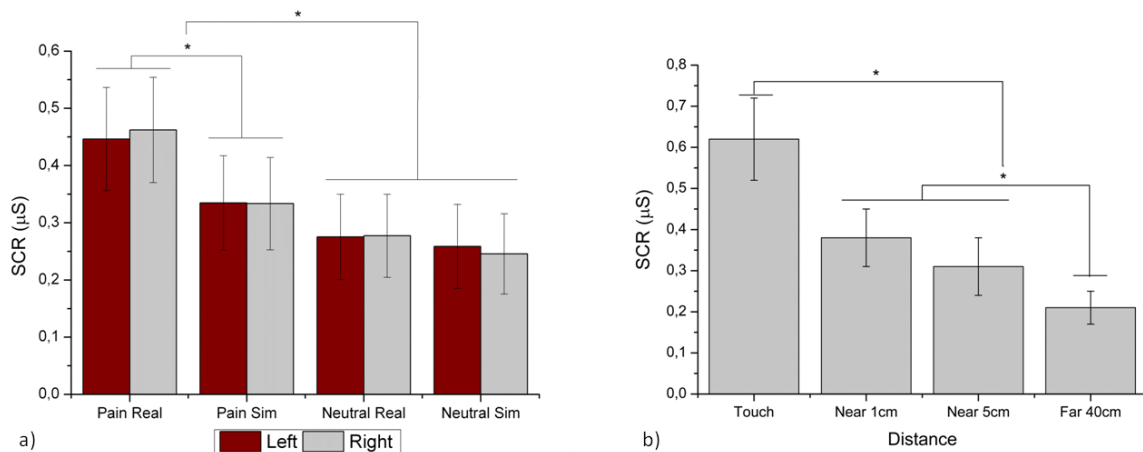
Data were analyzed using STATISTICA 6.0 (StatSoft, Italy, <http://www.statsoft.it>) and G\*Power 3.1 (<http://www.pscho.uni-duesseldorf.de/abteilungen/aap/gpower3/>). To verify whether a threatening stimulus induced a different pattern of activation due to its distance from the observer's body, the mean peak-to-base SCR in the different experimental conditions was analyzed in a repeated-measure ANOVA design, with 2 within-subject factors: axis (horizontal/vertical) and distance (touch, near 1cm, near 5cm, far 40cm). When appropriate, post-hoc tests were calculated using the Fisher test. Finally,

we measured the effect size in the ANOVA, by calculating the partial Eta Squared ( $\eta^2$ ), and we also reported the achieved power.

## Results

The ANOVA showed a significant main effect of the factor axis ( $F(1,13)= 8.35$ ,  $p< .05$ ,  $\eta^2= .39$ , power= .759): the SCR was higher when the stimulus moved along the vertical axis ( $.43\mu s \pm$  standard error  $.09$ ), as compared to the horizontal axis ( $.34 \pm .08\mu s$ ). Crucially also the main factor distance reached significance ( $F(3,39)= 24.15$ ,  $p< .001$ ,  $\eta^2= .65$ , power $> .999$ ), showing that SCR was modulated by the distance of the needle from the observer's body.

Indeed, when the needle touched the subject's index finger, the SCR was significantly higher ( $.62 \pm .1\mu s$ ) as compared to all other conditions: 1cm=  $.38 \pm .08\mu s$ ,  $p< .001$ ; 5cm=  $.32 \pm .07\mu s$ ,  $p< .001$ , 40cm=  $.21 \pm .04\mu s$ ,  $p< .001$ . There was no difference between the two near conditions (i.e., stimulus presented at 1cm and at 5cm,  $p= .22$ ). Instead, SCR was significantly lower when the needle was presented at 40cm from the body as compared with all other distances (all  $p< .05$ ). The axis by distance interaction did not reach significance level ( $F(3,39)= .7$ ,  $p= .5$ ,  $\eta^2= .05$ , power= .279).



**Fig.1.2** Columns represents the mean SCR, thin bars indicate standard errors, asterisks highlight significant differences. a) Experiment 1.2 results. In red there are the responses for the left hand stimulations in gray responses for stimuli directed to the right hand. b) Experiment 1.3 results, SCR as a function of stimulus distance from the hand.

## **Discussion**

This experiment shows that pain anticipatory responses depend on the distance of the threatening stimulus from the body. First, we found an overall greater SCR when the stimulus was presented along the vertical axis. This result might be explained by the fact that stimuli delivered along this axis were closer to the body (head and trunk) and that their final distance from the hand was more unpredictable, thus more alerting overall.

Moreover, the more the painful stimulus approached the hand, the greater the SCR. The response was indeed maximal when the needle touched the hand, but was also higher for the positions immediately near the hand (1cm, 5cm), as compared to the far distance (40cm). Taken together, these results suggest that when a threatening stimulus enters into the space close to the hand, it increases the level of arousal in the observer. The arousal response, therefore, increases not as a mere reaction to a salient, threatening object but is specifically linked to the potential danger of that approaching object to body integrity.

## **General Discussion**

The aim of the first study was to build and validate a novel experimental paradigm that allows measuring the involuntary responses to painful stimuli and is effective in dissociating the cognitive aspects of pain processing from the nociceptive component.

First we aimed at measuring whether the SCR could be considered as a confident index of autonomous nervous system activation to pain experience and not just to the somatosensory, noxious stimulation.

We found that, in the absence of any experimental manipulation, SCR measured in response to a painful stimulation, specifically a needle pinprick, was strongly predicting for the subsequent rating of stimulus intensity and unpleasantness. It is known that SCR is sensitive to different intensities of noxious stimuli especially for thermal and mechanical pain (Breimhorst et al., 2011), moreover it is known that pain ratings increase with the increasing intensity of the noxious stimuli (Longo et al., 2012). However it was not clear whether also the more emotional experience of pain unpleasantness, measured through

explicit ratings, and not the mere intensity of the stimulus, was related to the automatic response of skin conductance. In other words our intentions were to check the relationship between automatic responses to painful stimuli and the explicit judgments for that stimulations. This was a sort of pre-condition necessary to consider SCR as an index of pain experience.

The second experiment represents the novel paradigm that we mainly used throughout the entire thesis dissertation, and that aimed at dissociating the cognitive aspects of pain from nociception. In order to do so we designed a paradigm where, in half of the trials, the stimulation was simulated, thus any eventual response recorded would have been caused by the anticipation of sensory consequences of such stimulation, depending upon the cognitive aspects involved in that stimulation, without accompanying nociceptive analysis. Pain anticipation is considered as a good clue for the cognitive aspects of pain experience (Colloca et al., 2006; Hsieh et al., 1999; Ploghaus, 1999). Moreover both the right and the left hand were tested and the same level of SCR was recorded.

Another point of interest was that we tested the pain anticipation paradigm in two different populations: one of young participants and another of elder volunteers. Interestingly the young and elder people showed the same pattern of responses, thus demonstrating that the paradigm is sensitive also in elder people (See Study 2) while it is known that SCR may show different responses in elder people (Drory & Korczyn, 1993).

A final methodological remark is about statistical analysis. We analyzed data either in their raw scale (Armel & Ramachandran, 2003) and after intrasubject normalization, that transformed it in Z-scores (Rhudy et al., 2010). Either the raw SCR and the standardized measures were sensitive enough to capture pain anticipation and dissociate it from the nociceptive contribution, thus both methods can be adopted. Standardized data analysis seemed to be more powerful to capture the effect (see the effect size), however given that both methods were sensitive enough to capture significant effects with either 12 and 24 people of sample sizes, the specific choice can be adopted depending on specific characteristics of each experimental procedure considering the pros and cons that each

method takes with. A further validation of the paradigm came from the third experiment of the Study 1. Indeed one can argue that the anticipatory response recorded for simulated stimuli was not due to a threat specifically targeting the body, but rather to the mere appearance of an intrinsically salient stimulus (Gläscher & Adolphs, 2003). Experiment 1.3 shows that the anticipatory response to pain is highest when the stimulus actually touches the skin, but still larger when it reaches a distance close to the body, namely at 1cm and 5cm, as compared to a farther distance (i.e., 40cm). This result supports the notion about the existence of an area of space near the body, where threatening events are more likely to affect the observer's arousal. This is reminiscent of evidence showing that somatosensory stimuli approaching the monkey's skin elicit avoidance movements, as recorded both at electromiographic and behavioral level (Cooke & Graziano 2003). These responses were interpreted as automatic defensive reaction movements to potentially dangerous stimuli, suggesting for the existence of a defensive area (Cooke, et al., 2003, Graziano, et al., 2002).

Overall the present study showed that painful stimuli induce an anticipatory alertness response which is selective for approaching harmful stimuli. Moreover this anticipatory response is recordable either in young and elder participants, making this novel paradigm suitable for people of different ages. This is of particular interest to the present thesis, since it makes the paradigm useful to test post-stroke patients. In fact, it is known that SCR may show different responses in elder participants (Drory & Korczyn, 1993) which constitutes the typical post-stroke population. Finally, the present results are compatible with the existence of an area of safeguard in humans, which is close to the body and is reminiscent of the "flight zone" described by Hediger (1955), within which dangerous approaching stimuli can induce defensive responses (Graziano et al. 2006). Increased arousal to approaching threats may be crucial for preparing defensive responses in order to protecting one's own body, or for triggering empathic pro-social behaviors, when other people are in danger (Bufalari et al. 2007; Forgiarini et al. 2011).

## **- Study 2-**

### **Arousal responses to noxious stimuli in somatoparaphrenia and anosognosia: Clues to body awareness**

#### **Introduction**

Somatoparaphrenia is a well known disorder of body representation often associated with right brain lesions (Bottini et al., 2002; Vallar & Ronchi, 2009; Invernizzi et al., 2013) and represents a challenging situation for studying the sense of body ownership. This pathological disorder is defined as the acquired delusions and confabulations about the contralesional side of the body (Vallar & Ronchi, 2009). Patients affected by somatoparaphrenia typically deny the ownership of their contralesional limbs, which they attribute to others, such as the nurse, the doctor, relatives not even present (Bottini et al., 2002), or in general to someone else who could not possibly be there (Pugnaghi et al., 2011). Somatoparaphrenia is typically found in the acute post-ictal phase after right brain damage (Gandola et al., 2011), consequently limbs on the left side of the body are more often affected.

Although somatoparaphrenia holds high clinical relevance, it has been the subject of formal experimental group studies rarely (Feinberg et al., 2010; Gandola et al., 2011). Rather, it is often reported in multiple single case description studies (Fotopoulou et al., 2011; Cogliano et al., 2011; Invernizzi et al., 2013), in single case experimental studies (Bottini et al., 2002; van Stralen et al., 2011), and in anecdotal descriptions as well (Nightingale, 1982; Halligan et al., 1995; Pugnaghi et al., 2011).

The sense of ownership for one's own body has been extensively investigated by means of several techniques and experimental paradigms in healthy participants, such as the rubber hand illusion (RHI) (Botvinick & Cohen, 1998; Tsakiris, 2010), the mirror box (Romano et al., 2013a), the Full Body Illusion (Ehrsson, 2007; Lenggenhager et al., 2007; Ionta et al., 2011), and Virtual Reality environment (Perez-Marcos et al., 2009). The sense of body ownership modulates the perception and localization of sensory

stimuli, as well as the reaction to incoming threatening stimuli. In the RHI paradigm (Botvinick & Cohen, 1998), the modified feeling of ownership for the fake hand, which is usually assessed through subjective scales (Botvinick & Cohen, 1998; Ijsselstein et al., 2006; Longo et al., 2008; Petkova & Ehrsson, 2009; Bekrater-Bodmann et al., 2012), typically correlates with the level of emotional activation for sudden threats directed to the fake hand (Armell & Ramachandran, 2003; Ehrsson et al., 2007; Guterstam et al., 2011).

It is worth noting however that the clinical picture of body disownership is much stronger, and therefore likely to be more informative, than those induced by experimental manipulations in normal people (e.g. the aforementioned RHI) (de Vignemont, 2011).

A critical question unanswered by previous experimental studies is if somatoparaphrenia corresponds to a mere confabulation or whether it is characterized by coherent behavioral and physiological correlates. Behavioral effects consistent with the somatoparaphrenic confabulations have been shown. For example, one patient showed an increase in tactile sensitivity after she was told that the touch would have been delivered to the arm of the person to whom she was attributing the limb ownership (Bottini et al., 2002). In a different study two patients recovered a normal ownership sensation when they looked at themselves from an allocentric perspective such as in a frontal mirror (Fotopoulou et al., 2011), moreover it has been shown also that self-touch of impaired hand can increase the sense of ownership over it (van Stralen et al., 2011). However, the physiological markers of such striking confabulations were not investigated at all.

In the current study we sought for the experimental evidence that the behavior of somatoparaphrenic patients is associated with a specific physiological pattern when noxious somatosensory stimuli are directed towards the limb for which patients experience a reduced sense of ownership. To this aim we measured the Skin Conductance Response (SCR) to threatening stimuli directed either towards the affected arm or the contralateral arm and we compared these responses with the responses elicited by neutral stimuli. Given that SCR can be used as a measure of the automatic affective response to



approaching harmful and neutral stimuli (Lykken & Venables, 1971; Armel & Ramachandran, 2003; Guterstam et al., 2011;) and can be strongly modulated by the degree of ownership felt for an external alien limb (Armel & Ramachandran, 2003; Petkova & Ehrsson, 2009; Guterstam et al., 2011), we reasoned that patients with deranged ownership for contralesional limbs should show reduced or absent anticipatory responses to approaching stimuli threatening the affected limbs, as if those limbs were excluded from the body representation.

Critically, in the current study, noxious stimuli were delivered in both real and simulated conditions. Simulated stimuli were introduced for studying anticipatory response to pain that is considered a reliable index of the purely cognitive component of pain processing (Rhudy et al., 2008; Rhudy et al., 2010; Forgiarini et al., 2011) and allow to directly compare the reaction to pain in patients with and without somatosensory deficits.

We selected two separate control groups of patients for this study. As a first control group we selected patients without somatoparaphrenia but presenting with anosognosia for hemianaesthesia, which is recognized as a productive symptom of a disrupted body representation and usually follows a right brain damage (Vallar et al., 2003; Spinazzola et al., 2008; Bottini et al., 2009). Anosognosic patients typically deny their acquired somatosensory deficit which is instead undoubtedly observed after a clinical examination. Somatoparaphrenia and anosognosia for hemianaesthesia are associated frequently (i.e. somatoparaphrenic patients can still overestimate the sensory-motor ability of the limb they still believe to have, even if they does not recognize the shown hand as their own) however they reflect somewhat opposite manifestations of disrupted body representation concerning somatosensory expectations; while in the former case patients deny the ownership of the impaired arm and consequently to perceive an eventual sensory stimulus on that hand, in the latter case they overestimate their actual sensory functions. As a second control group we selected hemiplegic patients (i.e., patients without any deficit of ownership or awareness). Hemiplegic patients are considered as a suitable control group for an eventual general effect of RBD on SCR. For the control groups, we predicted that

in the presence of intact body ownership anticipatory responses to painful sensations will be found for both hands.

## Methods

### *Patients:*

Fifteen right handed (evaluated with the Edinburgh inventory. Oldfield, 1971) right brain damaged patients took part in the study (six females, age=72.06 (mean)  $\pm$ 9.2 (Standard Deviation) education=9.2 $\pm$ 5.9), after giving their informed consent. They were all recruited at the Stroke Unit of Niguarda Ca' Granda Hospital in Milan. The experiment was conducted according to the principles of "Declaration of Helsinki" (World Medical Organization, 1996) and was approved by the ethical committee of the Hospital.

Demographic details					Neurological Deficit				Anosognosia					NPS	
Patients	Age	Edu	Gen	Group	H	S	VF	P	H	S	VF	SP	PN	MMSE	N
p1	66	11	M	SP	2	3	3	+	1	2	2	+	-	23	+
p2	82	5	F	SP	3	3	3	+	2	2	2	+	+	21	+
p3	65	18	M	SP	3	3	3	+	0	2	0	+	-	20	+
p4	69	5	F	SP	3	3	3	+	2	2	2	+	+	20	+
p5	84	11	F	SP	3	1	3	-	0	0	1	+	+	20	+
p6	62	5	M	A	3	3	1	+	3	3	0	-	-	23	+
p7	67	17	M	A	3	3	0	+	0	3	0	-	-	21	+
p8	81	8	F	A	3	3	1	+	2	3	0	-	+	20	-
p9	77	5	M	A	1	3	1	-	0	3	0	-	-	22	+
p10	72	4	M	A	1	3	3	-	0	3	3	-	-	20	+
p11	67	5	M	H	3	0	0	-	0	0	0	-	-	25	-
p12	63	23	M	H	3	0	0	-	0	0	0	-	-	29	+
p13	65	5	F	H	3	1	0	-	0	0	0	-	-	23	-
p14	73	5	M	H	2	0	0	-	0	0	0	-	-	30	-
p15	74	11	F	H	2	1	1	-	1	0	0	-	-	25	+

**Tab. 2.1.** Main demographical and clinical features of patients.

Patients were divided into three groups of five individuals each, according to their clinical diagnosis, namely: somatoparaphrenia (three females, age=73.2 $\pm$ 9 scholarship=10 $\pm$ 5.4); anosognosia for somatosensory deficit (one female, age=74.6 $\pm$ 4.3 scholarship=7.8 $\pm$ 5.4) and left hemiplegia without anosognosia and somatoparaphrenia (two females, age=68.4 $\pm$ 4.9 scholarship=9.8 $\pm$ 7.8) (Tab.2.1). Patients were at their first stroke event in

the acute or subacute phase (less than 30 days from the stroke) and none of them reported any previous neurological or psychiatric disease nor presented any general delusional state.

### *Neurological Assessment*

Every patient received a standardized neurological assessment of basic motor, somatosensory and visual functions according to the procedure proposed by Bisiach and colleagues (Bisiach et al., 1986). Furthermore, we performed a preliminary evaluation of the subjective experience following the delivery of the experimental painful and neutral stimuli. We administered (in a random sequence) three stimulations for each stimulus type on either hand plus three additional catch trials, with the same setup used for the experimental procedure (see below). During this evaluation patients were blindfolded and were asked to detect each stimulus. Patients with anosognosia and somatoparaphrenia did not detect any tactile stimulation nor any painful stimulation on the left hand, while hemiplegic patients had a fully preserved somatosensation.

To the aim of testing proprioception, the examiner placed the patient's impaired hand in two different positions (palm up and palm down) for ten times. At each time, he asked the patient to place his/her unimpaired hand in the same position while keeping his/her eyes closed. Proprioception was also tested by positioning the patient's contralesional index finger in two different positions (up or down) for ten times. At each time, the examiner asked the patient to mimic that position with the ipsilesional homologous finger. Personal neglect was assessed following the procedure proposed by Bisiach and colleagues where the patient is asked to touch his/her left hand using his/her right hand (Bisiach et al., 1986).

### *Assessment of Anosognosia*

Patient's awareness of neurological deficits (i.e., anosognosia) was assessed by means of a standardized four-points scale (Bisiach et al., 1986). In this scale patients score 0 (full awareness) if they report their deficit after a general question about their illness; 1 if they report their deficit after a specific question about their strength, somatosensation or visual

functions; 2 if they recognize their deficit only after it is shown by the examiner (mild anosognosia); 3 if the acknowledgment of the disorder cannot be achieved in any way (severe anosognosia).

#### *Assessment of Somatoparaphrenia*

Somatoparaphrenia was investigated by interviewing patients about the presence of any delusional feeling referred to their contralesional upper limb. The interview started by placing the patient's contralesional left hand in front of him/her and included the following questions in this sequence: "What is this? Whose hand is this? Where is your hand? Why is there an alien hand here?". The first question was always asked, while each of the following ones was proposed only if patient reported any delusion in the preceding question. Patients were considered somatoparaphrenic in case they denied the ownership of the contralesional limb and attributed it to someone else (Invernizzi et al., 2013) (Tab.2.2).

<b>Patients</b>	<b>Examiner: "whose hand is this?"</b>
<b>p1</b>	<i>"It is your hand (i.e. the neuropsychologist hand), I am sure. My hand is bigger, mine is like a shovel, this is too tiny."</i>
<b>p2</b>	<i>"This is my sister hand, yes my sister's hand. My hand is on my belly but I am too fat I cannot see."</i>
<b>p3</b>	<i>"I do not know. It is not mine. It is just the two of us, so I guess it is your hand (i.e. the neuropsychologist hand)."</i>
<b>p4</b>	<i>"This is my niece hand. She works here (i.e. in the hospital), I do not know why her hand is here, she should be around."</i>
<b>p5</b>	<i>"This is not my hand. I do not know whose hand is this. Maybe someone working here who examined me before left it here."</i>

**Tab. 2.2** Verbalization of somatoparaphrenic patients to the question: "whose hand is this?"

Anosognosia and somatoparaphrenia were tested either during the neurological evaluation and just before the beginning of the experimental procedure to ensure that patients were showing the symptom during the experiment.

#### *Neuropsychological Screening*

A short neuropsychological screening was performed in order to test for the presence of neglect, which is typically associated with somatoparaphrenia and anosognosia, and in order to rule out any general cognitive impairment being the cause of somatoparaphrenic

confabulations and anosognosia. In the Mini Mental State Examination (Folstein et al., 1975) all patients obtained a score >20 thus discounting the presence of a general cognitive impairment. The screening of the neglect was assessed with the Albert cancellation task (Albert, 1973) and the clock drawing test (Mondini et al., 2003). 11 out of 15 patients showed neglect in at least one of these tasks (five somatoparaphrenic, four anosognosic and two hemiplegic patients, see Tab.1). For this reason the experimental paradigm was specifically designed in order to avoid any possible confound due to neglect (see experimental procedure section below).

