Empathy for pain and prosocial behaviour

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Publication date
2020
Document Version
Final published version
License
Other

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Empathy for pain and prosocial behaviour

EMPATHY FOR PAIN AND PROSOCIAL BEHAVIOUR

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor

aan de Universiteit van Amsterdam

op gezag van de Rector Magnificus

prof. dr. ir. K.I.J. Maex

ten overstaan van een door het College voor Promoties ingestelde commissie,

in het openbaar te verdedigen

op vrijdag 24 april 2020, te 14.00 uur

door Selene Gallo

geboren te Ragusa, Italië

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List of abbreviations

ACC: Anterior Cingulate Cortex

AI: Anterior Insula

ANOVA: Analysis of variance

BF: Bayesian Factor

BF_{01}: Baysian Factor given the alternative hypothesis

BF_{10}: Bayesian Factor given the null hypothesis

BF_M: Bayesian Factor of the Model

BOLD: Blood-oxygen-level-dependent

CC: Cingulate Cortex

CI: Confidence Interval

DC: Direct Current

dSI-L: dorsal Primary Somatosensory Cortex Left

EEG: Electroencephalography

EPIs: echo planar images

ERP: Event-Related Potential

FDI: first dorsal interosseous

FEM: Finite Element Method

fMRI: functional Magnetic Resonance Image

FOV: Field Of View

FWHM Gaussian kerne:l Kernel With Full Width At Half Maximum

GLM: General Linear Model

H_+: Positive tail of statistical test

$H_{1:}$ Alternative Hypothesis

HD-tDCS: High-Definition transcranial Direct Current Stimulation

H_o :Null Hypothesis

ICA: Independent Component Analysis

IFG: Inferior Frontal Gyro

IFG: Inferior Frontal Gyrus

JASP: software for statistical analyses

L: Left

MATLAB: software

MCC: Medial Cingulate Cortex

MEPs: Motor-evoked potentials

MNI: Montreal Neurological Institute

MRI: Magnetic Resonance Image

MVPA: Multi Voxel Patter Analysis

N/A: Not Applicable

OSP: Optimal Scalp Position

PF: Prefrontal Cortex

p_{unc}: Uncorrected P-Value

qFDR: False Discovery Rate

R: Right

rMT: Resting Motor Threshold

ROI: Region Of Interest

RP: Riccardo Paracampo (author)

rTMS: repetitive Transcranial Magnetic Stimulation

SD: standard deviation

SEP: Somatosensory Evoked Potentials

SG: Selene Gallo (author)

SI: Primary Somatosensory Cortex

SII: Secondary Somatosensory Cortex

SPM12: Statistical Parametrical Mapping 12

$t0_{active}$:Experimental Contidion Time0 under active brain stimulation

T0 t1…: Time 0, time 1 … (experimental conditions)

$t1_{active}$: Experimental Contidion Time1 under active brain stimulation

tDCS: transcranial Direct Current Stimulation

TE: Echo Time

TMS: Transcranial Magnetic Stimulation

TR: Repetition Time

vSI-L: ventral Primary Somatosensory Cortex Left

Chapter 1:
General introduction

Prosocial behaviour and empathy

Witnessing another person struggling with physical pain or emotional distress does not leave us indifferent. Often, the unpleasant sensation observed in the other also rises in the witnesses, together with the urge to do something to help and to stop the other's suffering. This mechanism is thought to exist in individuals of many species, and it can be so powerful that the action to terminate the co-specific's distress might be carried out even when doing so incurs risk to oneself (Preston *et al.* 2002). Actions that achieve a positive effect for an individual in distress and a negative effect (a 'cost') for the individual performing the act are referred to as 'costly helping behaviour'. Prosocial behaviour is a key aspect of our social life and one of the building blocks of our community.