### *Lesion Mapping*

Brain lesions were identified by computerized tomography (CT) and mapped in the stereotactic space of the Montreal Neurological Institute (MNI) using a standard MRI volume (voxels of 1 mm<sup>3</sup>) that conformed to that stereotactic space. Lesion reconstruction was performed using the free software MRIcro (Rorden & Brett, 2000; [www.mricro.com](http://www.mricro.com)). The mapping procedure included the following steps (see Gandola et al., 2012 for further details): (1) *Adaptation of the MRI template to the patient's CT scan*; (2) *Lesion mapping*: A skilled rater manually mapped the lesion onto each correspondent template slice by using anatomical landmarks. A second skilled rater double-checked for the accuracy of the tracings for each patient. In cases of disagreement an intersection lesion map was used; (3) *Lesion re-orientation*. The lesion maps were then transformed back into the standard space by using the inverse of the transformation parameters formerly used for the adaptation of the MRI template to the patient's brain scan; (4) *Lesion analysis*. We used the overlay lesion plots technique and the subtraction method (see review in Rorden & Karnath 2004), implemented in the software MRIcron (Rorden et al., 2007), to illustrate differences in the distribution of the lesion between groups.

The anatomical localization of the lesions was assessed using the Automated Anatomical Labelling map (template AAL; Tzourio-Mazoyer et al., 2002) which classifies the anatomical distribution of digital images in stereotactic space.

### *Stimuli*

A series of 64 mechanical stimuli were administered to each patient in a single session and simultaneously the SCR was recorded. The entire session took around 30 minutes.

Two types of stimuli were used: noxious stimuli (delivered through a needle) and neutral stimuli (delivered through a cotton swab) (Cheng et al., 2007; Forgiarini et al., 2011; Höfle et al., 2012). All participants flawlessly distinguished the needle from the cotton swab both by visual inspection and during the stimulation of their ipsilesional hand without seeing it.

Critically, the stimuli were administered under two conditions: real and simulated. In the real condition the needle and the cotton swab touched the back of the hand (between the thumb and the index finger) for about 0.5 seconds in an area of approximately 1 cm<sup>2</sup>. In the simulated condition the stimulus approached the same area of the skin, but stopped at a distance of approximately 0.5 cm from the skin where the stimulus stayed still for about 0.5 seconds and then retracted.

Stimuli were alternatively delivered to the ipsilesional right hand, which served as control, or the contralesional left hand. Globally, eight different stimulation conditions were used: Painful Real Right, Painful Real Left, Painful Simulated Right, Painful Simulated Left, Neutral Real Right, Neutral Real Left, Neutral Simulated Right, Neutral Simulated Left.

The 64 stimuli were divided into 8 independent blocks of 8 stimuli each (1 per condition); stimulus sequence was randomized within each block except in the first block where a pseudorandom sequence was used instead. In this pseudorandom sequence the first two stimuli were always two neutral stimulations followed by four noxious stimuli and by two neutral stimulations. By doing so the two initial stimulations, where the SCR is usually extremely strong due to the novelty of the situation, never included the critical noxious stimulation. In addition, this blocked procedure allowed to control for the effect of habituation, which can occur quite rapidly, and also ensured that all stimulus types

were delivered within each block. A pause was introduced after 4 blocks, or at the end of any block if the patient asked for a rest.

#### *Setting and procedures*

Patients comfortably sat at a table in front of the experimenter and their hands were resting on the table, palm down. The experimenter extended the patients hands on the table and radially aligned them along the mid-sagittal plane, with the aim of minimizing any neglect-induced unbalance in the visual monitoring during stimulus delivery. The hand closer to the body was the left hand for three patients in each group and the right hand for the remaining two. With this arrangement all patients reported to have unoccluded vision towards both hands.

Patients were then asked to relax, remain as still as possible and keep a regular breathing, while gazing towards a fixation point drawn at the center of an opaque screen, which was placed at a distance of 50 cm in front of them. The screen shielded both the experimenter's hands and the stimuli. The experimenter was trained to use the same trajectory at each stimulation. Stimuli emerged behind the screen and unpredictably approached one of the patient's hands. Patients were instructed to gaze at the stimuli for the whole trajectory.

#### *SCR apparatus*

Skin Conductance data were acquired with the same hardware, parameters set, and software of Experiment 1.2 following standard guidelines (Dawson et al. 2007).

#### *Data pre-processing*

The peak-to-base measure has been used in pain related SCR experiments (Lykken & Venables, 1971; Rhudy et al., 2010; Breimhorst et al., 2011; Rhudy et al. 2007; Ehrsson 2007) and here was computed for each trial as the difference between the maximum value detected in a 5-second post-stimulus time window and the baseline calculated as the average value of a 0.3 second pre-stimulus time window. Triggers coding for the stimulus type were manually sent to the SCR trace through the computer keyboard at the moment when the stimulus became visible to the participant from behind the opaque panel.

It is well known that right brain damaged patients show more frequently somatosensory deficit than left hemisphere damage patients possibly due to a more or less evident form of neglect that could lead to the unawareness of the stimulation despite the signal reach the somatosensory cortex (Sterzi et al., 1993; Vallar et al., 2003). To avoid any contribution of unaware somatosensory processing only responses to simulated stimuli were analyzed .

The peak-to-base measures were then normalized within-subject and converted in Z-scores (Rhudy et al., 2007; Rhudy et al., 2008; Williams & Rhudy, 2009; Rhudy et al., 2010), in order to obtain comparable measures among the patients, given the well-known large inter-subject variability of SCR (Lykken & Venables, 1971; Fowles et al., 1981) especially in RBD (Critchley, 2005).

#### *Data analysis*

Data were analyzed with SPSS 21 (IBM® SPSS® Chicago, Illinois). A General Linear Model was used on SCR data, factoring: Stimulus (painful/neutral), Hand (left/right), as within subject factors and group (hemiplegia/anosognosia/somatoparaphrenia) as between subject factor. This resulted in a 2\*2 (within) \*3 (between) ANOVA mixed design.

Achieved power and effect size, measured with the partial eta squared ( $\eta^2$ ) have been reported. Significant interactions have been explored by looking at the Confidence Intervals (Cohen 1990, 1992, 1994; Masson & Loftus 2003), we set at 90% level of the interval. Confidence Intervals show the range of probability where a datum could be found in that condition, consequently an interval without overlapping with another shows its independency and suggests for a relevant difference.

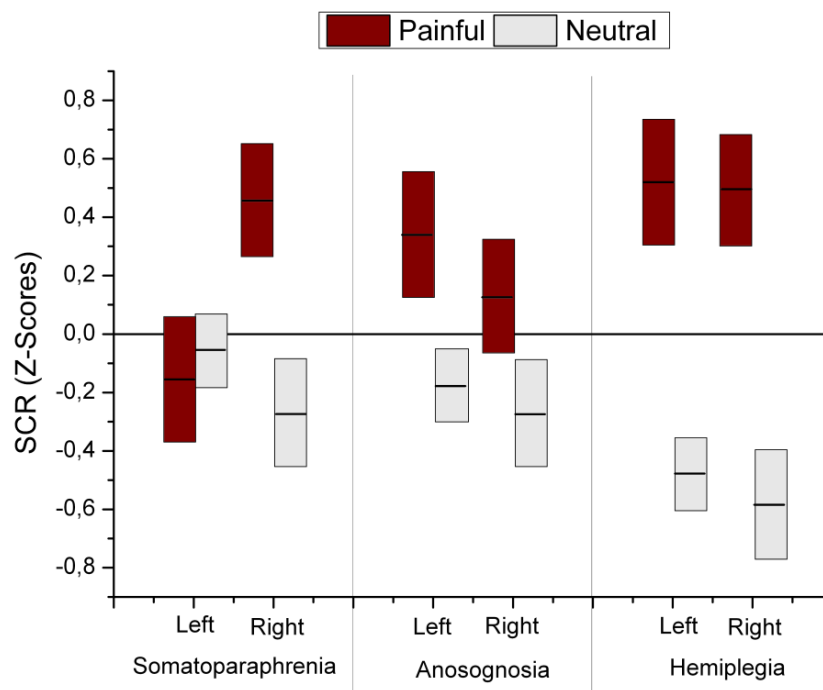
Assumptions to properly use parametric tests were tested and we did not find violations: all four conditions show normal range of skewness and kurtosis (all values  $<|1|$  and Kolmogorov-Smirnov  $p=n.s.$ ), F-test for the equality of variance between the four conditions and the three groups were not significant, suggesting that the normal distribution and equality of variance in our data can be assumed.



## Results

### *SCR results*

A three-level interaction was found between all factors of the model ( $F(2,12)= 4.576$ ,  $p< .05$ , power= .969;  $\eta^2= .433$ ). In patients with Somatoparaphrenia, Confidence Intervals show that responses to painful stimuli are different from neutral stimuli only in the right ipsilesional hand (painful right= .265 to .653 (z-scores for the 90% Confidence Interval), .459 (mean z-score); neutral right= -.457 to -.087, mean= -.272), while in the left impaired hand the anticipatory response to pain was lacking (painful left= -.369 to .059, mean= -.155; neutral left= -.178 to .070 mean= -.054).



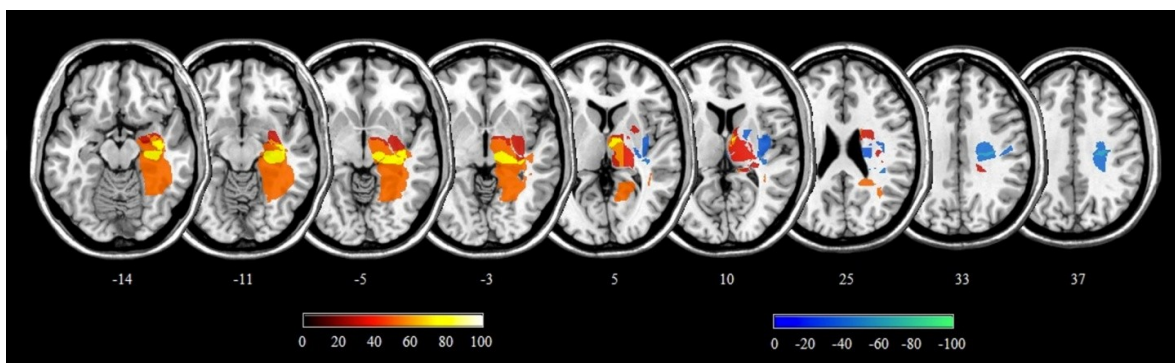
**Fig.2.1** SCR results of the three clinical populations to simulated painful (light gray columns) and neutral (dark gray columns) stimuli. Columns represent the 90% Confidence Interval of peak-to-base SCR expressed in Z-scores, horizontal bars indicate average values.

In the Anosognosia group Confidence Intervals show that the anticipatory response to pain was recordable in both hands (painful left= .124 to .552, mean= .338; neutral left= -.303 to -.055 mean= -.179; painful right= -.070 to .318, mean= .124; neutral right= -.457 to -.087, mean= -.272).

Likewise, in the hemiplegic group we found that both hands had comparable SCR that was larger for approaching painful, than neutral stimuli (painful left= .304 to .732, mean= .518; neutral left= -.605 to -.358 mean= -.482; painful right= .298 to .686, mean= .492; neutral right= -.770 to -.399, mean= -.584).

### *Lesion Mapping Results*

All patients presented a right brain ischemic or hemorrhagic lesion. Two patients were excluded from the lesion mapping because CT scans were not available (case p3 and case p12, Tab. 2.1). In patients with somatoparaphrenia the center of the overlap (defined as those voxels that were damaged at least in 3 of 4 patients) is localized in the right white matter (including the posterior limb of the internal capsule and the corona radiata), in the basal ganglia (caudate, putamen and pallidum) and in the thalamus. The overlap extended into the hippocampus and the amygdala. The lesions of patients with anosognosia for the somatosensory deficit overlapped in the right rolandic operculum and the insula, in the basal ganglia (caudate and putamen) and in the white matter including the posterior limb of the internal capsule, the corona radiata and the external capsule. Finally, in patients with left hemiplegia without anosognosia and without somatoparaphrenia, the centre of overlay (50% of patients) is localized in the sensorimotor cortex (precentral and postcentral gyri), in the parietal, frontal and insular cortices. The white matter in the right hemisphere is also damaged.



**Fig.2.2** Subtraction analysis. Regions frequently damaged in patients with somatoparaphrenia but spared in patients without this symptom (anosognosic group) are illustrated with warm colours, from dark red to white. The cold colours, from dark to light blue, show the opposite subtraction. MNI z coordinates of each section are reported under each slice. Reconstructions were performed using the software MRICron.

To distinguish the brain regions frequently damaged in patients with somatoparaphrenia but spared in patients with anosognosia for hemiplegia without feeling of disownership, we used the subtraction methods (Rorden & Karnath, 2004), a good alternative to objective voxel-wise statistical analyses when the sample size is small as in our study. We found that the same regions highlighted in the lesion overlap of the patients with somatoparaphrenia were at least 75% more frequently damaged in this group than in the anosognosic group (yellow in Figure 2.2). The subtraction analysis between patients with anosognosia for somatosensory deficits and patients with somatoparaphrenia showed that only few voxels in the subcortical white matter, in the rolandic operculum and in the insula were injured 55% or more frequently in the Anosognosic group compared with the Somatoparaphrenic group (light blue in Figure 2.2).

## **Discussion**

In the present work we characterized the physiological correlates of the processing of threatening stimuli in neuropsychological patients affected by somatoparaphrenia. Somatoparaphrenia typically follows a right brain lesion and is a condition in which patients feel that their paralyzed limb does not belong to their body. In particular we exploited the notion that pain is a multifactorial experience depending, among other factors, on the analysis of incoming stimuli in relation to the mental representation of one's own body (Longo et al., 2009; Gallace et al., 2011; Mancini et al., 2011b). The monitoring of incoming threats gives rise to cognitive and emotional anticipatory reactions (Ploghaus, 1999; Brown & Jones, 2008; Clark et al., 2008; Rhudy et al., 2008) that alert the subject for possible noxious stimuli directed towards his/her own body and activate defensive behaviors (Cooke & Graziano, 2003; Graziano & Cooke, 2006).

Results show that in all groups of patients the physiological reaction to an approaching stimulus changes depending on the salience of the stimulus, as typically found in the literature (Jensen et al., 2003; Breimhorst et al., 2011). In fact, the physiological reaction is stronger for noxious stimuli than for neutral tactile stimuli that approach the body. The

crucial finding of this study is that anticipatory responses to threatening stimuli are strictly dependent on the sense of ownership for the threatened body part.

SCR to threatening stimuli in patients with somatoparaphrenia was coherent with the referred delusion because it was reduced for the paralyzed limb. Indeed our patients verbally reported a sense of non-belonging for their left arm, that they ascribed to relatives, doctors or nurses (Bottini et al., 2002; Vallar & Ronchi, 2009; Pugnaghi et al., 2011; Invernizzi et al., 2013). Critically, such delusional subjective reports were strictly linked to reduced physiological responses to incoming threats. In particular, the anticipatory SCR to simulated noxious stimulations, which reflects the cognitive/affective component of pain perception (Brown et al., 2008b; Rhudy et al., 2008), was absent when the stimulus was directed to the hand that the patient attributed to someone else. Conversely the anticipatory response was present when the stimulus was directed to the hand that they identified as their own that exclude a general disruption of the SCR in the patients we tested.

However a possible explanation for the observed reduction of the response calls into play a general decrease in SCR for all stimuli directed to the contralesional body part in RBD patients. This possibility was discarded by the finding of normal anticipatory responses for stimuli directed to both hands in the control groups.

Alternatively, one could argue that patients with somatoparaphrenia did not show any pain anticipation on the left arm/hand because of an insensitivity to the stimuli on the left side, and not because of a disrupted body representation. In other words, an autonomic response might be not observed when noxious stimuli are no more detected. If this were the case, however, we should expect patients with anosognosia for hemianaesthesia to have the same pattern of responses despite their false belief of an intact somatosensory processing. By contrast, we found that anosognosic patients show an anticipatory response to noxious stimuli on both hands. This finding clearly rules out the potential alternative explanation based on insensitivity to stimuli on the left side.

Furthermore, it is well established that the level of processing of contralesional somatosensory stimuli is difficult to assess in RBD patients due to the possible contribution of neglect (Sterzi et al., 1993; Vallar et al., 2003), as witnessed by the presence of implicit processing of unreported somatosensory stimuli (Vallar et al., 1991). Therefore, in all our groups of RBD patients the SCR to simulated stimuli, where actual somatosensory stimulation was absent, was the most appropriate measure to compare the responses to actual threatening stimuli, excluding possible confounds depending on the unaware sensory processing. Our data are reminiscent of the results obtained in healthy participants following bodily illusions. In the RHI, where the feeling that a fake hand belongs to one's own body rise from congruent visuo-tactile stimulations on the fake and the participant's hand (Botvinick & Cohen, 1998; Tsakiris, 2010; Rohde et al., 2011), has been well established that the more the sense of ownership for the rubber hand the more the activation for an unexpected threatening stimulus directed to that fake hand (Armell & Ramachandran, 2003; Guterstam et al., 2011) suggesting that ownership sensation is relevant to determine autonomic responses. Congruently it has been reported recently (Pia et al., 2013) that in a group of patients, that after stroke used to incorporate in their own body representation external limbs, the vision of a threat directed to the arm that they embodied was judged to be as painful as when it was directed to their biological impaired hand.

Further clues about the nature of the deficit in our patients groups can be gathered from the anatomical analysis of brain lesions. The distribution of the brain lesions of patients with somatoparaphrenia confirms the anatomical pattern previously associated with this disorder (Gandola et al., 2012; Invernizzi et al., 2013; Jenkinson et al., 2013). The four patients with somatoparaphrenia for which we could reconstruct brain lesions had lesions overlapping in the subcortical white matter of the right hemisphere, in the basal ganglia and in the limbic circuit (i.e. hippocampus and amygdala). This localization pattern, which is predominantly subcortical, may cause a deficit in integrating bottom-up information with higher-order body representation, with a consequent feeling of

disownership for that part of the body (see also Gandola et al., 2012 for further discussion). Furthermore, the lesions of the right hippocampus and amygdala may reduce the sense of familiarity for the affected body part (Gandola et al., 2012) and contribute to the reduction of the emotional response to approaching threatening stimuli.

Four out of five patients with anosognosia for somatosensation presented a lesion of the insular cortex, basal ganglia and periventricular white matter, in agreement with the observation of Spinazzola and colleagues (Spinazzola et al., 2008). In patients with anosognosia the sense of body ownership was not impaired and the anticipatory response to pain was still present. This observation is particularly interesting because it suggests that, in patients with equivalent sensory impairment (all but one patients - case p5, Table 1 - in both groups presented a severe left hemianaesthesia, as assessed to the standardized neurological procedure), an anticipatory response to pain is preserved only if the lesion did not affect the sense of body ownership. Finally, the lesions of patients with hemiplegia did not impair body ownership for the paretic limb, as well as the emotional responses to pain. However, our anatomical results warrant great interpretative caution given that the small sample size did not allow statistical analysis.

Our data suggest that patients affected by disrupted ownership for contralesional limbs show a reduced monitoring of incoming threatening stimuli when these stimuli are directed towards the affected body part.

This finding selectively holds for patients with somatoparaphrenia, thus confirming that anosognosia and somatoparaphrenia are two distinct disorders of awareness of body representation, although they are frequently associated with right brain damage and often occur together. This is the first demonstration of the physiopathological correlates of somatoparaphrenia, which has been predominantly investigated by means of interviews because it manifests as a confabulation. The SCR pattern of somatoparaphrenic patients reflects a profound modification of automatic arousal responses to threats directed towards the affected limbs. Such a derangement of body representation also suggests specific caution for these patients, given the highly protective value of monitoring

peripersonal space for incoming threats. Our data suggest that somatoparaphrenia does not represent a mere disruption of body representation. Our data rather pinpoint somatoparaphrenia as a more general alteration of body/space interactions, which includes a relevant reduction in the reactivity to harmful stimuli. In line with the concept of a “safety region” surrounding our body (or *flight zone*, as outlined by Hediger (Hediger, 1955)), somatoparaphrenia may reduce the monitoring of such region of space and in turn significantly impair the patients’ interaction with the world around them. This deficit potentially adds to other coexistent neuropsychological deficits, such as neglect. Further investigations are needed and should aim both to prevent potential damage to the patients and to increase their level of interaction with surrounding space. This is particularly relevant for patients whose disorders of bodily awareness might be slightly different (Zeller et al., 2011) or present in a mild form and may then remain undiagnosed (Baier & Karnath, 2008).





### **- Study 3 -**

## **Arousal responses to noxious stimuli in patients with Body Integrity Identity Disorder (BIID): when the feeling of ownership is lost for healthy limbs.**

### **Introduction**

Body Integrity Identity Disorder (BIID), (Berlucchi & Aglioti, 2010; First, 2005) is a pathological condition characterized by an intense desire for amputation of one's own healthy limb (First, 2005; Sedda, 2011). It has been recently proposed that the sense of incompleteness reported by BIID patients may be underpinned by a dysfunctional activity of the right parietal lobe (McGeoch et al. 2011), congruently with the idea that BIID is not a mere paraphilia or psychological disorder (First 2005; Everaerd 1983; Money et al. 1977), but a true neurological syndrome (Blanke, et al., 2009; Sedda, 2011).

McGeoch and colleagues found reduced activity in the right Superior Parietal Lobule (SPL) when comparing the somatosensory responses for the affected leg with that of the unaffected leg or the leg of control participants. Moreover any other modulation of brain activity were found in areas typically associated with body representation, such as the insula (Berlucchi & Aglioti, 2010). In partial agreement, Hilti and colleagues, in a magnetic resonance morphological study, found differences in cortical architecture between BIID patients and controls in the right SPL, but also in right primary and secondary somatosensory cortices, and anterior insula (Hilti et al., 2013).

It has been proposed that BIID patients are able to perceive the affected limb because of spared visual and somatosensory cortices, but they have a disrupted representation of that specific body part due to parietal lobe dysfunction, attributing this dysfunction to the body image representation (Mcgeoch et al., 2011), however the very recent data from Hilti showed that the assumption of fully spared SI and SII cortices in BIID patients should be taken carefully (Hilti et al., 2013).

The parallelisms between the BIID syndrome and somatoparaphrenia (Vallar & Ronchi, 2009) has also been proposed (Berti, 2013; Brang, et al., 2008), although the two syndromes are not really overlapping. Indeed, BIID individuals can present with the desire for the amputation of left, right or bilateral limbs (First 2005; McGeoch et al. 2011); conversely somatoparaphrenic patients typically manifest disownership sensations limited to one side of the body (Vallar & Ronchi 2009; Bottini et al. 2009).

Interestingly it has been shown in two patients presenting with BIID symptoms an increased skin conductance response (SCR) for noxious stimuli contacting the body segment below the line of desired amputation (Brang et al., 2008). Similarly, in the current study, we compared SCR to both noxious and neutral stimuli. Crucially, following the novel experimental paradigm, presented in the previous study, we presented approaching stimuli in two different contact conditions: in the real contact condition the stimuli really touched the body part; in the simulated contact, stimuli approached the body and stopped just before contacting, thus assessing anticipatory responses. In the first contact condition we expect to replicate the findings of Brang, corresponding to an increase in the SCR for the limb the patients desired to amputee (Brang et al., 2008); in the latter contact condition, conversely, we expect reduced SCR for the unwanted limb following the parallelism with somatoparaphrenia, i.e. the idea of an under-representation of that body part.

## **Materials and Methods**

### *Subjects*

8 volunteers presenting with Body Integrity Identity Disorder (BIID) took part in the study (one female, age= 29 – 53, education= 13 - 18), after giving their informed consent. They were all recruited at Centre for Cognitive Neuropsychology of Niguarda Ca' Granda Hospital in Milan and were part of a larger European study on BIID.

Patients received the first psychiatric screening evaluation at the university hospital of Zurich (Switzerland) and those who were fulfilling the BIID characteristics (Ryan et al.,

2010) were engaged for the study after giving their informed consent. The experiment was conducted according to the principles of Declaration of Helsinki (World Medical Organization, 1996) and was approved by the ethical committee of the Niguarda Hospital.

	Age	education	gender	Limb to remove	side
<b>p1</b>	42	13	Male	leg	left
<b>p2</b>	29	18	Male	leg	right
<b>p3</b>	36	13	Male	leg	left
<b>p4</b>	43	18	Female	arm	left
<b>p5</b>	36	13	Male	leg	left
<b>p6</b>	45	18	Male	leg	left
<b>p7</b>	29	13	Male	leg	left
<b>p8</b>	53	18	Male	arm	left

**Tab. 3.1.** Main demographical and clinical features of patients.

### *Stimuli*

A series of 64 mechanical stimuli were administered to each patient in a single session and simultaneously the SCR was recorded. The entire session took around 30 minutes.

Following the paradigm designed in the previous study, two types of stimuli were used: noxious stimuli (delivered through a needle) and neutral stimuli (delivered through a cotton swab) (Cheng et al., 2007; Forgiarini et al., 2011; Höfle et al., 2012). All participants flawlessly distinguished the needle from the cotton swab by both visual inspection and touch.

Patients presenting with BIID are able to clearly indicate the exact line of the desired amputation, thus we identified a single symmetrical site on both legs or hands (depending on the desired amputation) that was below the desired line of the amputation of the unwilling limb. The two patients desiring arm amputation indicated the line above the elbow, thus the stimulation spot was identified on the dorsum of the hand for both patients. The other six, who wanted lower limb amputation, always indicated the knee or above it, so the lateral part of the calf was the target of stimulation for all of them.

Critically, the stimuli were administered under two conditions: real and simulated. In the real condition the needle and the cotton swab touched the skin on the identified spot for about 0.5 seconds. In the simulated condition the stimulus approached the same area of

the skin, but stopped at a distance of approximately 0.5 cm from the skin where the stimulus stayed still for about 0.5 seconds and then retracted.

Stimuli were alternatively delivered to the right and left limb. Globally, eight different stimulation conditions were used: Painful Real Right, Painful Real Left, Painful Simulated Right, Painful Simulated Left, Neutral Real Right, Neutral Real Left, Neutral Simulated Right, Neutral Simulated Left. The 64 stimuli were divided into 8 independent blocks of 8 stimuli each (1 per condition).

### *Setting and procedures*

Patients were comfortably laying on a medical bad in front of the experimenter and they were resting. Patients were then asked to relax, remain as still as possible and keep a regular breathing, while gazing at the at the point where stimuli emerged from the bed surface and became visible to the patient, namely close to the feet. Patients who desired upper limb amputation were tested following the same setup and procedure of Experiment 1.2 (see Exp.1.2 for the details), thus they were sitting at a table with both hands on it with the palm facing down, and the experimenter in front of them.

On each trial a stimulus was presented by the experimenter who was trained to use the same trajectory on each stimulation. Stimuli emerged from below the bed (or the opaque screen in the table setup) and unpredictably approached one of the patient's legs (or hand if the desired amputation was for the upper limb). Patients were instructed to gaze at the stimuli for the whole trajectory.

### *SCR apparatus*

Skin Conductance Response was recorded, following standard guidelines (Dawson et al. 2007), with the same hardware, parameters set, and software of Experiment 1.2.

### *Data pre-processing*

The peak-to-base measure (Breimhorst et al., 2011; Ehrsson 2007; Lykken & Venables, 1971; Rhudy et al., 2010; Rhudy et al. 2007) was computed for each trial as the difference between the maximum value detected in a 6-second post-stimulus time window and the baseline calculated as the average value of a 0.3 second pre-stimulus time window.

Triggers coding for the stimulus type were manually sent to the SCR trace through the computer keyboard at the moment when the stimulus became visible to the patients.

The peak-to-base measures were then normalized within-subject and converted in Z-scores (Rhudy et al., 2007; Rhudy et al., 2008; Williams & Rhudy, 2009; Rhudy et al., 2010), to reduce the effect of the well-known large inter-subject variability of SCR (Lykken & Venables, 1971; Fowles et al., 1981) and also to reduce the effect of stimulations on different body districts in different patients.

#### *Data analysis*

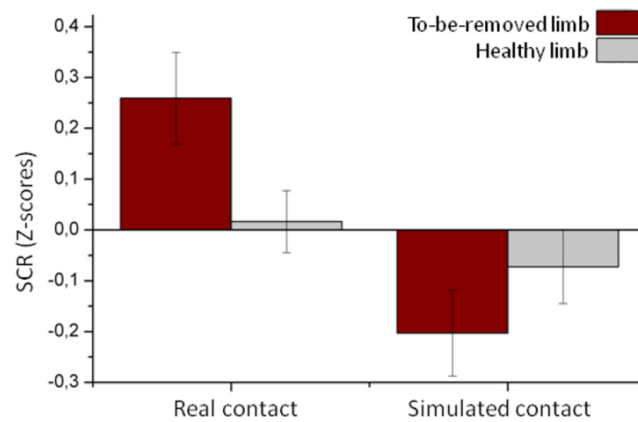
Data were analyzed with SPSS 21 (IBM® SPSS® Chicago, Illinois) and STATISTICA 6.0 (StatSoft, Italy, <http://www.statsoft.it>). A General Linear Model was used on SCR data, factoring: Stimulus (painful/neutral), Contact (real/simulated), and side (to-be-removed limb / healthy limb), as within subject factors. This resulted in a 2\*2\*2 repeated measures ANOVA design. Achieved power and effect size, measured with the partial eta squared ( $\eta^2$ ) have been reported.

### **Results**

The ANOVA showed a significant main effect of stimulus ( $F(1,7)= 73.985$   $p<.001$ ,  $\eta^2=.914$ , power $>.999$ ; painful $= .329 \pm .04$ , neutral $= -.329 \pm .04$ ), the interaction between stimulus and contact ( $F(1,7)= 5.741$   $p<.05$ ,  $\eta^2=.451$ , power $=.541$ ; painful real $= .6 \pm .14$ , painful simulated $= .06 \pm .12$ , neutral real $= -.324 \pm .06$ , neutral simulated $= -.334 \pm .06$ ) and, critical to our purpose, the interaction between contact and side ( $F(1,7)= 20.983$   $p<.01$ ,  $\eta^2=.749$ , power $=.973$ ; real to-be-removed $= .259 \pm .09$ , real healthy $= .016 \pm .06$ , simulated to-be-removed $= -.203 \pm .08$ , simulated healthy $= -.073 \pm .07$ ). The main effects side ( $F(1,7)= 1.988$   $p=.201$ ,  $\eta^2=.221$ , power $=.231$ ), contact ( $F(1,7)= 3.622$   $p=.1$ ,  $\eta^2=.341$ , power $=.376$ ) and the other interactions were not significant.

Interestingly Fisher post-hoc analysis on the critical significant interaction side by contact showed that simulated stimulations induced larger SCR on the healthy side than the to-be-

removed ( $p = .05$ ), conversely when the stimuli actually touched the limb, the SCR was stronger on the to-be-removed side than the healthy ( $p < .01$ ).



**Fig.3.1** Columns represents the mean standardized SCR, thin bars indicate standard errors. Red columns represent data for stimuli directed toward the to-be-removed limb, while in gray are represented responses to stimuli directed toward the healthy limb.

## Discussion

BIID is a striking condition where people feel a limb as foreign, the feeling is so strong that they desire to remove that healthy limb, despite it holds intact on the sensory and motor aspects. Individuals affected by this condition are fully aware that it is an unusual condition and that the amputation would have serious consequences (First, 2005; Sedda, 2011).

BIID is an extreme example of body representation disruption because of the serious consequences and the ethical issues implied by this clinical condition (Müller, 2009; Sedda, 2011). In this population we studied the role of the feeling of ownership for the anticipatory alert response to noxious sensory stimuli approaching the body. Eight patients presenting with BIID symptoms were recruited from the University Hospital of Zurich (Switzerland), and the experiment presented here is part of a larger study on BIID, these patients were administered with the pain anticipation paradigm. Our working hypothesis was based on a parallelism with somatoparaphrenia, hypothesizing that that BIID is caused by a deficit in body representation (Berti, 2013), and more specifically in

the feeling of ownership (de Vignemont, 2011). Akin to patients with somatoparaphrenia, the working hypothesis was that decreased sense of ownership for the affected limb would result in reduced anticipatory responses to threatening stimuli approaching that limb.

Congruently we found that the SCR to stimuli approaching, but not contacting, the unwanted limb was smaller than when stimuli approached the healthy side, suggesting for a diminished anticipatory response for stimuli on the affected side; conversely when stimuli actually touched the body the SCR was stronger for the contact with the to-be-removed limb than the other, in line with previous findings (Brang et al., 2008). Such an increased arousal could be interpreted as an altered processing of sensory stimuli.

Somatosensory system in BIID patients could be altered (Brang et al., 2008), but not disrupted as people are sensitive for stimuli on the to-be-removed limb. Our findings suggest that the altered response to noxious stimuli contacting the body was not due to a distorted somatosensory response per se, rather it is depending on the ability to anticipate the sensory consequences for stimuli directed to the body part they want to remove. This is compatible with the idea that BIID symptoms would be caused by an underrepresentation of the limb they want to remove, so penetrating in the body representation, that would impact the feeling of ownership (de Vignemont, 2011). The feeling of ownership was defined as a primitive sensation that a body part belongs to oneself, and was differentiated from the judgment of ownership and even beliefs (de Vignemont, 2007). It has been proposed that the feeling of ownership is an exclusive sensation for biological body parts (de Vignemont, 2007), while judgment of ownership and beliefs could be induced by means of bodily illusions (de Vignemont, 2011).

The strong disownership felt by BIID patients would produce, in turn, the fail to anticipate physiological responses to incoming noxious stimuli properly, despite the limb is an healthy limb with a preserved somatosensation; so the noxious stimulation, when really contacted the limb, would be processed without preparation, generating a stronger alertness reaction. In other words, the lack of anticipation impact the response when

stimuli actually contacted the body, possibly inducing a feeling of surprise or disorganized response that was recorded finally as an increased SCR.

Congruently with this hypothesis it has been shown that expectations and preparation to the noxious stimuli can interact with the subsequent processing of the painful stimuli (Brown et al., 2008a; Brown et al., 2008b; Porro et al., 2002) (see also Study 4 results).

The results we found confirmed the parallelism with somatoparaphrenia especially for the idea that an integer body representation is necessary to anticipate properly the sensory consequences of incoming stimuli. However the current results go even further into the discussion about the concept of ownership. Indeed BIID patients have the sensation that the limb is like an alien body part, but still they believe and judge that body part as a part of their body. This peculiar aspect differentiates BIID patients from people with somatoparaphrenia. By using the taxonomy of de Vignemont (2011), BIID patients have a preserved judgment of ownership but they do not have the feeling of ownership, conversely in somatoparaphrenia both the feeling and the judgment of ownership are compromised, given that the core deficit of somatoparaphrenia is the disownership sensation for one's own limb and the explicit attribution of the impaired arm to someone else. Our results strongly witness that the feeling of ownership, and not the mere judgement of ownership, critically determines the ability to anticipate incoming sensory stimuli.

The emotional reaction following the threatening of a body part is strictly dependant from the feeling of ownership felt over that body part. This is reminiscent of the results in healthy people with the RHI paradigm, where a stronger alertness response is usually associated with the degree of ownership rated by participants for the fake hand (Armél & Ramachandran, 2003; Ehrsson et al., 2007; Guterstam et al., 2011). Thus while questionnaires surely reflect the explicit judgment of ownership felt for the rubber hand (Botvinick & Cohen, 1998; Longo et al., 2008), the emotional activation might be an index of the more deep feeling of ownership felt for the fake body part.



It is still unquestionable that the disrupted sense of ownership reported by patients for their biological body part is stronger, and thus more informative, than any artificial embodiment sensation for external objects that can be induced in healthy participants (de Vignemont, 2011). However, it is also true that inducing a stronger sense of ownership, and not merely a judgment of ownership for extracorporeal objects, could be an important challenge for experimental studies, for example those assessing the compliance to functional prostheses.



## **- Study 4 -**

# **The visual size of one's own hand modulates pain anticipation and perception.**

### **Introduction**

Pain is a complex sensation, that seems to emerge from the co-activation of a complex network in the brain, which comprises a large neural network brain areas related to primary discriminative-somatosensory analysis, namely S1 and S2 as well as associative multimodal areas including the posterior parietal cortex, anterior insula and anterior cingulate cortex (ACC) (Iannetti & Mouraux, 2010; Price, 2000).

Pain has been proved to be modulated through a large range of experimental manipulations (Gallace, et al., 2011; Longo, et al., 2009; Brown & Jones, 2010; Rhudy, et al., 2010; Babiloni et al., 2008; Brown & Jones, 2008; Porro et al., 2002; Avenanti, et al., 2010; Forgiarini, et al., 2011).

Notably to our purpose pain experience has been shown to be modulated also by vision (Longo, et al., 2012; Longo et al., 2009). In particular, the distortion of the visual feedback relative to the body part affected by pain can strongly modulate painful sensations and has been proposed as a candidate for the reduction of pain in clinical conditions (Moseley, et al., 2008; Ramachandran, et al., 2009). However the results of such a sensory distortion are still controversial. While in some work the magnification of the visual size of a hand targeted by a painful stimulus has increased the level of perceived pain (Moseley et al., 2008) in other cases this has led to pain reduction (Mancini, et al., 2011b). Furthermore, the neurophysiological underpinnings of such modulations are still to be clarified.

In the current study we sought for further evidence about the effect of visual bodily distortion on subjective pain experience as well as the physiological correlates of such distortion. The working hypothesis was that the vision of an enlarged body part may increase the preparation of the sensory system to the consequence of the incoming painful

stimulus, leading to subsequent analgesia. To this aim we designed an experimental paradigm where we measured the anticipatory physiological response of participants exposed to an incoming harmful stimulus, as well as the somatosensory response when the stimulus eventually touches the skin. Therefore, Skin Conductance Response (SCR) was recorded following the application of painful stimuli – i.e. a needle (Cheng et al., 2007; Forgiarini et al., 2011) - that touched the hand or simply approached it without contacting, according to the experimental procedure set up in Study 1. In the former situation we expected, at baseline, a response, due to the sensory reaction to the nociceptive stimulation, while In the latter we expected a smaller response only due to the affective/cognitive anticipatory response to pain (Clark et al., 2008). Critically these measures were taken both under real-size or distorted vision of the participant's hand, in order to measure the effect of visual distortion on the anticipatory and sensory aspects of pain processing.

Moreover, in separate experiments, we assessed the explicit experience of pain in terms of intensity and unpleasantness under the same circumstances of visual distortion.

## **Experiment 4.1 – Pain processing modulation through the vision of an enlarged hand**

### ***Materials and Methods***

#### *Subjects:*

38 healthy participants, recruited among the students attending the Università degli Studi di Milano-Bicocca took part in the Experiment 4.1 after giving their informed consent and received course credits for their participation. The experimental protocol was explained in detail, but the participants were blind to the purpose of the experiment. The experiment was conducted according to the principles of the Declaration of Helsinki ("Declaration of Helsinki," 1996). A total of 18 (14 females, mean age=24.09 s.d.=2.25) naïve subjects took part in Experiment 4.1a; 20 different volunteers took part in the Experiment 4.1b (14 females, mean age=23.85 s.d.=1.84).

### Experiment 4.1a (Big Hand – SCR)

#### *Somatosensory Stimuli*

A painful stimulus was delivered using a needle with a blunt end (Cheng et al., 2007; Forgiarini et al., 2011; Höfle, et al., 2012).

The stimulus could be administered in two contact conditions (Factor: Contact): real or simulated following the same procedure of Experiment 1.2. Stimuli were either delivered to the right and left hand, thus four different conditions were available: Painful Real Right, Painful Real Left, Painful Simulated Right, Painful Simulated Left.

#### *Visual distortion of the hands*

Participants saw one of their hands, either the right hand, for half of the participants, or the left hand, for the other half, through a transparent screen, with the exclusion of the fingertips which were shielded from view by a small opaque cardboard screen. The contralateral hand was seen through a lens with a magnification factor of 2X, again with the exclusion of the fingertips, which were invisible. With this arrangement one hand resulted in a visual magnification while the other one was perceived at normal size.

#### *SCR Hardware and Software*

The hardware, parameters set, and software of Experiment 1.2 was used to collect Skin Conductance Responses following standard guidelines (Dawson et al. 2007).

#### *Experimental Procedure:*

Participants sat comfortably at a table with the experimenter sitting in front of them. They were asked to put both hands on the table with the palm facing up under either the transparent screen or the distorting lens. Each trial started with participants gazing at the fixation point placed at the center of a 40-cm tall vertical opaque board placed at 50 cm distance in front of them. A trained experimenter delivered the stimulus to one of the two hands (Factor: Hand), by approaching it with a smooth, continuous movement. Painful real and simulated stimuli emerged, in random sequence, from behind the opaque board and participants were instructed to gaze at them along their entire trajectory. The stimuli remained always visible except for the last 3 cm of their trajectory. With this

arrangement, the final contact between the hand and the needle was invisible in order to avoid any distortion of the visual stimulus occurring in the case this passed below the magnifying lens.

A total of 32 painful stimuli were administered to the participants in a single session, while the Skin Conductance Response (SCR) was recorded.

The 32 stimuli were divided into 8 independent blocks of 4 stimuli each (1 per condition); the stimuli were randomized within each block. A pause was introduced after 4 blocks, or at the end of any block if the volunteer asked for a rest. The entire session took around 30 minutes.

#### *Data pre-processing:*

The SCR peak-to-base measure (Breimhorst et al., 2011; Lykken & Venables, 1971; Rhudy et al., 2010) was computed for each trial as in Experiment 1.2.

Manual markers identifying each stimulus type were added to the SCR trace by the computer keyboard, at the moment that the stimulus became visible to the participant.

#### *Data analysis*

Data were analyzed with STATISTICA 6.0 (StatSoft, Italy, <http://www.statsoft.it>).

The analysis consisted in a 2\*2 repeated-measures ANOVA conducted on the SCR data with the factors: stimulus contact (real/simulated) and hand (big/normal), in order to assess the effect of visual distortion on the anticipatory and somatosensory stages of pain processing.

Significance level was set at  $p < .05$ , Fisher's LSD post hoc test was used to explore significant interaction.

#### *Experiment 4.1b (Big Hand – Pain Ratings)*

The experimental procedure was the same of Experiment 4.1a, except for the following differences. As in this case we were interested in the explicit pain ratings reported by the participants under different visual feedback conditions, only the real contact stimulations were administered following the same procedure of Experiment 4.1a.

### *Pain Ratings*

We investigated the subjective experience of pain by asking participants to judge the intensity and the unpleasantness of each stimulation, separately. Participants answered to each question through a Visual Analogue Scale (VAS) ranging from 0 to 100 mm where 0 indicated “no pain” and 100 corresponded to “the worst imaginable pain” (Longo et al., 2009).

### *Data analysis*

The data from each question underwent an intra-subject standardization by means of an ipsatization procedure, in order to neutralize the effect of response set (Broughton & Wasef, 1990; Cattell, 1944). Ipsatization transformed questionnaire ratings in Z-scores with a normal distribution, allowing a proper use of parametric tests (Broughton & Wasef, 1990; Cattell, 1944). The repeated measures ANOVA was conducted on ipsatized values of painful stimuli resulting in a 2\*2 within subject design factoring scale (intensity/unpleasantness) and hand (big/normal).

Significance level was set at  $p < .05$ , as in Experiment 4.1a for an eventual significant interaction Fisher LSD test was used for post-hoc comparisons.

## **Results**

### *Exp 4.1a Skin Conductance Response*

The ANOVA conducted on the SCR to painful stimuli revealed significant differences for the main factor stimulus contact ( $F(1,17) = 32.368$ ,  $p < .001$ ) with a larger response for real ( $.71\mu s \pm .1$ ) than for simulated ( $.48\mu s \pm .08$ ) painful stimuli, while the main factor hand, was not statistically significant ( $F(1,17) = .288$   $p = .598$ ). Furthermore, and critical to our aim, there was a significant interaction for contact\*hand ( $F(1,17) = 11.911$   $p \leq .01$ ).

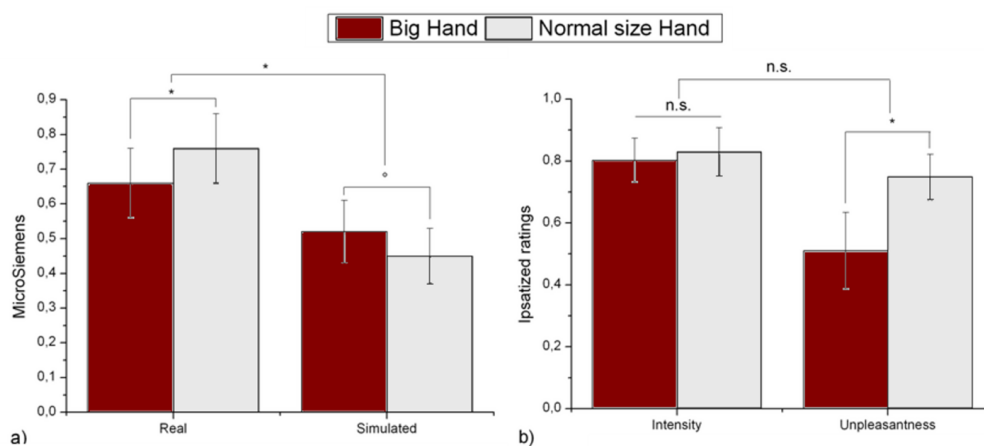
The post-hoc tests revealed that the real contact stimulation had a smaller SCR in the visually distorted hand ( $p = .009$ ; Big =  $.66\mu s \pm .1$ , Normal =  $.76\mu s \pm .1$ ). By contrast an opposite trend was observed for the simulated contact conditions ( $p = .066$ ; Big =  $.52\mu s$

$\pm .09$ , Normal =  $.45 \mu s \pm .08$ ). In this case, the vision of an enlarged hand induced a stronger response than the normal size hand.

#### Exp 4.1b VAS Questionnaire

The ANOVA on pain ratings did not show any significant main effect (scale:  $F(1,19) = 2.899$   $p = .105$ ; hand:  $F(1,19) = 1.741$   $p = .203$ ), while the hand\*scale interaction was significant ( $F(1,19) = 4.279$   $p \leq .05$ ).

Post hoc tests showed that the unpleasantness scale rating for the big hand stimulation ( $.51 \pm .12$ ) was significantly lower than the other three ratings (all  $p < .01$ ; unpleasantness scale-normal size =  $.75 \pm .07$ , intensity scale-big size =  $.8 \pm .07$ , intensity scale-normal size =  $.83 \pm .08$ ), suggesting that our participants referred a less unpleasant experience for the stimulation delivered on the enlarged hand, despite they reported a similar pain intensity for both hands.



**Fig.4.1** Columns represent the mean responses to painful stimuli, thin bars indicate standard errors, asterisks highlight significant differences. a) Experiment 4.1a results, columns represent mean SCR. b) Experiment 4.1b results, columns indicate average ipsatized pain ratings.

## **Experiment 4.2 - Pain processing modulation through the vision of a shrunken hand**

### ***Materials and Methods***

#### *Subjects:*

38 new healthy participants were recruited from students attending the Università degli Studi di Milano-Bicocca, took part in this experiment, after giving their informed consent



and received course credits for their participation. The experimental protocol was explained in detail, but the participants were blind to the purpose of the experiment. The experiment was conducted according to the principles of the Declaration of Helsinki (“Declaration of Helsinki,” 1996). 18 new participants (12 females, mean age=23.77 s.d.=3.77) took part in experiment 2a; another different sample of 20 participants took part in the experiment 2b (16 females, mean age=25.05 s.d.=5.93).

#### Experiment 4.2a (Small Hand – SCR)

The experimental procedure, data processing and analysis were the same as Experiment 4.1a except for the type of visual distortion introduced. Here the visual feedback from one hand was systematically minified (instead of magnified) with a 2X factor.

To achieve the desired visual distortion a wooden structure was crafted, which held a reverse telescope (Moseley et al., 2008) on one side and a very similar cylinder with neutral lens on the other one. As in Experiment 4.1a, the arrangement allowed the participant to see both hands through the cylinders, one minified and one with normal size, with the exception of the fingertips that were not visible. Either the left or right hand was visually distorted, following a counterbalanced order among participants.

#### Experiment 4.2b (Small Hand – Pain Ratings)

The experimental procedure, data processing and analysis were the same as experiment 4.1b except for the type of visual distortion, now consisting in a minified view.

## **Results**

#### Exp 4.2a Skin Conductance Response

In the ANOVA, a significant difference was found for the main factor stimulus contact ( $F(1,17)= 16.381$   $p< .001$ ) with the real contact stimulus inducing a larger response ( $.70\mu s \pm .1$ ) than the simulated pinprick ( $.58\mu s \pm .1$ ). The main factor hand was also significant ( $F(1,17)= 4.708$   $p= .044$ ) with the small hand eliciting an overall increased SCR ( $.67\mu s \pm .1$ ), than the normal size hand ( $.62\mu s \pm .1$ ). Differently from experiment 4.1a, and

crucially, the interaction between the two factors was not significant ( $F(1,17)= 1.085$   $p=.312$ ).

#### Exp 4.2b VAS Questionnaire

The ANOVA showed a main effect of the scale factor ( $F(1,19)= 6.69$   $p\leq .01$ ; intensity =  $.82 \pm .04$ , unpleasantness =  $.59 \pm .09$ ); while the other main factor hand ( $F(1,19)= .49$   $p=.492$ ) and the hand\*scale interaction ( $F(1,19)= 2.332$   $p=.143$ ) were not significant.

### **Discussion**

In the present study we investigated how pain processing can be modulated by the ongoing visual distortion of one's hand targeted by a harmful stimulus. In particular we searched for physiological and behavioral effects of visual magnification or shrinking of the participant's hand receiving a visible, acute noxious stimulus, at different stages of pain experience, namely the anticipatory and the nociceptive response.

Our crucial interest was the modulation of pain processing and experience following the visual distortion of one's own hand. While many previous studies on pain processing used to investigate only the response to noxious stimuli by means of explicit pain ratings (Brown et al., 2008a; Longo et al., 2009; Mailis, 1996; Moseley et al., 2008; Ramachandran et al., 2009) or by the assessment of subjective pain thresholds (Hänsel, et al., 2011; Mancini et al., 2011b), here we evaluated both the conscious pain experience and the physiological, autonomic responses to noxious stimuli. In addition, the paradigm used led us to differentiate the cognitive/affective contribution from the global response to painful stimulation, in order to shed light on the physiological underpinnings of the effect of visual body distortion on different level of pain processing.

Specifically, in Experiment 4.1a we found a reduced SCR for the real contact stimulation delivered on the visually magnified hand. This reduced physiological response suggests that the visual magnification of the body can reduce the sensory response to a noxious stimulus, in line with recent findings by Mancini and colleagues, (Mancini et al., 2011b), who showed that the increased visual size of one's own hand can lead to an increased

pain threshold. However, our results go beyond previous findings by showing that the reduced SCR to real contact noxious stimuli, in the condition of visual magnification, is coupled with an increased anticipatory SCR when the stimuli approach the skin without touching it. We hypothesized that looking at a visually increased body part might increase the sensory analysis of the visual scene. In line with this view, it is known that a higher SCR can be found in response to enhanced attentional (Babiloni et al., 2004) and cognitive (Critchley, et al., 2000; Critchley, 2005) load; congruently, it is known that also the processing of tactile non-painful stimuli are modulated by the vision of one's own hand (Longo & Sadibolova, 2013), increasing the sensitivity in presence of a visual magnification of the stimulated hand (Kennett, et al., 2001; Pavani & Zampini, 2007). The larger anticipatory arousal response to the approaching painful stimuli could then mediate the subsequent effect observed when the noxious stimulus eventually contacts the body. Different mechanisms, at present still speculative, could be responsible of this effect: on one hand the anticipatory response may induce the activation of endogenous analgesic descending neural pathways (Fields, et al., 2006), thus producing a reduced sensory input at subcortical level, following the stimulation. On the other hand, the expectation of the incoming painful stimulus may induce a pre-activation of early somatosensory regions with a subsequent modulation of painful sensation (Porro et al., 2002). Finally, a better preliminary visual analysis in the anticipatory phase may increase the expectancy relative to the forthcoming sensory experience of pain, but then decrease the general saliency, and thus somatosensory response, of the subsequent painful stimulation (Brown et al., 2008a; Iannetti & Mouraux, 2010; Legrain, et al., 2011; Rhudy et al., 2008).

Whatever the mechanism, it is noteworthy that a different pattern of SCR was found in Experiment 4.2a, where a minified vision of the hand was provided: here we found a general increase of SCR for both real and simulated contact. In line with this results, Mancini and co-workers found opposite effects between visual enlargement and reduction of the stimulated hand in a pain threshold paradigm (Mancini et al., 2011b). The pattern

of results of Experiment 4.2a excludes that the reduced response to real contact stimuli, observed for the enlarged hand, was due to a generic effect of the visual distortion, such as a generic feeling of reduced ownership from the visually distorted hand (Moseley et al., 2008). Indeed, while both visual distortions produced an increased anticipatory response, the response to the real contact stimulation induced a reduced SCR with the magnified hand but an increase SCR with the minified hand.

The increased somatosensory response following visual reduction of hand size is likely to be due to a general feeling of uneasiness or non-familiarity induced by the vision of one's own body parts at a reduced size (e.g. see discussion in Moseley et al., 2008). Interestingly, at the behavioral level, the vision of a shrunk body part is less likely to induce perceptual illusions (de Vignemont, et al., 2005; Pavani & Zampini, 2007) as well as kinematic effects in visuo-motor tasks (Bernardi et al., 2013; Marino, et al., 2010). Overall, the brain might be less prone to use the visual information coming from the minified hand in order to adequately anticipate the incoming experience of pain.

Noteworthy, besides the specific reduction of SCR to painful stimuli following the visual magnification of the hand, the response to the pain ratings showed that the visual magnification of the hand selectively reduced the unpleasantness of the pain stimulation and not its perceived intensity, suggesting that the analgesic effect is more related to the affective/cognitive component of pain than to its strict sensory analysis. Although our results are in line with recent experimental findings (Mancini et al., 2011b), they are in sharp contrast with other previous outcomes showing analgesic effects induced by an opposite visual distortion of the body size (i.e. a reduction). In one patient with chronic phantom limb pain Ramachandran and colleagues (Ramachandran et al., 2009) were able to modulate the painful sensation by providing a distorted visual feedback through a Mirror Box apparatus (Ramachandran, et al., 1995; Ramachandran & Rogers-Ramachandran, 1996). With this technique, the intact hand is reflected on a parasagittal mirror thus providing an image compatible with that of the absent limb. Critically, the painful sensations were reduced to a higher degree following visual reduction than visual

magnification of the reflected image of the hand. In another study Moseley and co-workers (Moseley et al., 2008) found that a visual magnification of the hand produced an increased pain and swelling sensation in patients with chronic pain. The differences, between our study and those by Ramachandran and Moseley (Moseley et al., 2008; Ramachandran et al., 2009), might be explained in terms of experimental populations and protocols proposed, which comprised healthy participants, receiving acute painful stimulations in our study and patients affected by chronic pain in the above cited works. Different neural mechanisms are likely to underpin chronic and acute pain (Moseley, et al., 2005); moreover chronic pain induces long lasting modification of the body representation (Moseley et al., 2005; Moseley, 2005) that could, per se, change the patterns of response induced by distorted visual feedback from the body.

We suggest that the vision of an enlarged hand would favor the monitoring of any incoming somatosensory experience, including pain, as shown by the higher preparatory autonomic response to approaching harmful stimuli. Such a greater anticipation would result in a less pronounced response once the harmful stimulus actually touches the skin.

By contrast, if the body part is visually reduced, the observer merely shows a generally increased level of arousal during the stimulation. These novel finding contribute to uncover the physiological and behavioural effects of multisensory body representation for pain processing and suggest that the manipulation of the former could be strategically used to modulate the latter in clinical populations.



## **- STUDY 5 -**

### **Illusory self-identification with an avatar reduces automatic pain responses.**

#### **Introduction**

Bodily self-consciousness is not considered anymore a unitary inviolable concept. Recent experimental evidences suggest that it is rather a result of multisensory bodily signal integration in the brain. In the rubber hand illusion (RHI), the visuo-tactile congruent stimulation of one's own hidden hand and a visible anatomical compatible fake hand induces the sensation that the prosthetic limb belongs to oneself (Botvinick & Cohen, 1998; Tsakiris, 2010). Similarly, the full body illusion (FBI) can be induced: thus congruent visuo-tactile stimulation at the trunk can induce self-identification and self-location changes with respect to a virtual or fake body (Aspell, et al., 2009; Ehrsson, 2007; Lenggenhager et al., 2007; Petkova et al., 2011).

Previous works have shown that looking at one's own body, but not to an object or at another person's body, while receiving a painful stimulus, produces analgesic effects (Longo, et al., 2012; Longo et al., 2009). Starting from this observation, we aimed at investigating the relationship between pain processing and body ownership, here we sought for evidence that reduced responses to nociceptive stimuli can be obtained not only by looking at one's own biological body (Longo et al., 2009), but also when looking at another person's body or avatar, under conditions of illusory self-identification with the virtual body. Thus, we asked whether changes in illusory self-identification following the induction of the FBI would be associated with a reduction of pain responses.

In two experiments, we combined robotic stimulation and virtual reality technology in order to induce the FBI (Duenas et al., 2011; Ionta et al., 2011; Pfeiffer et al., 2013). We then investigated the response to acute noxious stimuli delivered to the participant's hand, through the recording of the SCR (Armel & Ramachandran, 2003; Forgiarini, et al., 2011; Guterstam, et al., 2011). Since the response to a noxious stimulus starts before skin

contact, as a consequence of anticipatory evaluation of the sensory consequence to the approaching stimulus (Clark, et al., 2008), we also studied the modulation of such an anticipatory response to pain following FBI. We induced the FBI by manipulating the congruency of visuo-tactile stroking between the virtual body and participants' own body (Stroking factor) and we manipulated whether the volunteers saw a virtual body or a control picture on their head-mounted display (visual feedback configuration factor).

### **Exp. 5.1 – Full Body Illusion (FBI) reduces SCR to painful stimuli.**

#### **Materials and Methods**

##### *Participants*

Fourteen right-handed healthy volunteers took part in Experiment 5.1 (Mean Age  $\pm$  Standard Deviation:  $24.87 \pm 2.82$  years; 3 females). All participants had normal vision and were naive to the purpose of the experiment. All participants gave their written informed consent before the inclusion in the study. The study was approved by the local ethics committee — La Commission d’Ethique de la Recherche Clinique de la Faculté et de Médecine de l’Université de Lausanne — and was conducted in accordance with the ethical standards of the “Declaration of Helsinki” (World Medical Organization, 1996).

##### *Experimental Setup*

The experiment was conducted in a light-shielded room where a robotic device for tactile stroking was installed (Duenas et al., 2011). The robotic device had 200 cm x 90 cm x 10 cm dimensions and a soft foam cover that permitted participants to lie comfortably on their back. Stroking units were integrated in the robotic device that allowed to separately stroke the left and right upper back of participants. A stroking unit consisted of an ultrasonic motor (Shinsei, USR60-E3N, Japan, <http://www.shinsei-motor.com>) that actuated via a pinion-hole mechanism movable end parts on which a spring blade and a plastic sphere were mounted. Plastic spheres reached through gaps in the foam cover of the robotic device to touch the upper back of a participant and via the spring blades adapted to the curvature of participants' back during stroking.



Visual stimuli were presented on a head-mounted display (Virtual Realities, Virtual Viewer 3D, Houston Texas, [www.vrealities.com/virtualviewer3d.html](http://www.vrealities.com/virtualviewer3d.html)) with 800 x 600 pixel resolution and 35 degrees of visual angle. On earphones white noise was presented to participants in order to prevent them from hearing acoustic cues from the robotic stroking.

A serial keypad (Targus Numeric Keypad AKP10US, Anaheim CA, [www.targus.com](http://www.targus.com)) was used to record participants' button press responses, which were given with participant's right hand. In-house software (ExpyVR, Lausanne Switzerland, <http://lnc0.epfl.ch/expyvr>) was used for visual and acoustic stimulus presentation and recording of responses and LABview software (National Instruments Corporation, version 2010b, Austin Texas, [www.ni.com/labview](http://www.ni.com/labview)) was used for robotic device control.

### *Stimuli*

Tactile stroking by the robotic device was specified by pre-programmed stroking sequences. A total of four random sequences were created before the experiment with Matlab (MathWorks, version R13, Massachusetts US, <http://www.mathworks.ch>). These sequences specified the position of a stroking unit at 100 Hz sampling rate, within 0-20 cm distance range, and 2-12 cm/s velocity range. Within these limits, the four sequences had respectively random direction, timing, relative position, and speed.

The head-mounted display showed an image of a human body (male or female, according to participant's gender) wearing a white t-shirt and blue jeans against a gray background (virtual body, Fig. 5.1a) or a white rectangle, as a control condition (virtual object, Fig. 5.1b). The virtual body held a prone posture and was seen in bird's eye view (as in Pfeiffer et al., 2013).

### *SCR device*

The ActiveTwo Biosemi system (ActiveTwo, BioSemi B.V., Amsterdam, Netherlands) was used as signal amplifier with specific GSR sensors consisting of 2 passive Nihon Kohden electrodes. The sensors were applied on the distal phalanx of the index and middle finger of the left hand, while the two references electrodes were applied to the left

forearm. A saline conductive paste was applied to the electrodes, in order to improve the signal-to-noise ratio.

Data were digitalized on a dedicated computer through optic connection with a sample rate of 2048 Hz and then data were re-sampled offline at 200 Hz.

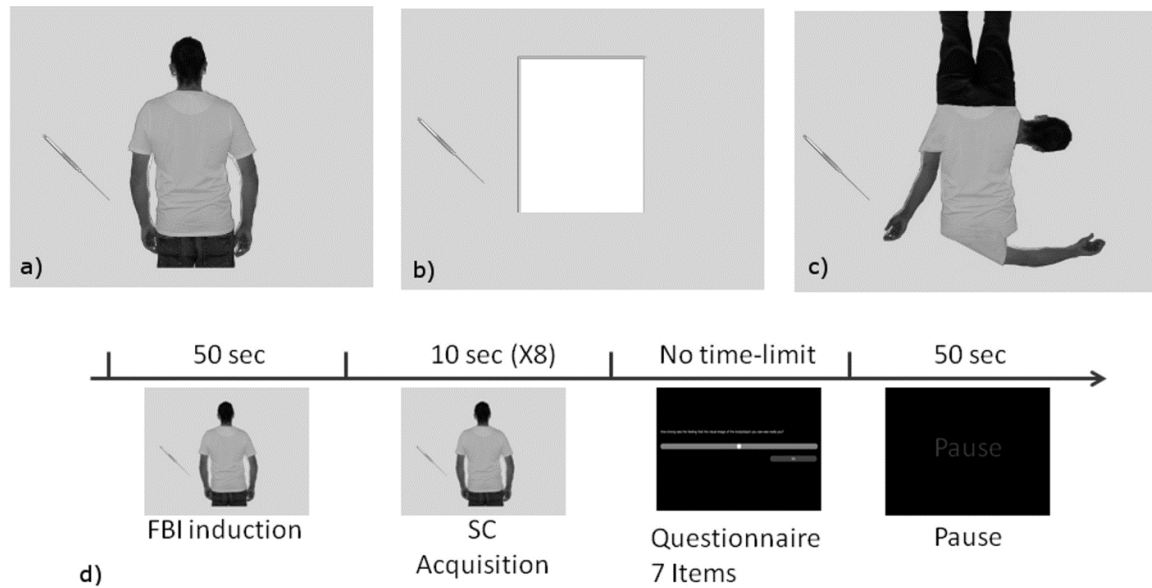
### *Procedure*

An experimental run consisted of an FBI-induction phase, followed by a pain-stimulation phase, questionnaire ratings, and a resting period.

The FBI-induction phase consisted of 50 sec visuo-tactile stroking in synchronous or asynchronous fashion (stroking factor) seen on an avatar or object (visual feedback factor).

A total of 8 trials were presented during the pain-stimulation phase. A trial began by visually presenting a needle that moved toward the body/object eventually contacting the target (“virtual puncture”) lasting 5 sec and was followed by a fixed interstimulus interval of 5 sec after which the next trial was presented. During the pain-stimulation phase visuo-tactile stroking was continuously presented. The picture of a big static needle was displayed on the left side of the virtual body/object during either the induction phase and the stimulation phase. For half of the trials, the biological left hand of the participant was hit with a needle synchronously with the contact of the virtual needle with the visual target. This corresponded to the real contact condition. In the other half of trials the participant did not receive any stimulation during the vision of the virtual pinprick. This condition (simulated contact) was used to assess the presence of any SCR response to the vision of the noxious stimulus hitting the target, in the absence of any somatosensory painful stimulation.

This procedure resulted in 8 different conditions: body congruent real; body congruent simulated; body incongruent real; body incongruent simulated; object congruent real; object congruent simulated; object incongruent real; object incongruent simulated. After the pain-stimulation phase, the questionnaire was administered; 50 seconds of rest separated the different condition runs.



**Fig. 5.1:** Visual stimuli and procedure. (a) A human body image stimulus seen from the back was used in both experiments. (b) A control object stimulus was a white rectangle and used in Experiment 5.1, and (c) a scrambled body stimulus was used as a control for the body stimulus in Experiment 5.2. (d) Sequence of events for an experimental run of the full-body illusion, starting with visuo-tactile stroking for 50 sec, followed by 8 painful stimuli with SCR acquisition for 80 sec, followed by questionnaire ratings and a resting period of 50 sec.

## Measures

### Skin Conductance

The Skin Conductance Level was recorded at DC level. An off-line digital high pass filter set at 0.05 Hz was applied to obtain phasic Skin Conductance Responses (Andreassi, 2000). This filter is effective to get back at level 0 the SCR after 1-3 seconds post-peak and highlights the event related responses in the skin conductance signal. The maximum amplitude of the SCR was used as measure of autonomous nervous system responses. For each trial, the maximum amplitude recorded in the time window of 7 seconds starting with the initial movement of the needle was extracted. The measures were intra-subject normalized (Rhudy, et al., 2010; Rhudy, et al., 2008; Williams & Rhudy, 2009) in order to obtain comparable measures among the participants, given the well known large inter-subject variability of SCR (Fowles et al., 1981; Lykken & Venables, 1971). In addition the mean skin conductance level (SCL) during each condition was calculated to evaluate the basal sympathetic tone (Nagai, et al., 2004).

### *Questionnaire ratings.*

During each condition run and immediately after the pain-stimulation phase, participants were asked to complete a questionnaire comprising items adapted from previous studies on bodily illusions (Botvinick & Cohen, 1998; Lenggenhager et al., 2009; Lenggenhager et al., 2007) and pain (Longo et al., 2009) (Tab. 5. 1).

<b>Question:</b>	
<b>Q1</b>	<i>How strong was the feeling that the visual image of the body/object you saw was really you? (SELF-IDENTIFICATION)</i>
<b>Q2</b>	<i>How strong was the feeling that you were drifting downwards or upwards? (SELF-LOCATION).</i>
<b>Q3</b>	<i>How Strong was the feeling that you could control the movement of the body you saw? (AGENCY OVER THE VISUAL OBJECT)</i>
<b>Q4</b>	<i>How strong was the feeling that you cannot move your own body? (LOSS OF AGENCY)</i>
<b>Q5</b>	<i>How strong was the feeling that you had more than two bodies? (CONTROL QUESTION)</i>
<b>Q6</b>	<i>How much intense was the pain inflicted by the needle? (PAIN INTENSITY)</i>
<b>Q7</b>	<i>How much unpleasant was the needle stimulation? (PAIN UNPLEASANTNESS)</i>

**Tab. 5.1: questionnaire items.** From Q1 to Q4, questions inquiring about bodily illusory sensations, Q6 and Q7 asking for explicit pain experience, while Q5 is a control question. Questions sequence were fully randomized and under computer control.

Responses were given through a 7 points visual analogue scale (VAS), and were coded by the experimental software with a score ranging from -3 to +3.

Participants were asked to move the cursor along a horizontal axes by pressing buttons with the index and ring fingers (left/right movement) with their right hand, while they confirmed their choice pressing the button in the center with the middle finger. The random sequences of the questions were under computer control, as the random sequences of the conditions.

The data from each question underwent an intra-subject standardization by means of an ipsatization procedure, in order to neutralize the effect in responses set (Broughton & Wase, 1990; Cattell, 1944). Specifically, each rating was subtracted by the mean rating of the subject responses in all questions and conditions and then divided by the standard deviation of subject's responses in all questions and conditions.

### *Data analysis*

Data were analyzed with STATISTICA 6.0 (StatSoft, Italy, <http://www.statsoft.it>) and G\*Power 3.1 (<http://www.psych.uni-duesseldorf.de/abteilungen/aap/gpower3/>).

A repeated measure ANOVA was run on SCR data in a 2\*2\*2 within subject design. The main factors were: visual feedback configuration (body/ object); stroking (congruent/ incongruent) and stimulus contact (real/ simulated). When a significant effect was found the  $\eta^2$  effect size, and the achieved power were computed.

Ipsatization transformed questionnaire ratings in Z-scores with a normal distribution, allowing a proper use of parametric tests on questionnaire data (Broughton & Wasel, 1990; Cattell, 1944). Separated repeated measures ANOVAs were conducted for each different question on ipsatized values and on SCL data with a 2\*2 within subject design factoring visual feedback configuration (body/object) and stroking (congruent/incongruent).

Significance level was set at  $p < .05$ , when a significant interaction was detected, post-hoc analysis were conducted with Fisher LSD test.

In addition, the Pearson's  $r$  correlation was calculated between questionnaire responses and SCR.

## **Results**

### *Skin Conductance*

The ANOVA showed a main effect of contact ( $F(1,13) = 20.589$   $p < .001$ ,  $\eta^2 = .613$ , power = .987) and an interaction between visual feedback and stroking ( $F(1,13) = 6.111$   $p < .05$ ,  $\eta^2 = .320$ , power = .942). The other main factors and interactions were not significant. Fisher's post-hoc tests showed that the real contact ( $Z = .3 \pm .08$ ) induced a greater SCR than the simulated contact ( $Z = -.4 \pm .08$ ). The interaction between visual feedback and stroking notably showed that, during the visual feedback of the body, the SCR was lower for congruent versus incongruent stroking while for the object visual feedback the trend was in the opposite direction. Post-hoc testing revealed that the body congruent condition

( $Z = -.28 \pm .05$ ) had different responses from body incongruent ( $Z = .03 \pm .09$ ;  $p = .026$ ) and object congruent conditions ( $Z = .08 \pm .13$ ;  $p = .013$ ). At the same time the other three conditions did not show statistically significant differences in any direct comparison.

The ANOVA on SCL did not show any significant effect nor for the main effects (visual feedback:  $F(1,13) = 1.288$ ,  $p = .277$ ; stroking:  $F(1,13) = 3.049$ ,  $p = .104$ ), neither for their interaction ( $F(1,13) = 1.765$ ,  $p = .207$ ).

### *Questionnaire*

The analysis of self-identification ratings (Q1) showed a main effect of visual feedback ( $F(1,13) = 15.81$ ,  $p < .001$ ,  $\eta^2 = .549$ , power = .956) but no main effect of stroking and no visual feedback x stroking interaction. Thus, self-identification was rated significantly higher in the body ( $Z = .91 \pm .26$ ) conditions than in the object conditions ( $Z = -.22 \pm .23$ ).

The ANOVA for self-location ratings (Q2) showed a visual feedback x stroking interaction ( $F(1,13) = 5.29$ ,  $p < .05$ ,  $\eta^2 = .29$ , power = .904). Post hoc comparisons showed that values were lower in the object incongruent condition ( $Z = -1.79 \pm .39$ ) than in the other three conditions (Body Congruent =  $-1.28 \pm .54$ ; Body Incongruent =  $-1.28 \pm .49$ ; Object Congruent =  $-.86 \pm .51$ ), which were at the same level (all  $p \leq .05$ ). There was no main effect of visual feedback and no main effect of stroking for self-location ratings (Q2).

Questions about agency (Q3 and Q4), the control question (Q5), and questions about pain experience (Q6 and Q7) revealed no significant main effects and no interactions. The absence of significant differences for the questions about pain experience (Q6 and Q7) suggests that our experimental manipulation did not result in consciously reportable effects on pain experience.

### *Correlation analysis*

Correlation analysis showed a significant negative correlation between self-identification ratings (Q1) and real contact SCR ( $r = -.27$ ,  $p < .05$ ), that is, a high degree of self-identification was associated with low SCR. None of the other questions showed significant correlations with the implicit measures.

## **Exp. 5.2 – Only anatomical avatars are effective to induce FBI and SCR reduction.**

### **Materials and Methods**

#### *Participants*

Sixteen right-handed healthy volunteers participated (Age  $\pm$  SD: 23.56  $\pm$  2.50 years, 4 females), who had not participated in Experiment 5.1 and were thus naïve to the purpose of the experiment. All participants had normal vision and gave their written informed consent before the inclusion in the study.

#### *Procedures*

The materials, methods, procedures, and analysis were the same as in Experiment 5.1, except for the following differences.

In Experiment 5.2 we investigated whether any visual effect of self-identification on SCR needed the visual observation of a realistic, anatomically intact body. To this purpose we presented participants with either a virtual human body (similar to Experiment 5.1), or with a scrambled version of the same body, shaped with anatomically impossible limb configuration. More precisely, the trunk of the virtual body was presented at the center of the image, similarly to the original image, and the other body segments were presented at incongruent positions.

The scrambled body image was created with GIMP software (GIMP 2.6.10; [www.gimp.org](http://www.gimp.org)) and was a modification of the avatar image, in such a way that we provided a unitary picture that could be processed also as a whole and not just as a fragmented summation of smaller figures. We named this condition scrambled body.

The experimental design resulted in a 2\*2\*2 within subjects design factoring: visual body configuration (anatomical / scrambled body), stroking (congruent / incongruent) and stimulus contact (real / simulated) for SCR data and a series of 2\*2 within subjects design factoring: visual body configuration (anatomical / scrambled body), stroking (congruent / incongruent) for SCL and questionnaire.

## Results

### *Skin Conductance*

The ANOVA showed a main effect of contact ( $F(1,15)= 68.15$   $p< .001$ ,  $\eta^2= .819$ , power $> .999$ ) and a main effect of visual feedback ( $F(1,15)= 34.91$   $p<.001$   $\eta^2= .699$  power $> .999$ ) moreover the interaction between visual feedback and stroking factors was significant ( $F(1,15)= 6.46$   $p< .05$ ,  $\eta^2= .301$ , power $= .95$ ) as well as the interaction between visual feedback and contact ( $F(1,15)= 11.22$   $p< .01$ ,  $\eta^2= .428$ , power $= .997$ ). The main factor stroking ( $F(1,15)= .417$   $p= .528$ ) and the other interactions were not significant.

The real contact ( $Z= .37 \pm .04$ ) induced a greater SCR than the simulated contact ( $Z=-.38 \pm .04$ ). The visual feedback main effect showed that independently from the stroking main effect, seeing a virtual body in anatomical configuration ( $Z=-.19 \pm .03$ ) induced lower SCR to painful stimuli than seeing a scrambled body ( $Z=.18 \pm .03$ ). The interaction between visual feedback and stroking factors, congruently with Experiment 5.1 and our prediction, showed that, using the anatomical configuration as a visual feedback, the SCR was lower for congruent than for incongruent stroking while for the scrambled body there was an opposite trend. Post hoc comparisons showed that the body congruent condition ( $Z=-.33 \pm .05$ ) differed significantly from scrambled congruent condition ( $Z=.31 \pm .09$ ,  $p<.001$ ) and scrambled incongruent ( $Z=.05 \pm .09$ ,  $p<.05$ ), moreover a difference close to significance level was found between anatomical body congruent and anatomical body incongruent ( $Z=-.05 \pm .06$ ,  $p=.08$ ) contrast.

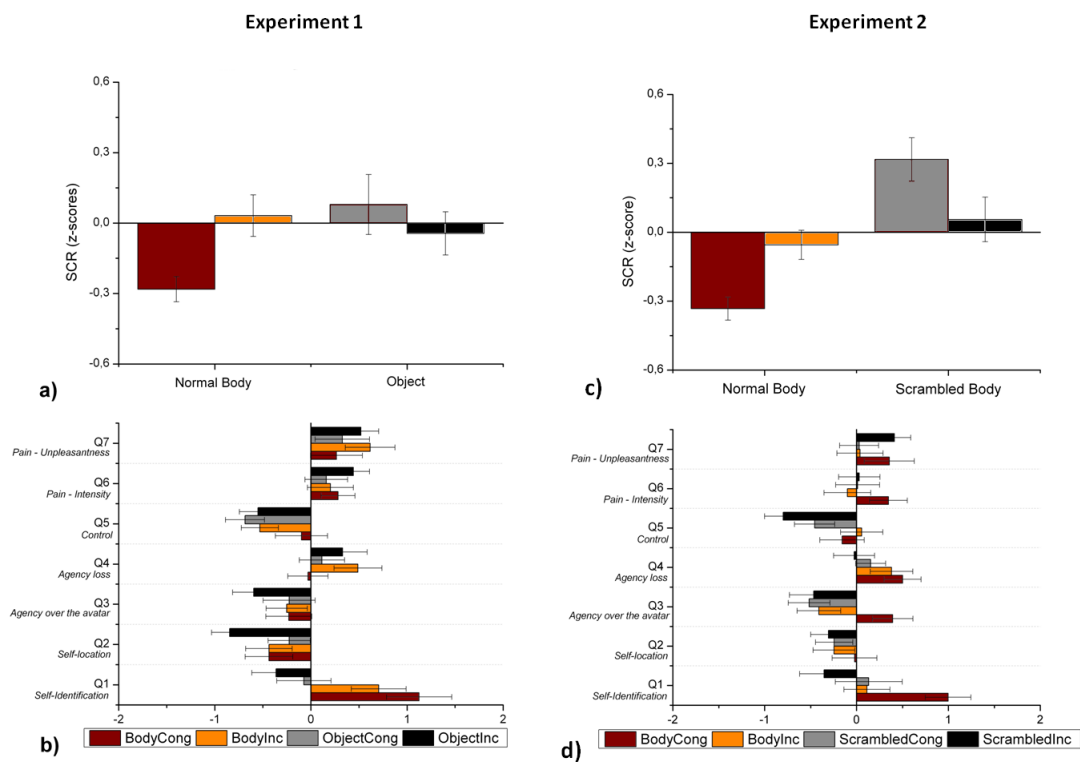
The post hoc analysis for the visual body configuration by contact interaction showed that the main effect of visual body configuration was driven by differences in the real contact conditions as the real touch during anatomical body conditions ( $Z= .06 \pm .07$ ) differed significantly from the real touch during scrambled body configurations ( $Z= .68 \pm .08$ ,  $p<.001$ ). Moreover, both were stronger than the simulated contact conditions (all  $p< .001$ ), which did not show significant difference for the two visual body configurations (simulated anatomical body  $= -.45 \pm .05$ , simulated scrambled body  $= -.31 \pm .06$ ;  $p= .178$ ).



Consistently with Exp. 5.1 the ANOVA on SCL did not show significant results nor for the main effects (visual feedback:  $F(1,15)= .647$ ,  $p= .434$ ; stroking:  $F(1,15)= .067$ ,  $p= .798$ ), neither for the interaction ( $F(1,15)= .096$ ,  $p= .761$ ).

### Questionnaire data

The ANOVA for self-identification ratings (Q1) showed significant main effects of visual body configuration ( $F(1,13)= 5.99$   $p< .05$ ,  $\eta^2= .285$ , power= .629) and stroking ( $F(1,13)= 5.04$   $p< .05$ ,  $\eta^2= .251$ , power= .556). There was no visual body configuration by stroking interaction.



**Fig.5.2.** In the first row SCR results from experiments 5.1 (a) and 5.2 (c), columns represent standardized mean SCR. In the second row questionnaires results from experiment 5.1 (b) and 5.2 (d), bars represent average ipsatized ratings. Thin bars indicate standard errors. In red are represented conditions of normal avatar stroked synchronously, in orange are represented normal bodies stroked in an incongruent fashion; in gray and black there are the control conditions of visual feedback stroked in a congruent way (gray) or incongruent (black), which were a white rectangle and a scrambled body in experiment 5.1 and 5.2 respectively.

The anatomical body ( $Z= .55 \pm .18$ ) conditions induced higher self-identification ratings than the scrambled body conditions ( $Z= -.11 \pm .26$ ). Moreover, the congruent stroking ( $Z=.56 \pm .24$ ) induced higher ratings compared to the incongruent ( $Z= -.12 \pm .22$ );

suggesting an additive, not interactive effect which identified the anatomical body congruent stroking condition as the one with the stronger effect of self-identification with the human picture.

Questions about self-location (Q2) and agency (Q3 and Q4) did not reveal any significant main effect and no interaction. The control question (Q5) showed a significant main effect of the visual feedback ( $F(1,13)= 10.23$   $p< .01$ ,  $\eta^2= .406$ , power= .997; body=  $-.05 \pm .19$ ; scrambled=  $-.63 \pm .18$ ). Consistently with Experiment 5.1, none of the questions about pain experience (Q6 and Q7) showed significant main effects or interactions.

### *Correlation*

Correlation analysis showed a significant negative correlation between self-identification ratings (Q1) and real contact SCR ( $r= -.31$ ,  $p< .05$ ). No other questionnaire items showed a significant correlation with the SCR.

## **Discussion**

In the present study we found that changes in self-identification with a virtual body modulate automatic responses to acute painful stimulations, as reflected by a decreased SCR. Although, external stimuli that evoke nociceptive afferent signals are a main contributor to pain experience, other internal factors take part to the genesis and the modulation of the pain experience, such as generically defined cognitive or affective components. Among these factors, it has been possible to differentiate affective-motivational components, such as emotions or meditation-induced states (Brown & Jones, 2010; Rhudy et al., 2010), from more cognitive factors, such as attention and expectations related to incoming stimuli features (Brown & Jones, 2008; Brown, et al., 2008a; Clark et al., 2008). Moreover, perceptual factors, such as proprioception (Gallace, et al., 2011) and the visual size of a body part play a role (Mancini, et al., 2011b).

Looking at one's own body, but not looking at a neutral object or at another person's body, has been reported to induce analgesia for acute painful stimulation (Longo et al., 2012; Longo et al., 2009). Here we sought for a similar modulation of pain responses

induced by the vision of a virtual body, that also depended upon the level of illusory self-identification with that body during a full-body illusion (Lenggenhager et al., 2007) induced by controlled robotic stimulations (Duenas et al., 2011; Ionta et al., 2011; Pfeiffer et al., 2013).

In the first experiment we compared automatic responses to acute painful stimulations while a virtual body was shown in back-view on an head mounted display. These responses were compared with those obtained when seeing a control neutral object (Armel & Ramachandran, 2003; Hänsel, et al., 2011; Hohwy & Paton, 2010; Lenggenhager et al., 2007; Tsakiris et al., 2010). Our results revealed that the SCR to painful stimuli decreased for real and simulated noxious stimuli, specifically under congruent visuo-tactile stimulation and when the body, but not the object, was seen.

This result was further qualified by the second experiment, where another control condition was added, consisting of a scrambled human figure, typically used in studies testing body and face perception (Hershler & Hochstein, 2005; Reed, et al., 2003; Reed, et al., 2006). The specific aim of Experiment 2 was to assess the importance of a realistic body configuration (Reed et al., 2003) for inducing the FBI and the reduction in the SCR to painful stimuli. The physiological responses to the applied pinpricks, both real and simulated, showed, consistently with Experiment 5.1, a selectively decreased SCR only when the body was shown in the anatomical configuration and under congruent visuo-tactile back stimulation. Moreover negative results from SCL analysis suggested that the changes in arousal responses that we observed were more related to the transient event related response, than a modification of an altered basal sympathetic tone (Nagai et al., 2004).

The present results add to previous findings on the modulation of pain experience by vision. Vision of noxious stimuli seen as approaching another person's body induces arousal responses in healthy humans (Forgiarini et al., 2011). It has been argued that such responses are mainly based on the cognitive evaluation of the approaching stimulus, which would produce an automatic anticipatory response (Clark et al., 2008; Ploghaus,

1999) that has been hypothesized to be mediated by emphatic sharing of the affective component of pain (Hein & Singer, 2008; Singer et al., 2004). Furthermore, previous works have reported that the vision of one's own stimulated body parts can modulate pain thresholds (Longo et al., 2009; Mancini et al., 2011b) or the rating of pain intensity (Longo et al., 2012), suggesting an analgesic effect when looking at one's own body. The present study goes beyond such previous results by linking the visual response to an incoming threat directed towards another person's body, to illusory self-identification experienced towards an observed virtual body.

We hypothesized that the increased self-identification, when seeing the virtual body stroked in a congruent fashion, would be reflected in changes in the processing of painful stimuli akin to those described during the direct observation of one's own body, typically consisting of a reduced response to painful stimuli (Longo et al., 2012; Longo et al., 2009; Mancini et al., 2011b).

Questionnaire data showed that higher self-identification was recorded with the avatar only when presented with an anatomically correct body configuration and when stroked congruently, according to the literature (Botvinick & Cohen, 1998; Ehrsson, et al., 2004; Guterstam et al., 2011; Lenggenhager et al., 2007; Tsakiris et al., 2010).

Further investigating the relationship between the self-identification and reduced SCR, we found that the overall correlation between the magnitude of illusory self-identification and the magnitude of SCR was significant. The correlation was negative in both experiments, sustaining that the more self-identification with the avatar our participants reported, the stronger was the reduction of the SCR for painful stimuli. Although in the correlation analysis all conditions were considered together, with the potential risk of an autocorrelation bias, it is noteworthy that illusory self-identification was the only item from a total of 7 items that showed the same negative correlation with SCR in experiments 5.1 and 5.2.

Our physiological SCR data thus corroborate earlier studies reporting elevated pain thresholds when seeing a body part or when self-identifying with a virtual body (Hänsel

et al., 2011; Longo et al., 2009), but also extend these data on visual analgesia described earlier. In particular, we here show that such visual analgesia is tuned by bodily self-consciousness and also modulates anticipatory levels of pain processing and thus is not strictly dependant on the somatosensory nociceptive input. However whereas these implicit pain data have recently been extended with explicit ratings of pain experience (Longo et al., 2012), we did not observe similar effects in explicit pain ratings in experiment 5.1 and 5.2. Consistently with our results, a recent study (conducted in two different laboratories) showed that explicit pain ratings for stimuli delivered to the biological hand did not change during the RHI (Mohan et al., 2012). It is worth noting that we assessed pain ratings only once for each condition during the questionnaire phase, and not on trial by trial basis after each stimulation, in order to reduce possible interference with the induced illusory state and Skin Conductance recording. However this procedure might weakened the confidence with pain ratings as several external confounding effects, like memory or other post-perceptual processes, might have interfered with the judgment of stimulations which was delayed.

It has also been proposed that the body is processed as a whole, as suggested by the reported advantage for a global processing of body pictures shown in an upright posture as compared to upside-down or non-anatomical postures (Bosbach, et al., 2006; Reed et al., 2003). In the current study we showed that in order to induce the FBI the picture of the avatar needs to be presented in its correct anatomical configuration. Although modulation of pain experience for an isolated body part was found when looking at the body part (Longo et al., 2009; Mancini et al., 2011b), the present data – comparing the observed effects for the full normal versus scrambled body - suggest that global bodily processing of a seen human body impacts self-identification and pain processing. Interestingly we proposed avatars that matched the gender of our participants, but still presented many differences from specific aspect of the volunteers as for example the hair style, or the skin colour; however it seems that was the anatomical configuration of the avatar that defined the possibility to increase the self-identification with it, and not its

actual similarity with participant's body, congruently with the finding that even an opposite gender avatar could induce embodiment effects (Slater, et al., 2010).

In conclusion we found that it is possible to reduce the implicit physiological response to acute painful stimuli throughout the full body illusion. This effect is already available during the anticipatory response to the incoming expected painful stimulation, it is related to the degree of self-identification with the stroked picture and would be achieved only for pictures of human body presented with a normal anatomical configuration. However this implicit physiological reduced response is not transferred to an aware reduced experience of pain.

## **- STUDY 6 -**

### **Visual size modulation of an embodied avatar impact SCR to painful stimuli.**

#### **Introduction**

Bodily self-consciousness has been proposed to comprise self-identification – the *sense of ownership* - (the experience that ‘I’ identify with a body), self-location (the experience of where ‘I’ am located), and a first-person perspective (from where ‘I’ experience the world), but does also relates to the sense of agency (the experience that ‘I’ am the agent causing ‘my’ actions) (Blanke & Metzinger, 2009; Blanke, 2012; Ferri, et al., 2012).

Bodily self-consciousness can be manipulated by means of several experimental paradigms (Botvinick & Cohen, 1998; Ehrsson, 2007; Newport & Gilpin, 2011; Romano, et al., 2013a; Tsakiris, 2010), critical to the purpose of this study in the full body illusion (FBI) congruent visuo-tactile stimulation of one’s own body and a mannequin can induce self-identification and self-location changes with respect to a virtual or fake body (Aspell, et al., 2009; Ehrsson, 2007; Lenggenhager et al., 2007; Petkova et al., 2011).

Also the pain experience has been proved to be modulated through a large range of experimental manipulations (Gallace, et al., 2011; Longo, et al., 2009; Brown & Jones, 2010; Rhudy, et al., 2010; Babiloni et al., 2008; Brown & Jones, 2008; Porro et al., 2002; Avenanti, et al., 2010; Forgiarini, et al., 2011).

Interestingly previous works have shown that looking at one’s own body, but not to an object or at another person’s body, while receiving a painful stimulus, produces analgesic effects (Longo, et al., 2012; Longo et al., 2009); congruently in the previous study (Study 5) we brought evidences for analgesic effects induced by the vision of another person body under the illusory state of the FBI, suggesting for the representation of the body as a mediator for the analgesic effects and not the vision of biological body itself. Moreover the distortion of the visual feedback relative to the body part affected by pain can strongly modulate painful sensations as the magnification of the visual size of a hand targeted by a

painful stimulus led to acute pain reduction (Mancini, et al., 2011b; see also Study 4 presented here).

In the current study we used virtual reality technology in order to induce the FBI in a first person perspective. We then investigated the response to acute noxious stimuli delivered to the participant's calf, through the recording of the SCR (Armel & Ramachandran, 2003; Forgiarini, et al., 2011; Guterstam, et al., 2011) and congruently with previous studies we also assessed the anticipatory response to pain (Brown et al., 2008a; Ploghaus, 1999).

Here we sought for replicating findings from Study 5 of a reduced responses to nociceptive stimuli when looking at an avatar, under conditions of illusory self-identification with the virtual body. Differently from Study 5, here we induced the FBI by congruent visuo-tactile stroking of the right leg that was seen in a first person perspective. Moreover we applied a visual modulation of avatar size, known to be effective for changing pain responses when applied on biological body, hypothesizing that, when the ownership for the avatar is increased, the same effect of visual size modulation as when looking at one's biological body, should be detected. Finally, in order to modulate the ownership sensation felt for the avatar, we manipulated the orientation of the virtual body that was provided in an anatomical (0° of rotation) or non-anatomical (90° rotated) position and in three different sizes, namely: normal body, big body (legs width increased of 30%), small body (legs width decreased of 30%); while the congruency of the visuo-tactile stroking was kept constantly synchronized.

## **Materials and Methods**

### *Participants*

21 right-handed healthy volunteers took part in the Experiment (Mean Age  $\pm$  Standard Deviation:  $22.95 \pm 1.96$  years; 9 females). All participants had normal vision and were naive to the purpose of the experiment. All participants gave their written informed consent before the inclusion in the study. The study was approved by the local ethics



committee — La Commission d’Ethique de la Recherche Clinique de la Faculté et de Médecine de l’Université de Lausanne — and was conducted in accordance with the ethical standards of the “Declaration of Helsinki” (World Medical Organization, 1996).

### *Experimental Setup*

The experiment was conducted in a light-shielded room. Visual stimuli were presented on a head-mounted display (Virtual Realities, Virtual Viewer 3D, Houston Texas, [www.vrealities.com/virtualviewer3d.html](http://www.vrealities.com/virtualviewer3d.html)) with 1280 x 1024 pixel resolution and 60 degrees of visual angle. On headphones white noise was presented to volunteers in order to prevent them from hearing acoustic cues from external environment.

A serial keypad (Targus Numeric Keypad AKP10US, Anaheim CA, [www.targus.com](http://www.targus.com)) was used to record participants’ responses to the questionnaire.

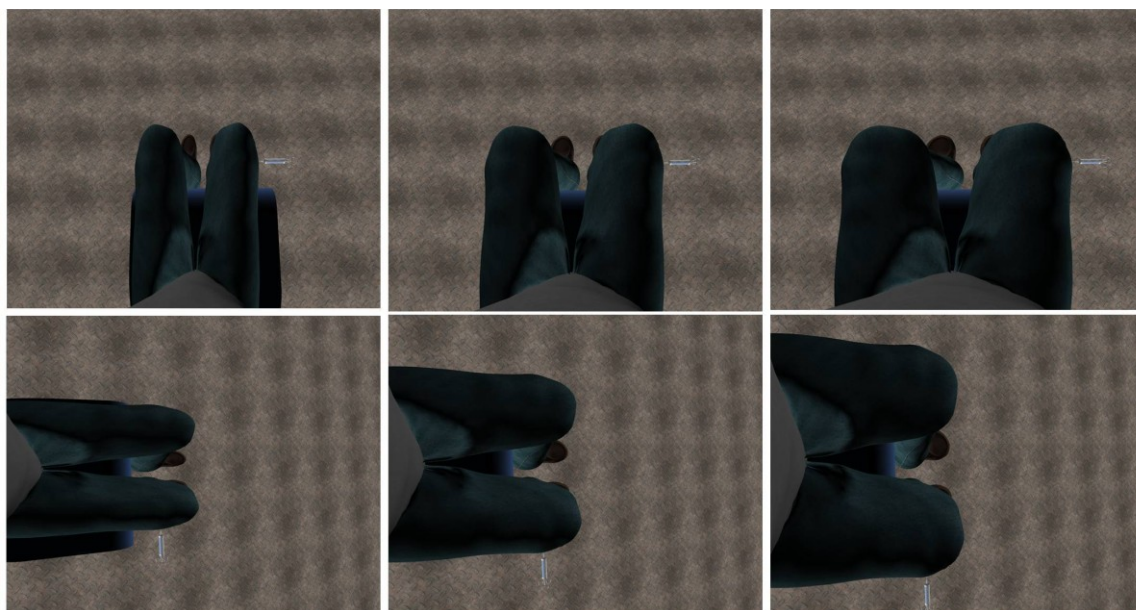
In-house software (ExpyVR, Lausanne Switzerland, <http://lnc0.epfl.ch/expyvr>) was used for visual stimuli presentation and recording ratings.

### *SCR hardware*

Skin Conductance data were acquired with the same system and parameters set for Study 5. The sensors were applied on the distal phalanx of the index and middle finger of the left hand, while the two reference electrodes were applied to the left forearm.

### *Visual Stimuli*

The head-mounted display showed an image of a human body, perceived in first person perspective, wearing a grey t-shirt and blue jeans. Critically the body was oriented in two possible orientation. In 0° orientation the virtual body was turned in such a way that when participants was looking down the saw the virtual legs in place of their own in an anatomical location. In 90° orientation the virtual body was rotated of ninety degrees on the left, in this condition when volunteers looked down they saw two legs that were not anatomically compatible with their own. Moreover a visual size modulation was introduced by increasing and reducing of 30% the width of the standard virtual body resulting in six possible visual conditions: small 0°, small 90°, normal 0°, normal 90°, big 0°, big 90°.



**Fig. 6.1:** In the first row there are the 0° rotated anatomical legs; in the second row there are the 90° rotated legs, resulting in a non-anatomical orientation. The three columns show the different sizes: reduced of 30% in the first, standard in the second, enlarged of 30% in the third.

### *Procedure*

An experimental run consisted of an induction phase, followed by a pain-stimulation phase, questionnaire ratings, and a resting period.

The illusory induction phase consisted of 90 sec visuo-tactile stroking always in synchronous fashion where participants saw a red dot scratching the right leg of the virtual body while a trained experimenter touched participants right leg with a wooden stick in a synchronous fashion (Lenggenhager et al., 2007; Pfeiffer et al., 2013).

Then 8 trials were presented during the pain-stimulation phase. A trial began by visually presenting a syringe moving toward the right virtual leg contacting the target (“virtual puncture”), lasting 3 sec, and was followed by a fixed interstimulus interval of 7 sec after which the next trial was presented. In four trials, the real right leg (lateral part of the calf) of the participant was hit with a needle synchronously with the contact of the virtual needle (real contact condition). In the other four trials the participant did not receive any stimulation during the vision of the virtual pinprick. This condition (simulated contact) was used to assess the anticipatory component of pain (Colloca et al., 2006; Hsieh et al., 1999; Ploghaus, 1999).

This procedure resulted in 12 different conditions: small 0° real, small 90° real, normal 0° real, normal 90° real, big 0° real, big 90° real; small 0° simulated, small 90° simulated, normal 0° simulated, normal 90° simulated, big 0° simulated, big 90° simulated. After the pain-stimulation phase, the questionnaire was administered; a rest of 90 seconds separated the different condition runs.

### ***Measures***

#### *Skin Conductance Response:*

The Skin Conductance Level was recorded at DC level. An off-line digital high pass filter, set at 0.05 Hz, was applied to obtain phasic Skin Conductance Responses (Andreassi, 2000). The SCR peak-to-peak measure (Bellodi et al., 2013; Benedek & Kaernbach, 2010; Tronstad et al., 2013) was then computed for each trial as the difference between the maximum and the minimum value detected in the time window of 7 seconds starting with the initial movement of the needle. The measures were intra-subject normalized (Rhudy, et al., 2010; Rhudy, et al., 2008; Williams & Rhudy, 2009).

#### *Questionnaire ratings.*

During each condition run, participants were asked to fulfill a questionnaire comprising items adapted from previous studies on bodily illusions (Botvinick & Cohen, 1998; Lenggenhager et al., 2009; Lenggenhager et al., 2007) and pain (Longo et al., 2009). The questions were the same of Study 5 (Tab. 5.1) except Q2 which was substituted by the item: “How strong was the feeling that you were in the location of the body you saw”. Moreover a question about perceived size of the virtual legs was added with the specific purpose of investigating the subjective judgments of the introduced visual size modification (Q8: “The size of the body you saw was the same as your body (left=smaller; right=bigger; middle=the same”). Responses were given through a 7 points visual analogue scale (VAS), and were automatically coded by the experimental software with a score ranging from -3 to +3. The random sequences of the questions were under computer control, as the random sequences of the conditions.

Data from each question underwent an intra-subject standardization by means of an ipsatization procedure, in order to neutralize the effect in responses set (Broughton & Wasef, 1990; Cattell, 1944).

#### *Data analysis*

Analysis were conducted with, STATISTICA 6.0 (StatSoft, Italy, <http://www.statsoft.it>), SPSS 21 (IBM® SPSS® Chicago, Illinois) and G\*Power 3.1 (<http://www.psych.uni-duesseldorf.de/abteilungen/aap/gpower3/>).

A repeated measure ANOVA was run on SCR data in a 2\*3\*2 within subject design. The main factors were: body orientation (0°/ 90°); size (small/ normal/ big) and stimulus contact (real/simulated). Effect size ( $\eta^2$ ), and achieved power have been reported.

Eight separated repeated measures ANOVAs were conducted for each different question on ipsatized values. Each ANOVA resulted in a 2\*3 within-subject design factoring body orientation (0°/ 90°) and size (small/ normal/ big).

Significance level was set at  $p < .05$ , when a significant interaction was detected, post-hoc analysis were conducted with Fisher LSD test.

As additional analysis we run a correlation analysis between the question about perceived size of the virtual legs and SCR dividing anatomical conditions (0° orientation) from the non-anatomical (90° rotated). We expected a negative correlation between size and SCR only when the avatar would have been embodied thus in 0° orientation. Two participants were excluded from SCR analysis due to technical problems during the signal acquisition.

## **Results**

### *Skin Conductance Response*

The ANOVA showed a main effect of contact ( $F(1,18) = 144$   $p < .001$ ,  $\eta^2 = .889$ , power  $> .999$ ; real =  $.337 \pm .04$ , simulated =  $-.466 \pm .03$ ) and orientation ( $F(1,18) = 11.168$   $p < .01$ ,  $\eta^2 = .383$ , power =  $.885$ ; 0° =  $-.164 \pm .03$ , 90° =  $.035 \pm .03$ ), importantly the interaction between orientation and size factors was significant ( $F(2,36) = 3.162$   $p \leq .05$ ,  $\eta^2 = .149$ , power =  $.861$ ). The main factor size ( $F(2,36) = 1.664$   $p = .2$ ,  $\eta^2 = .085$ , power =  $.327$ ) and the

other interactions were not significant. Fisher's post-hoc tests showed that the normal ( $-.285 \pm .05$ ) and big ( $-.279 \pm .05$ ) legs in  $0^\circ$  conditions were close to the same level ( $p = .957$ ) and statistically different from all other four conditions (all  $p \leq .01$ ; small  $0^\circ = .07 \pm .09$ , small  $90^\circ = .03 \pm .09$ , normal  $90^\circ = .007 \pm .1$ , big  $90^\circ = .064 \pm .1$ ).

### *Questionnaire*

The analysis of self-identification ratings (Q1) showed a main effect of orientation ( $F(1,20) = 8.554$   $p < .01$ ,  $\eta^2 = .299$ , power = .793;  $0^\circ = .14 \pm .12$ ,  $90^\circ = -.33 \pm .12$ ) but no main effect of size and any significant interaction.

The ANOVA for self-location ratings (Q2) showed a main effect of orientation ( $F(1,20) = 27$   $p < .001$ ,  $\eta^2 = .574$ , power = .998;  $0^\circ = .626 \pm .14$ ,  $90^\circ = -.512 \pm .15$ ) and a main effect of size ( $F(1,20) = 3.329$   $p < .05$ ,  $\eta^2 = .143$ , power = .598; small =  $.038 \pm .15$ , normal =  $.277 \pm .12$ , big =  $-.144 \pm .13$ ), and their interaction was not significant.

Questions about agency over the virtual body (Q3) highlighted a main effect of orientation ( $F(1,20) = 8.155$   $p < .01$ ,  $\eta^2 = .29$ , power = .776;  $0^\circ = .015 \pm .15$ ,  $90^\circ = -.489 \pm .12$ ), while the other main factor and the interaction were not significant.

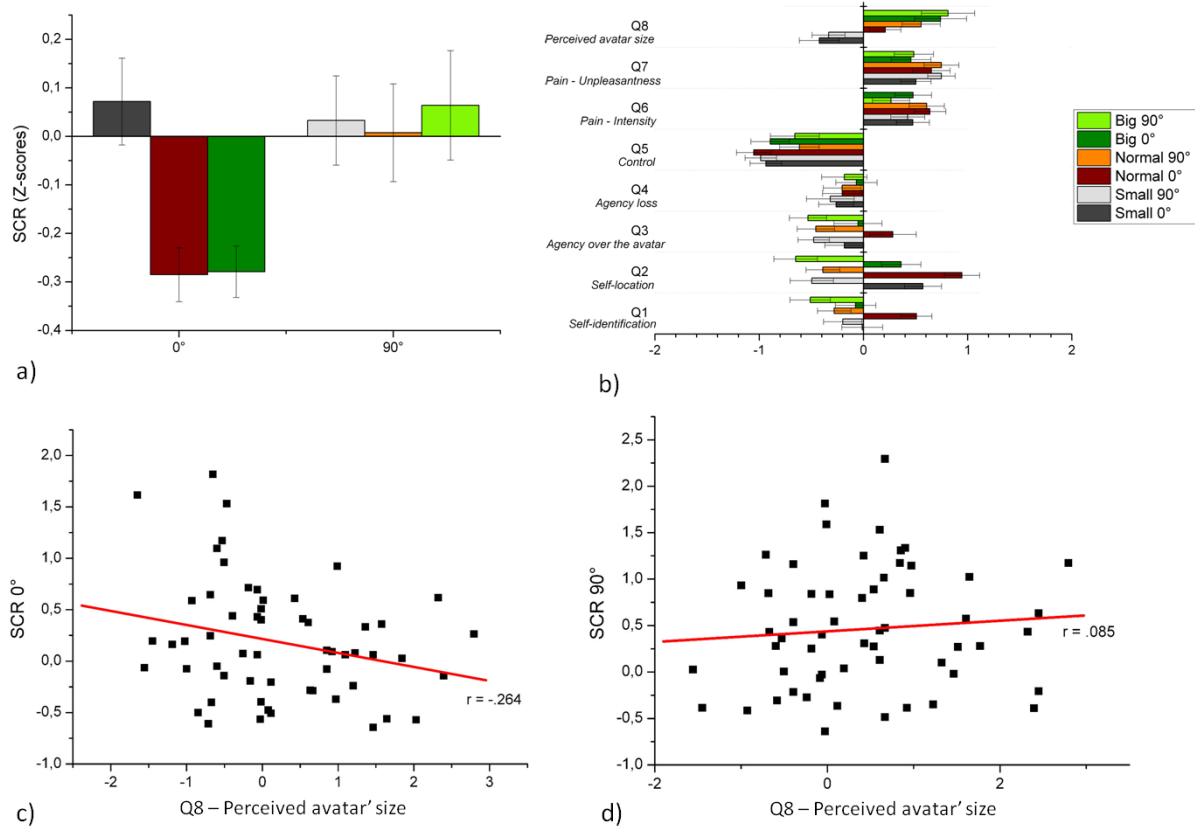
The question about perceived size (Q8) showed a significant main effect of factor size ( $F(1,20) = 13.95$   $p < .001$ ,  $\eta^2 = .398$ , power = .996; small =  $-.379 \pm .16$ , normal =  $.381 \pm .15$ , big =  $.776 \pm .23$ ), but no main effect of orientation neither a significant interaction. It is worth to note here that in Q8 positive values indicate that the virtual legs were perceived as bigger than biological legs, 0 means same size and negative values addressed for virtual narrow legs.

The question about the loss of agency (Q4), the control question (Q5), and questions about pain experience (Q6 and Q7) revealed no significant main effects and no interactions.

### *Correlation Analysis*

Correlation analysis showed a significant negative correlation between the rating of perceived size (Q8) and SCR ( $r = -.264$   $p < .05$ ) when the legs were in anatomical position

(0° conditions), conversely when the legs were rotated (90°) the correlation was null and not significant ( $r = .085$ ,  $p = .527$ ).



**Fig. 6.2. Study Results.** (a) Columns represent standardized average SCR; (b) bars represents ipsatized mean ratings. Thin bars indicate standard errors, colour legend is reported aside. The second row shows correlations between SCR and perceived avatar size in anatomical 0° rotated legs (c) and non-anatomical 90° rotated legs (d).

## Discussion

In this study we proposed a new version of the Full Body Illusion (FBI) where a virtual body was perceived from a first person perspective, in particular participants were wearing the head mounted display where the avatar was projected. In this condition when they looked downward in the direction of their own legs, they saw a pair of legs superimposing their biological limbs. The virtual body was presented in two possible orientation: one anatomical – i.e. the virtual legs corresponded in orientation with biological legs -, and one non-anatomical - i.e. rotated of 90° in such a way that the virtual legs crossed the position of the real legs -. Moreover we were interested at evaluating whether a change in the visual size of the avatar was effective for modulating

the responses to noxious stimuli in a similar way to the changes in SCR and ratings obtained with the visual size changes of one's biological hand, corresponding to an analgesic effect for enlarged body and no-effect or opposite effect for the shrunken body (see Study 4). In order to do so the avatar was provided in a standard size, enlarged and restricted. A main issue with this setup was that the virtual legs could not really match the real size of biological legs of any participants, thus the three sizes of the virtual body could be perceived very differently from each participant. In order to control for the subjective experience of the different sizes we introduced a specific item in the questionnaire where volunteers were asked to rate on a 7 points scale if they experienced the seen legs as large as their own, bigger or smaller than their biological legs.

The main hypothesis was that an increase in ownership could be induced only during the condition of anatomical legs orientation, in this condition the ownership experience should induce the visual analgesia (Longo et al., 2012; Longo, et al., 2009) in a similar way to the results observed in Study 5. Moreover only under condition of illusory ownership the virtual legs size should affect the processing of noxious stimuli boosting the analgesic effect with the bigger bodies (Mancini, et al., 2011b) and similar to the results observed in Study 4.

Our results were in line with our expectations for the legs orientation prediction as we found reduced SCR for painful stimulation when the legs were presented in anatomical orientation, congruently in the same conditions participants reported increased embodiment sensations for the virtual legs. Critically the effects of orientation, interacted with the size of the virtual legs, showing that significantly smaller SCR was detectable selectively for standard size legs and enlarged legs, but not for the small legs in the anatomical orientation. Notably the visual reduction of the virtual body size was not effective for reducing the SCR and is likely that the brain might be less prone to use the visual information coming from the shrunken body in order to modulate the processing of the incoming noxious stimulation.

Standard and enlarged legs reached the same level of SCR, thus results on visual size modulation were apparently only partially in line with our expectations, as the working hypothesis predicted that big legs should show smaller SCR than normal size. A possible explanation might be related to the real legs size that changes the degree of perceived enlargement for the virtual legs. Notably we suppose that the boosting effect of enlarged body on visual analgesia rely on body representation modulation, thus it should be related on how do participants perceive the avatar as enlarged or reduced. This hypothesis was corroborated by showing that, only under conditions of illusory self-identification with the avatar, the SCR to painful stimuli was negative correlated with the perceived size of the virtual legs. In other words the more a participant judged the virtual legs bigger than his own, the less the SCR to painful stimulation, but only when virtual legs were in anatomical orientation.

On the other hand when the avatar was not in an anatomical position, thus it is less likely to be embodied, such a relationship between perceived size of the legs and SCR to painful stimuli was not detectable.

The results presented so far show two main novel findings. First, the embodiment of virtual bodies in first person perspective induces reduced physiological response to noxious stimuli directed to the biological body. This finding is reminiscent of results obtained in Study 5 for the FBI in third person perspective and analgesic effects obtained for the vision of one's own real body (Longo et al., 2012; Longo, et al., 2009), suggesting again, with a slight different illusion than Study 5, that visual analgesia is mediated by the sense of ownership felt for the seen body. Moreover in a strict analogy with Study 5 the physiological reduced response was already recordable at anticipatory level, without recording significant modulation of explicit pain ratings. The second main finding is that the application of an experimental manipulation on an embodied avatar, that is known to be effective on biological body, induces similar effects on the processing of noxious stimuli than the real body manipulation. The second main point is converging with the



first sustaining once more that the analgesic effect of visual manipulation of one's own body is mediated by the representation of such body, and its ownership assignment.

Interestingly the correlation analysis suggests that the effectiveness of body enlargement is tuned by the size and it is not an all-or-nothing effect that can be switched on. Further studies might be interested on the limit of magnification for being effective, also considering that is known that also the processing of tactile non-painful stimuli are modulated by the vision of one's own hand (Longo & Sadibolova, 2013), and that the visual magnification of the stimulated hand increase the tactile sensitivity (Kennett, et al., 2001), induce stronger perceptual illusions (de Vignemont, et al., 2005; Pavani & Zampini, 2007) and changes kinematic patterns in visuo-motor tasks (Bernardi et al., 2013; Marino, et al., 2010).

In contrast with our expectations we did not find different responses in anticipatory phases than in real contact conditions. It is known that in the bodily illusion paradigms, like the RHI, threatening the fake hand induces an increase response in SCR (Armél & Ramachandran, 2003; Guterstam et al., 2011). Our simulated contact condition was very similar to the classic threatening condition of the RHI, thus we predicted similar results of increased SCR for conditions of illusory ownership, while we found the opposite.

A possible explanation referred to the specific context where this threat comes. Indeed in our study the menacing conditions were in a context where half of the trials were really touching the body thus, a pain experience was really available and expected. This contextual factor might change the way the participants responded to the virtual puncture, reacting to the virtual pinprick on the avatar as if their own body was in pain and they were looking at it, thus reducing the physiological response (Longo, et al., 2009). Despite in contrast with the classic literature (Armél & Ramachandran, 2003; Ehrsson, 2007; Guterstam et al., 2011), this controversial aspect might prove once more that the fake body was processed as their own during illusory conditions. Indeed when a real painful stimulation is not expected (in the classic RHI paradigm is usually specified that participants will not receive any painful stimulation) the threatening of embodied fake

hand is felt as an unexpected menace inducing stronger SCR as for unexpected and more salient stimulations (Brown et al., 2008a; Gläscher & Adolphs, 2003); while when real painful stimulations are expected the virtual pinprick is processed as directed to one's own body reducing the response to pain (Longo et al., 2012; Longo, et al., 2009).

## **- Study 7 -**

### **Dynamic expansion of alert responses to incoming painful stimuli following tool use**

#### **Introduction**

As discussed in the previous chapters, the ability to anticipate incoming threatening stimuli is a crucial adaptive function of living beings. It allows us to understand potentially dangerous situations, in order to carry out appropriate defensive behavior. This function is particularly relevant with respect to the coding of potentially noxious stimuli that are within, and/or rapidly moving toward the space surrounding our body (Graziano et al. 2002; Graziano & Cooke 2006), the so called peripersonal space (Rizzolatti et al. 1981a; Rizzolatti et al. 1981b). Several studies support the existence of such a mechanisms for body-related crossmodal integration in humans (Macaluso & Maravita 2010). For instance, the investigation of right brain-damaged (RBD) patients with left tactile extinction has provided convincing evidence for the existence of an integrated visuo-tactile representation of peripersonal space in humans. These patients can typically detect a single touch on the left or right hand in isolation, but they fail to report the contralesional, left-sided, touch when it is presented simultaneously with an ipsilesional, right-sided, stimulus of the same (Bender 1952) or different sensory modality (di Pellegrino et al. 1997; Mattingley et al. 1997; Làdavas et al. 1998; Farnè & Làdavas 2000). Crossmodal extinction of touch to the left hand by right vision is usually more pronounced when the right visual stimulus is presented at a short, as opposed to long distance from the right hand (Làdavas et al. 1998). However, after a brief period of training with a tool allowing to reach for objects in the space far from the body, crossmodal extinction emerges even for visual stimuli placed far from the body, but near the tip of the tool (Farnè & Làdavas 2000; Maravita et al. 2001; Farnè et al. 2007), suggesting an expansion of crossmodal visuo-tactile interactions to the far space.

Studies in healthy people using the Crossmodal Congruency Task (CCT) (Driver & Spence 1998a; Driver & Spence 1998b; Maravita et al. 2003; Spence et al. 2004a) provide further evidence for the efficacy of tool use for expanding crossmodal responses to the far space (Maravita et al. 2002; Holmes et al. 2004; Spence et al. 2004a; Holmes et al. 2007a; Macaluso & Maravita 2010).

The functional meaning of having such a peculiar representation of peripersonal space is likely due to its importance for object manipulation, but also for the avoidance of incoming threats.

Graziano and colleagues further characterized Hediger' claims of a "defensive flight zone" (1950) in non-human primates, by reporting avoidance behaviors in response to stimuli rapidly approaching the body or air puffs directed to single bodily regions (Graziano et al. 2002; Cooke & Graziano 2003).

Given the plasticity of peripersonal space for action, as shown in the case of tool use, the present work investigates whether also the boundaries of such a "safety barrier" may be dynamically modulated by tool use experience. Notwithstanding the critical importance of the defensive role assumed by the peripersonal space (Graziano et al., 2002; Graziano & Cooke 2006), this issue has not yet been investigated.

To this aim, we assessed the possibility for modulating the spatial pattern of the spatial organization of automatic responses to the vision of approaching noxious stimuli by measuring the Skin Conductance Response (SCR), following a training with a tool.

Here, we addressed the issue of whether SCR to incoming painful stimuli can be modulated by the expansion of peripersonal space boundaries that follows tool use. This would be an indication that the safety region surrounding our body has not a fixed extension, but can be plastically expanded following contingent experience, specifically adapting to the novel extension that peripersonal space acquires following the use of space-probing tools.

## **Exp. 7.1 - Increasing the alert response to incoming noxious stimuli presented far from the body by means of active training with a tool.**

### **Materials & methods**

#### *Participants*

Twelve right-handed participants took part in Experiment 7.1 (2 males, mean age: 24±6), after giving their informed consent. The study was conducted according to the principles of the Declaration of Helsinki (“Declaration of Helsinki,” 1996).

#### *Experimental procedure*

Stimulus type, presentation and SCR recording were identical to Experiment 1.3, but now stimuli were only presented along the horizontal axis, while participants passively held a tool during the task. The tool was a 45cm long wooden stick, with a diameter of 2.5cm. At the tip of the stick a 5cm long nail was placed, which made the tool useful to collect objects during the following training phase (see below). Stimulation conditions were identical to Experiment 1.3. However, since in Experiment 1.3 no difference was found between the two nearer (i.e., 1cm and 5cm) distances from the hand, now different spatial distance were used, in order to explore the effect of tool on SCR response in space near the hand (2cm), between the hand and the tool tip (20cm), and at the tip (40cm) of the tool. Eight blocks of stimuli were given, each one containing one stimulus per condition, for a total of 32 stimuli.

To assess the effect of active tool use on SCR to painful stimuli, participants performed the experimental task under two conditions, namely before (baseline) and after a motor training with a hand-held stick to act in the extrapersonal space (post-tool) (Farnè & Làdavas 2000; Maravita et al. 2002; Holmes et al. 2007a; Sposito et al. 2012).

#### *Tool training*

Four different tool use tasks were used during the training in order to have participants performing a prolonged tool training, while avoiding a decrease of sustained attention due to habituation to a single task (Sposito et al. 2012). All subjects performed the training with their right hand.

Task 1: A number of 15 polystyrene targets were placed at a distance of about 70 cm from the participant's body, holding a colored number clearly visible. On each trial, participants were instructed to pick one of the targets displaying a number written in the color named by the experimenter, using the nail fixed at the tool tip. In order to pick it up participants had to hit the center of a cross drawn on the top face of the target using the nail placed at the tool tip. Once they hooked the target they had to bring it close to the body, pick it up with the left hand and place it on a grid drawn on the table, at the spatial position displaying the same number. Participants were instructed to make a continuous, fluid movement and to place the arm back on the arm-rest placed on their right side, at the end of each trial.

Task 2: The procedure was similar to the previous task, but now, on each trial, participants were instructed to pick up the target objects displaying the number named by the experimenter and put them into one of two boxes placed on the table, depending on their odd/even status.

Task 3: Now the stick was used as a rake in order to retrieve the target objects. To this aim a 15 X 10 X 1cm plastic plate was fixed on the distal nail. The targets were placed close to the participant's body in a random order. Participants had to push the target cubes, starting from number 1, over a paper template, fixed on the table, displaying the numbers from 1 to 15 in a domino-like sequence. There was no time constraint, but participants were required to be as accurate as possible.

Task 4: During this task participants were blindfolded. The experimenter scattered the targets all over the table and participants were asked to explore the space in front of them, trying to retrieve the targets and move them close to their body midline, using the same rake-tool as in the previous task.

#### *Statistical analysis*

For each spatial position, we calculated the difference ( $\Delta$ ) in SCR before and after the training (i.e., Post-tool training minus Baseline values), with positive values indicating an increased SCR after the training. The  $\Delta$  SCR was then analyzed via a one-way repeated

measures ANOVA with distance (touch, near, middle, far) as main factor. Fisher post-hoc test was used to investigate direct comparisons and the partial Eta Squared ( $\eta^2$ ) was calculated as measure of effect size.

## **Results**

The ANOVA showed a significant effect of distance ( $F(3,33)= 4.568$ ,  $p< .01$   $\eta^2= .29$ ). Post-hoc comparisons showed no significant differences ( $p=.85$ ) between the  $\Delta$ SCR in the touch condition ( $-.16\pm.0 \mu s$ ) and the near (2cm) condition ( $-.14\pm.06\mu s$ ). Moreover, the  $\Delta$ SCR for the middle ( $20cm=.07\pm.08\mu s$ ) and the far ( $40cm=.1\pm.06\mu s$ ) conditions showed positive values, and they were significantly different from the touch (both  $p< .05$ ) and the near (both  $p< .05$ ) conditions.

## **Discussion**

Experiment 7.1 shows that the spatial pattern of SCR to noxious stimuli can be significantly altered by active tool use. After the training, there was a reduction of the SCR in responding to a menacing stimulus presented near or on the hand.

This result likely reflects the habituation that normally affects galvanic responses for constant stimulation (Levinson & Edelberg 1985; Elie & Guiheneuc 1990).

Conversely, there was an increase of the galvanic response in the post training session for the middle and far locations, even in spite of the habituation process; therefore, after active use of the tool, the alertness response expanded as to include the full length of the tool.

### **Exp. 7.2 – Is the tool presence necessary to increase alert responses?**

#### **Materials & methods**

##### *Participants*

Twelve naive participants took part at Experiment 7.2 (all right-handed, 5 males, mean age:  $25\pm 6$ ), giving their informed consent.

### *Experimental procedure*

The experimental procedure was the same of that of Experiment 7.1.

The only difference was that now, during the pre- and post-training sessions, participants did not hold the tool but simply kept their hand closed, as if they were grasping the tool.

### **Results**

Data were analyzed with the same statistical models used in Experiment 7.1. The ANOVA on  $\Delta\text{SCR}$  showed significant main effect of distance ( $F(3,33)= 6.025$ ,  $p< .01$ ,  $\eta^2= 0.35$ ): indeed  $\Delta\text{SCR}$  for Touch condition was significantly greater ( $\Delta\text{SCR} .21\pm.06\mu\text{s}$ ) as compared to all the other conditions (near=  $\Delta\text{SCR} .09\pm0.05\mu\text{s}$ ,  $p<.05$ ; medium=  $\Delta\text{SCR} .02\pm0.07\mu\text{s}$ ,  $p<.01$ ; far=  $\Delta\text{SCR} -.02\pm.03\mu\text{s}$ ,  $p<.001$ ) which did not differ from themselves (all  $p>.05$ ).

### **Discussion**

In the Exp. 7.2 we aimed at investigating whether the increased SCR to far stimuli induced by tool-use is related to the presence of the tool itself during the testing session, or it can emerge even after the removal of the tool.

Results suggest that the presence of the tool during the SCR assessment is crucial for the spatial remapping effects in the spatial positions distant from the hand. Indeed, SCR did not increase in far positions when the tool was removed, while a significant reduction of SCR responses was found in the touch condition.

### **Exp. 7.3 – Projection of attention or extension of peripersonal space? Comparing attentional deployment in far space with active tool use.**

#### **Materials & methods**

##### *Participants*

Twelve naïve, right-handed participants took part in Experiment 7.3 (5 males mean age  $25\pm6$ ), giving their informed consent.

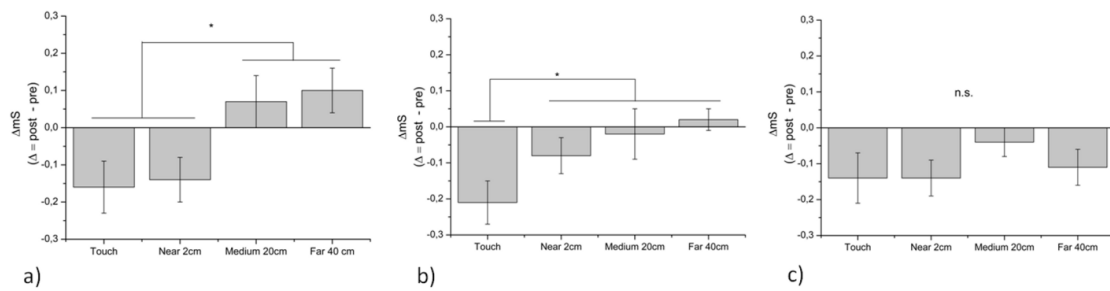


### Experimental procedure

The procedure was similar to that of Experiment 7.1, with the only difference that now the training did not require the use of the tool. Participants were blindfolded and held the tool passively. The experimenter displaced only 14 out of 15 cubes on the table, in random order, at about 40 cm away from the participant's hand. The participants were then un-blindfolded and asked to report which one of the cubes was missing, after silently reading the number labeling each single cube. This procedure was repeated for 15 minutes.

### Results

Data were analyzed with the same statistical model used in Experiment 7.1. The one-way repeated measures ANOVA with distance as factor was not significant ( $F(3,33)= 1.358$ ,  $p= .28$ ;  $\eta^2= .11$ ). Thus no difference between the two sessions was found as a function of distance of the noxious stimulus from the body in the far and in the near space, while only an overall decrease in SCR, likely due to habituation, was found ( $\Delta\text{SCR}$ : touch=  $-.14 \pm .2$   $\mu\text{s}$ , near=  $-.14 \pm .18$   $\mu\text{s}$ , middle=  $-.04 \pm .13$   $\mu\text{s}$ , Far=  $-.11 \pm .16$   $\mu\text{s}$ ).



**Fig. 7.1. Study Results** from experiments 7.1(a), 7.2 (b), and 7.3 (c). Columns represented mean difference in the peak to base index of the SCR between post and pre training measurements ( $\Delta\text{SCR}$ = post-pre), thin bars expressed standard errors and asterisks show significant differences.

### Discussion

The last experiment aimed at confirming that the spatial remapping of SCR depends on the active use of the tool during the training, rather than on a mere attentional effect due to the prolonged monitoring of far positions for task execution. Hence we recorded SCR

before and after the participants were submitted to an attentional training during which they passively held the tool but, critically, they did not use it. Results suggest that the spatial pattern of responses to approaching threatening stimuli is not modulated by an attentional task that required to monitor targets at far space.

## **General discussion**

The present work provides two main findings. First, it confirms that physiological, anticipatory responses to the sight of a threatening stimulus approaching the observer's body are affected by the distance of that stimulus from the body. Second, the spatial constraints of such anticipatory responses can be dynamically changed, following the use of a tool that extends action space. Here we also show that the spatial extension of the putative protective space is not rigid in humans, rather it can be efficiently expanded by active tool use. As shown in Experiment 7.1, after a tool training in the far space, the SCR in response to a threatening stimulus emerges even in response to extra-personal, far, stimuli. Notably, this effect emerges despite the rapid SCR habituation typically occurring following repeated stimulations (Levinson & Edelberg 1985; Elie & Guiheneuc 1990). Overall, these findings extend previous work showing the modulation of the response to non-painful visual stimuli in the peripersonal space, following tool use in monkeys (Iriki et al. 1996; Maravita & Iriki 2004) and in humans (Berti & Frassinetti 2000; Làdavas & Farnè 2004; Maravita & Iriki 2004). Recently, Longo and Lourenco (2006) reported that when performing a line bisection task, participants committed a rightward error which was progressively larger going from near (30cm) to far (120cm) space, suggesting the existence of a gradual shift of space representation from near to far space (Cowey et al., 1999). Critically, the spatial boundary between near and far space could be shifted more distally by the use of a long tool (Longo & Lourenco, 2006). This reshaping of visual spatial processing in peripersonal space has been shown to be paralleled by a change in the brain representation of the body metrics, compatible with an extension of perceived

arm length following tool-use (Cardinali et al. 2009b; Cardinali et al. 2011; Sposito et al. 2012).

A likely reason for such a modification of the body representation by tool use may be a process of embodiment of the tool, i.e. the acquisition of the tool as a functional addendum to the body representation for perception and action (de Vignemont 2011). This process would bias both perceptual processing of stimuli delivered near the tool (Berti & Frassinetti 2000; Farnè & Ladavas 2000; Maravita et al. 2002), as well as the kinematic parameters of reaching actions (Cardinali et al. 2009b). The critical advance of the present experiment is that tool use enhances not only visual responses or multisensory integration with visual stimuli far from the hand, but also the alertness reaction to such stimuli along the space occupied by the tool. The spatial reshaping of visual responses, therefore, brings along both perceptual and affective components of sensory processing, qualifying as a form of sophisticated and multi-componential plasticity of space processing, that can be dynamically modulated by contingent factors.

In addition to the spatial modulation of alert responses, one may also speculate that the putative process of embodiment of the tool itself may extend to the affective domain (de Vignemont, 2011), thus making the tool sensitive to the same anticipatory, protective physiological reactions that are usually produced to safeguard real body parts, or embodied fake body parts (Armel & Ramachandran 2003; Ehrsson et al. 2007; Hägni et al. 2008; Guterstam et al. 2011). As compared to previous work, here we show that such a putative affective embodiment would also occur for an external object not resembling a body part, as a pure effect of visuomotor experience.

An alternative explanation for the results of Experiment 7.1 would be that the training with the tool has induced a shift of spatial attention from the effector to the tip of the tool (Holmes et al. 2004; Holmes et al. 2007b), more than an expansion of the visual responses across the peripersonal space, up to the far space. However, it is worth noting that our results do not fully support such a mere attentional account, given that the increased  $\Delta$ SCR values were found not only at the tip of the tool, but also along the tool

shaft, and because the results of Experiment 7.3 suggest that an attentional task does not lead to increased arousal responses neither at the tool tip, nor along the tool shaft, as compared to the critical active tool use task. Therefore, although attention may surely induce some enhancement or shift in spatial responses to visual stimuli at the tool tip, which is the mostly relevant part of the tool (Holmes, 2012), it does not seem to fully explain the increased arousal responses in the whole space occupied by the tool, following the training.

Overall, our data support the hypothesis that the use of a tool to operate in peripersonal space is effective for inducing not only an expansion of the visual properties of the multisensory space around the body, but also of the affective and defensive function of peripersonal space.

## **- General Thesis Discussion -**

In this thesis I tried to outline the question of how pain processing and its anticipation may be dependant from different conditions of manipulated or distorted body representation.

Along the entire experimental work, two main measures were consistently used: the skin conductance response (SCR) and the explicit rating of pain, thus assessing for both the autonomic, involuntary and explicit processing of pain experience.

In the first study I built and validated a novel experimental paradigm that measured the involuntary responses to painful stimuli and was able to dissociate the cognitive aspects from the global perceptual processing, comprising also nociceptive contributions, of pain experience.

I found that, in the absence of any experimental manipulation, SCR measured in response to a painful stimulus was strongly predictive of the subsequent explicit rating. This was a pre-condition necessary to consider the SCR as a reliable index of the autonomic nervous system response relative to the global pain experience induced by the experimental stimuli.

Furthermore, in order to assess the efficacy of the paradigm to differentiate cognitive and nociceptive components of pain experience, we measured SCR to painful and neutral stimuli in two different contact conditions. In one condition the stimulus actually touched the skin, thus activating the tactile or the nociceptive system, while in the other condition the contact was only simulated and the arousal response corresponded to the anticipation of the incoming stimulus. The latter response was ideal for my purpose to capture the cognitive aspects of pain processing (Colloca et al., 2006; Hsieh et al., 1999; Ploghaus, 1999) in isolation, being independent from any activation of the nociceptive system. Both the dominant and the non-dominant hands were assessed, with similar results. This is important since, in the series of studies conducted in the present thesis, I also aimed at assessing differences among the hands following selective ownership disruption of one

limb in neurological patients, or following experimental manipulations in healthy participants.

A further validation of the paradigm came from Experiment 1.3. Indeed one can argue that the anticipatory responses recorded for simulated stimuli were not due to a threatening situation, where the participant anticipated the incoming sensory stimulation, rather to the mere appearance of a salient stimulus per se (Gläscher & Adolphs, 2003). However results from Experiment 1.3 showed that the SCR increased much more when stimuli entered the peripersonal space, getting near to the body, than when they targeted farther spatial positions.

Moreover, I found that anticipatory responses to incoming threats is recordable both in young and elder participants. I was particularly interested in validating the paradigm in elder participants since I planned to study body ownership in the age range of typical post-stroke patients, at which SCR may be strongly reduced (Drory & Korczyn, 1993).

In Study 2 I investigated the pattern of anticipation of threatening stimuli in post-stroke patients presenting with disorder of body representation. Specifically I studied five patients presenting with somatoparaphrenia – i.e. an acquired disorder of body ownership, where patients deny the ownership of one limb, attributing it to someone else – five patients with anosognosia for hemianaesthesia and preserved ownership – i.e. a disorder of body awareness, where patients deny the acquired hemianaesthesia – and five patients with hemiplegia without deficit of body ownership and awareness. The general aim of these studies was to find a relation between the degree of body ownership and the processing of incoming stimuli threatening the body, with the idea that the latter is strictly modulated by the former. The working hypothesis was based on studies of body ownership in healthy participants. It has been shown that when a sense of illusory ownership is induced for a fake hand, as in the RHI paradigm (Botvinick & Cohen, 1998; Tsakiris, 2010), an increase SCR is recorded after threatening that fake hand (Armell & Ramachandran, 2003; Guterstam et al., 2011). Congruently with this evidence, I hypothesised that patients who attributed a part of their own body to someone else, have a

lack of ownership for that body part and thus they should show a reduced or even absent pain anticipation response when an harmful stimulus was directed toward that body part. Conversely when the sense of ownership is preserved, patients should anticipate the incoming threat, even in the presence of a focal neurological disorder affecting motor or sensory abilities.

Results overall agreed with my hypothesis, showing that patients with somatoparaphrenia had anticipatory responses only for the right, spared hand and not for the impaired left one. While either the patients with anosognosia and those with hemiplegia, without somatoparaphrenia, showed pain anticipation for both sides of the body. Study 2 suggested that the anticipation of sensory consequence for an incoming stimulation is strongly modulated by the body representation, and in particular from the ownership attribution of the threatened body part, no matter the preserved anatomical continuity with that body part. In other word, it is not sufficient that a body part is actually connected to the body in order to react to incoming painful stimuli, but a top-down representation of such a body part is necessary to make us alert about incoming threats.

An even more extreme example of the role of body representation to anticipate and react to sensory noxious stimuli came from Study 3. In this study a population of eight patients presenting with Body Integrity Identity Disorder (BIID) was administered with the pain anticipation paradigm. BIID is a striking condition where people feel that one of their limbs is extraneous. Such a disturbing feeling is so strong that they desire the physical amputation of that healthy limb (First, 2005; Mcgeoch et al., 2011; Sedda, 2011). The working hypothesis of my experiment was that BIID is caused by a deficit in body representation (Berti, 2013), and specifically in the feeling of ownership (de Vignemont, 2011). As a consequence, in a parallelism with somatoparaphrenia, I expected to find reduced anticipatory response for the stimuli approaching the limb that patients wanted to remove. Interestingly it was previously shown that BIID patients have increased SCR following noxious stimulations (Brang et al., 2008) and a general altered processing for sensory stimuli delivered on the limb they want to remove (McGeoch et al., 2011).

Congruently we found that when the stimulus actually touched the skin, SCR was stronger on the impaired side as compared to the normal limb, however this outcome of altered response is completed by the novel finding that such an increased response is preceded by a decrease anticipatory SCR. These findings suggest that the underrepresentation of the limb that they want to remove is so profound that they fail to anticipate physiological responses to incoming noxious stimuli. By contrast, since the limb is still a fully healthy limb in its sensory aspects, it produced a response when the stimuli actually contacted the skin. The fact that the incoming stimulus was not anticipated could have determined a subsequent increased nociceptive response, compared to the contralateral limb. Congruently with this hypothesis it was shown that expectation and preparation to an incoming noxious stimulus can interact with pain processing, by changing arousal responses and ratings (Brown et al., 2008a; Brown et al., 2008b; Galak & Meyvis, 2011). Study 3 confirmed the findings of Study 2 and specifically that an integer body representation is necessary to anticipate properly the sensory consequences of incoming stimuli. However this goes further into the discussion over the concept of ownership. Indeed BIID patients have the sensation that the limb is like a foreign body part, but still they acknowledge that body segment as a part of their body. In other words they have a disrupted feeling of ownership – i.e. the primitive sensation that a body part belongs to oneself (de Vignemont, 2007) – against a preserved judgment of ownership (de Vignemont, 2011). This differentiation, helps to distinguish that is the feeling of ownership the critical element to anticipate incoming sensory stimuli and not the mere judgement of ownership.

Moreover the findings of Study 2, and Study 3 might have a practical application in clinical context. Both somatoparaphrenia and BIID suffer a lack of insight in their clinical assessment, thus a paradigm similar to the one we used might be helpful to support with more objective data the diagnosis at list in borderline, or mild cases.

In Study 4, I aimed at assessing the pattern of pain processing following the artificial manipulation of body representation. Previous works have shown that the visual



distortion of the size of a body part modulates the global experience of pain (Mancini, et al., 2011b; Moseley, et al., 2008; Ramachandran et al., 2009). Controversial results showed that the visual magnification of a hand can either increase the pain threshold (analgesic effect) (Mancini, et al., 2011b) or increase painful sensation and the swelling in patients with chronic pain syndrome (Moseley, et al., 2008). The same contradiction was found for the reduction of visual size of the hand, showing opposite results: in healthy people the pain threshold decreased (Mancini, et al., 2011b), while in a patient with phantom pain, it induced analgesic effects (Ramachandran et al., 2009). I contributed to this discussion by using the pain anticipation paradigm in healthy participants under conditions of visual size manipulation of the hand. With the novel paradigm outlined in this thesis I not merely aimed at replicating results already present in the literature on nociceptive analysis of somatosensory stimuli, but, critically, at setting up a novel method to measure the anticipatory response to threatening stimuli approaching the body, that could help understanding the underlying mechanism of interaction between the (visual) representation of the body size, and pain processing. I found that when the visual size of the hand was enlarged, the SCR to real painful stimulation decreased, together with the rating for the unpleasantness of the stimulation, as compared with the hand viewed at real size, suggesting an analgesic effect for the enlarged hand, congruently with the findings by Mancini and co-workers (Mancini, et al., 2011b). Critically to my investigation, the observed decreased SCR was preceded by a stronger pain anticipation response for stimuli approaching the enlarged hand, than the real-size hand, suggesting that the cognitive component of pain, modulated by vision, impacts the global response to noxious stimuli (Brown et al., 2008b) and, more in general, sensory processing (White, et al., 2010). It is worth noting that the shrunken hand did not induce a selective modulation of pain processing, since we did not record any modulation of pain ratings and an overall small increase in SCR in both real and simulated contact conditions. This result was likely due to a general feeling of uneasiness induced by the vision of one's own body parts at a reduced size (Moseley, et al., 2008). In line with this finding, it is known that the vision

of a visually reduced body part is less likely to induce perceptual illusions (de Vignemont, et al., 2005; Pavani & Zampini, 2007) as well as kinematic effects in visuo-motor tasks (Bernardi et al., 2013; Marino, et al., 2010). The idea is that the brain might be less prone to use the visual information coming from the shrunken body part in order to anticipate the incoming sensory stimulation.

Our results on increased visual size of the body are in line with recent data from Mancini and co-workers (Mancini, et al., 2011b), but also congruent with the literature about non-painful stimulation (Bernardi et al., 2013; Marino, et al., 2010; Pavani & Zampini, 2007). However they are in contrast with results from visual size modification of the body in patients with chronic or phantom pain (Moseley, et al., 2008; Ramachandran et al., 2009), who report a general reduction of perceived pain with visual reduction, and not increase, of the body size. The difference, between our results and those by Moseley and Ramachandran (Moseley et al., 2008; Ramachandran et al., 2009), might be clarified in terms of experimental populations and different types of pain, which consisted of healthy participants, receiving acute painful stimulations in our study, while included patients affected by chronic or phantom pain in the above cited works. Different neural mechanisms are likely to underpin chronic and acute pain (Moseley, et al., 2005); moreover chronic pain induces long lasting modification of the body representation (Moseley, 2005; Moseley et al., 2005) that could, *per se*, change the patterns of response induced by distorted visual feedback from the body.

Overall, Study 4 suggests that is possible to interact with the processing of painful stimuli by manipulating the visual feedback coming from the body. The underlying hypothesis is that the visual feedback coming from one's own biological body can significantly affect the internal representation of the body which is critical for the processing of stimuli approaching and contacting the body, thus affecting the response to such stimuli.

While the first four studies were all based on direct visual feedback coming from the participant's own body, in the following Study 5 and 6 we used a bodily illusion in order

to evaluate the contribution of body representation in the processing of painful stimuli, in the absence of direct visual feedback from the participant's own body.

In Study 5 the response to painful stimuli in healthy participants was studied under conditions of illusory self-identification with a virtual body. In this well-known illusion, namely the Full Body Illusion (FBI), the congruent visuo-tactile stroking of participant's back and the virtual body seen in back view through a head mounted display, increases the illusory self-identification with that avatar, inducing a shift of perceived self-location toward the avatar as well (Lenggenhager et al., 2007), in a similar fashion to the effects induced by the famous Rubber Hand Illusion paradigm (Botvinick & Cohen, 1998; Tsakiris, 2010) with a singular body segment.

We hypothesized that, under condition of increased self-identification with the avatar, a painful stimulus contacting the biological body, during the simultaneous congruent visual stimulation on the avatar, should be processed as if seen approaching the participant's real body. In particular it was shown that looking at one's own body, but not to a neutral object or another body, induces analgesic effects (Longo et al., 2012; Longo, et al., 2009), thus we expected that the more the self-identification with the virtual body during the FBI the less the response to real painful stimuli. Indeed we found, at group level, that the SCR to painful stimuli was smaller under the condition associated with the stronger self-identification. Noteworthy we also found a negative correlation between the participant's ratings over self-identification and the SCR to painful stimuli, suggesting that the degree of ownership for the virtual body was indeed related to the arousal response to noxious stimuli. Interestingly we compared the anatomical virtual body not only with a neutral object, as typically done in FBI experiments (Aspell et al., 2013; Aspell et al., 2009; Lenggenhager et al., 2007; Palluel, et al., 2012), but also with a scrambled version of the same body. The body is a particular object that showed some advantages for its processing as a whole (Bosbach, et al., 2006; Reed, et al., 2003), but also the possibility to be processed as a sum of individual segments, as in the case of the illusory feeling of an extra-arm (Guterstam et al., 2011) or the selective impairment of body representation

limited to one hand (Invernizzi et al., 2013). We found that the illusory self identification is stronger when the avatar holds an anatomical configuration and is stroked synchronously with the participant's body. Congruently, the SCR for painful stimuli was smaller under conditions of anatomical than scrambled avatar, suggesting that the FBI takes advantages from the processing of the body as a whole.

This result extends previous findings on the modulation of pain experience by vision. In healthy humans the vision of a noxious stimulus approaching another person's body induces arousal responses (Forgiarini et al., 2011) that are based on the cognitive evaluation of the sensory consequences of that stimulation (Clark et al., 2008; Ploghaus, 1999). Such responses are supposed to be mediated by empathic sharing of the affective component of pain (Hein & Singer, 2008; Singer et al., 2004). Interestingly, in previous works it was reported that the vision of one's own stimulated body parts can modulate pain thresholds (Longo et al., 2009; Mancini et al., 2011b) or the rating of pain intensity (Longo et al., 2012), suggesting an analgesic effect when looking at one's own body. However, the vision of another person's body was not effective for inducing analgesia. Our physiological results corroborate earlier studies reporting increased pain thresholds when seeing a body part (Longo, et al., 2009) or when self-identifying with a virtual body (Hänsel et al., 2011; Longo et al., 2009), but also extend these findings. Here we showed that bodily self-consciousness induces analgesia by preparing the body to receive the visual input, prior to the activation of the nociceptive system. However, although such effects have recently been reported with explicit ratings of pain experience (Longo et al., 2012), we did not find a modulation in explicit pain ratings. Congruently with our results, a recent study (conducted in two different laboratories) failed to find a modulation in pain ratings for stimuli delivered to the biological hand during the RHI (Mohan et al., 2012), possibly suggesting that the modulation of pain at the level of explicit experience is harder to achieve by means of bodily illusions. This might be truth considering that the experience of ownership felt under illusory conditions is heterogeneous and, generally speaking, less strong than the feeling of ownership felt for one's own body and the

disorders of that feeling following pathological conditions such as somatoparaphrenia or BIID (de Vignemont, 2011). One may speculate that the physiological changes that follow the presentation of a threatening stimulus dissociate from the conscious experience of pain. Although the physiological response may precede a modulation of conscious experience of pain (see Study 2) it may also occur without any behavioural counterpart.

The illusion proposed in Study 5 presented the virtual body seen from a third person perspective at the classical visual distance of 2 meters (Lenggenhager et al., 2007; Pfeiffer et al., 2013). However it is known that the first person perspective plays a major role in bodily self-consciousness, and generally induces stronger illusory effects (Blanke, 2012; Slater, et al., 2010).

For this reason, in the Study 6, we built a new version of the FBI where a pair of virtual legs were perceived in first person perspective, by looking downwards at the position of the participant's real legs, through an head mounted display. The legs were provided in two possible orientation: one anatomical and one non-anatomical - i.e. rotated of 90° in such a way that the virtual legs crossed the position of the real legs -. We hypothesized that an increased ownership could be induced only by the vision of legs in anatomical position. Moreover in this study we aimed at evaluating whether the modulation obtained with the visual size changes in Study 4 was replicable also under conditions of illusory ownership. Thus the legs were provided in standard, enlarged and restricted size. The virtual legs model was the same for all participants, thus it did not perfectly match the real size of biological legs of each participant, even when presented at the non-distorted size. For this reason I introduced a specific item in the questionnaire, asking whether they experienced the virtual legs to hold the same, bigger or smaller size than their own. We found that a smaller SCR was recordable when the virtual legs were provided in an anatomical orientation as compared to the non-anatomically compatible one. Furthermore, in the same condition, participants reported an increased ownership sensation for the virtual legs. As expected, the effects of legs orientation interacted with the size of the virtual legs, showing significantly smaller SCR for standard size legs and enlarged, but

not shrunken view of the legs. However the expected difference between standard and big legs was not found. This may be due to the variable difference in size between the participant's legs and the regular-size virtual legs. This possibility is corroborated by showing that overall, under condition of illusory self-identification with the avatar, i.e. in the anatomical viewing condition, the SCR to painful stimuli was negatively correlated with the perceived size of the virtual legs; in other words, the more a participant judged the virtual legs as bigger than his/her own, the less the SCR. Conversely when the virtual legs were not embodied, because they were not in an anatomical compatible orientation, such a relationship between perceived size of the legs and SCR to painful stimuli was absent. Similarly to Study 5, in Study 6 the physiological modulation of pain responses induced by our experimental manipulations was not followed by a change in explicit ratings of pain experience.

Overall the Study 6 confirmed previous findings about the role of body representation in visual analgesia, extending the results to the first person perspective illusion. Moreover our results extend also previous findings about the modulation of autonomic reaction to threatening stimuli under visual size modulation of the body, now using a virtual body as visual input.

In the classic bodily illusion paradigms, like the RHI or the FBI, threatening the fake hand during conditions of illusory ownership, increases SCR levels (Armell & Ramachandran, 2003; Ehrsson, 2007; Guterstam et al., 2011). Since our simulated contact condition impinges on the cognitive evaluation of pain processing, it may be considered logically similar to the classic threatening of the fake hand in the RHI. However, in Studies 5 and 6, we found decreased responses also in simulated contact conditions and differently from classic RHI increased responses (Armell & Ramachandran, 2003; Guterstam et al., 2011). A possible explanation is that in our paradigm participants actually received real painful stimulations intermingled with simulated ones, while in classic studies with RHI the painful stimulus never contacts the participants body, and the participant is previously informed that he/she would never receive a painful stimulation (Armell & Ramachandran,

2003; Ehrsson, 2007). Although results are apparently in contrast with previous work (Armel & Ramachandran, 2003; Ehrsson, 2007; Guterstam et al., 2011), this may suggest that the virtual bodies was processed as the biological body during the illusory conditions. Indeed, when a real painful stimulation is not expected (as in the classic RHI) the threatening of an embodied fake hand is felt as an unexpected menace directed to one's own body, inducing stronger SCR as for unexpected and more salient stimulations (Brown et al., 2008b; Gläscher & Adolphs, 2003). Conversely, when real painful stimulations are known to be received in the experimental context (Brown et al., 2008b), the virtual noxious stimulus would be processed as if directed to one's own body, showing a response pattern similar to the visually-induced analgesic effect reported in previous works (Longo et al., 2009; Longo et al., 2012).

The effects found in Study 5 and Study 6 may also have an applicative value, if one thinks of clinical conditions such as the various forms of neuropathic pain. Although, as discussed above, chronic and acute pain are based on different mechanisms, the methodology used in the present work could be suitable to be adapted in both conditions. Taken together the studies described up to now investigated how the ownership affect pain processing under condition of visual feedback from the participant's own body or external bodily-like objects that undergo some degree of embodiment.

In the last study presented in this thesis, the aforementioned pain anticipation paradigm was applied to the embodiment of an external, non-bodily shaped object, namely a hand-held tool. First of all, in this study we confirmed that physiological, anticipatory responses to incoming threatening stimuli approaching the body are affected by the distance of that stimulus from the body. Moreover such a spatial boundary of the anticipatory response can be dynamically changed by means of an active training performed with a tool that extends the extension of the so-called peripersonal space. These results extend previous work showing the dynamic properties of peripersonal space following tool use in monkeys (Iriki et al. 1996), and in humans (Berti & Frassinetti, 2000; Làdavas & Farnè 2004; Maravita & Iriki 2004). It has been proposed

that, in a similar way to the fake hand in the RHI paradigm, following motor training, even a non-bodily object can go toward a process of embodiment, defined as the acquisition of the tool as a functional addendum to the body representation for perception and action (de Vignemont 2011). The embodied tool would bias either perceptual processing of stimuli delivered near the tool (Berti & Frassinetti 2000; Farnè & Làdavas 2000; Maravita et al. 2002), and the kinematic parameters of reaching actions performed by participants (Cardinali et al. 2009b). The novelty of Study 7 is that the tool use boosts not only visual responses or multisensory integration with stimuli in far space, as previously shown, but also the arousal responses to threatening stimuli presented in the space occupied by the tool and far from the body. This result shows that the spatial reshaping of visual responses would prove to be effective also in the affective components of sensory processing, not only for alien body parts (Armel & Ramachandran, 2003; Ehrsson et al., 2007; Guterstam et al., 2011; Hägni et al., 2008) but also for non-bodily shaped tools, following their acquisition in the body representation. A similar kind of affective embodiment for non-bodily objects was, so far, not described (de Vignemont, 2011). An alternative explanation for the results found so far would be that the tool training induces a shift of spatial attention from the hand to the tip of the tool (Holmes et al. 2004; Holmes et al. 2007b), rather than an expansion of the peripersonal space. Notably my results disagree with a full attentional hypothesis, as increased SCR values were found not only at the tip of the tool, but also along the tool shaft, and because the attentional training was not able to induce changes in SCR for distant stimuli. Therefore, although attention may surely induce some enhancement or shift in spatial responses to visual stimuli at the tool tip (Holmes, 2012) or maybe even drive to some extent the reshape of peripersonal space, it cannot fully explain the increased arousal responses in the whole space occupied by the tool that I showed only following active motor training. Overall, these data support the hypothesis that the use of a tool to operate in peripersonal space is effective for modulating not only the multisensory, but also the affective and defensive properties of peripersonal space.



## Conclusion

Bodily self-consciousness is a blooming field of research where a lot of questions are still unsolved. The agenda proposed as a reference for addressing such questions by current neuroscientific and philosophical research (de Vignemont, 2011) is to seek for clues about the fundamental role of body ownership, how the sense of ownership relates to bodily sensations, action and emotion and the possibility to transfer the sense of ownership towards external objects. I believe that the present thesis gives some original contribution to this debate, pinpointing some interesting results about the relations between sensations, emotions and body ownership, in relation to the processing of incoming threats directed to the body itself, to the space immediately around it and to real or virtual objects towards which we have acquired a sense of body ownership.

Taken together the studies presented show three main findings:

- an anticipatory response to incoming noxious stimuli is recordable when stimuli enter in the peripersonal space;
- an intact body representation is necessary to properly monitor noxious stimuli approaching to our own body;
- when the sense of ownership is transferred to an external object or a virtual body, a visual threat to that external object elicits anticipatory responses akin to those elicited by a menace directed to our own body.

The findings discussed so far suggest that body representation is strictly entangled with pain processing and that the sense of ownership seems to be a crucial determinant for the interactions between these two functions. Future studies might shed light also on the shared neural networks between the two functions, showing how the behavioural observations reported in the present work, and their putative mechanisms, are grounded in the brain. First we saw that the relation between ownership and sensation is so strict that having a proper feeling of ownership is fundamental to adequately process and anticipate the incoming sensory experience. Second, our data suggest that the feeling of ownership is involved in the construction of the multisensory representation of space around the body,

namely the peripersonal space (Macaluso & Maravita, 2010). This space may be conceptualized as the space of action, but also as a safety defensive area of space (Graziano & Cooke, 2006; Makin et al., 2009), acting as the true connection between body and space for proactive and defensive purposes. Interestingly, it has been recently proposed that the idea of body schema shares many features with the definition of peripersonal space, even if these two are not completely overlapping (Cardinali et al., 2009a), supporting the strict relationship between the body and its surrounding space.

A final contribution of my thesis is related to the possibility of expanding the boundaries of peripersonal space, together with those of perceived ownership, for self-protective purposes. In my experimental work I have shown that the anticipatory response to incoming noxious stimuli depends, in general, upon the feeling of ownership towards the threatened body part or external object. This is crucial since it has been proposed that the feeling of ownership could be only felt towards biological body parts (de Vignemont, 2007), but not for embodied external objects, during bodily illusions (de Vignemont, 2011). In other words I showed, to the best of my knowledge for the first time, that, after proper training, even non bodily-shaped external objects, start to be processed in a similar way to one's own body parts, congruently with the technical definition of embodiment (de Vignemont, 2011). This suggests that external non-bodily shaped object can be incorporated, to some extent, in the body representation, for both physical/spatial and emotional aspects.

Overall, the key contribution of the present work is to provide converging data, gathered from different models ranging from the study of pathological populations to the assessment of neurologically intact individuals by means of experimental manipulations, about the role of body representation for the efficient and safe interaction with the world around us, by correctly anticipating potentially dangerous incoming stimuli. This basic, vital and strongly adaptive function requires a more and more profound level of understanding to which the present work has to some extent, contributed.

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