A factor considered fundamental for motivating prosocial behaviour is empathy, the capacity to share the emotional states of others (Batson *et al.* 1981; Preston *et al.* 2002; FeldmanHall *et al.* 2015; Preston and De Waal 2017). There are many definitions of empathy. Here, we refer to the feeling of sharing another person's emotions, putting ourselves in their shoes. The role of empathy in motivating prosocial behaviour is intuitive: someone else's pain motivates us to care about and help that person. Many studies support this idea. In a series of experiments, Batson and his colleagues put their participants in situations in which they had the opportunity to help another person in different ways, such as donating money, taking over an unpleasant task, or cooperating at a cost to themselves. When subjects were encouraged to feel empathy for those in need of help, they were more likely to behave prosocially (Batson *et al.* 1981). The perception-action model of empathy, proposed in 2002 by Preston and De Waal, suggests that empathy is a key motivator and the mechanism behind altruistic behaviour (Preston *et al.* 2002): an individual perceives and shares the distress of another person and acts to reduce his or her own suffering (Preston *et al.* 2002; Preston and De Waal 2017). Helping others at a cost to the self is a function of the desire to minimise one's own discomfort when observing others in pain (Cialdini *et al.* 1987; de Waal 2008).

Interestingly, this kind of behaviour is not unique to human beings. A monkey chose to starve itself to prevent a shock being given to another monkey (Masserman, Wechking and Terris 1964), and rats choose to release trapped cage mates over accessing attractive food for themselves (Ben-Ami Bartal, Decety and Mason 2011; Sato *et al.* 2015). The central role of affect transfer in helping behaviour was demonstrated by showing that the administration of the anxiolytic midazolam reduced rats' helping responses without impairing the instrumental act required to obtain food (Ben-Ami Bartal, Decety and Mason 2011).

The fact that animals also show costly helping behaviour supports the idea that sharing another's suffering can motivate prosocial acts without the need for the higher cognitive function more typical of humans, indicating that it is an intrinsic characteristic of social animals. According to Preston and de Waal (2002), empathy is a term for a broad category of responses, comprising a continuum—from basic forms, such as sensorimotor or emotional contagion, to complex forms, such as perspective taking (de Waal and Preston 2017).

Other authors are more sceptical about the role of empathy in complex behaviours and warn against seeing it as a panacea. Empathy is narrow in its focus, rendering it subjects to bias. It can motivate cruelty and aggression and lead to burnout and exhaustion. It can lead to decisions that clash with our considered moral judgements and its promotion can have the opposite effect to that desired (Bloom 2017; Zaki 2018).

Neurocorrelate of social behaviour

A surprising discovery made by a team of Italian scientists in the 1990s provided a powerful framework for social behaviour. Rizzolatti and colleagues discovered that some neurons in the macaque monkey's ventral premotor cortex fired when the monkey performed a particular goal-directed action and when it observed another individual performing a similar action (Gallese *et al.* 1996; Rizzolatti *et al.* 1996). Thus, observation of an action caused the activation of parts of the same cortical neural network that are active during its execution. This finding led many to believe that the observer understood the action because they knew its outcomes when they performed it themselves. In this framework, action understanding depends on the 'penetration' of visual information into the experiential ('first person') motor knowledge of the observer (Gallese, Keysers and Rizzolatti 2004). The neurons showing this property have been named 'mirror neurons'. Several studies using different methodologies have shown that similarly in humans the observation of actions performed by others activates cortical motor representations (for a review see Urgesi, Candidi and Avenanti 2014) and neurons that fired for both action performance and observation have been described (Mukamel *et al.* 2010). Visual information emerging from lower-level visual areas is sent to temporal regions, from where it is relayed to parietal regions (including the inferior parietal lobe and the anterior intraparietal area) and ultimately to premotor regions (Rizzolatti and Craighero 2004; Blakemore and Frith 2005; Caspers *et al.* 2010; Keysers and Gazzola 2014; Urgesi, Candidi and Avenanti 2014). This temporal, parietal, and premotor network is often referred to as the action observation network. The primary somatosensory cortex (SI) is also consistently active during action perception and execution (Avikainen, Forss and Hari 2002; Buccino *et al.* 2004; Avenanti *et al.* 2007; Gazzola and Keysers 2009; Caspers *et al.* 2010; Turella *et al.* 2012; Jacquet and Avenanti 2015) and it is considered an additional sensorimotor node of the action observation network (Keysers, Kaas and Gazzola 2010).

Regions with this pattern of activation have been observed not only for observing another person performing an action, but also for observing feelings, such as touch (Keysers *et al.* 2004; Blakemore *et al.* 2005; Ebisch *et al.* 2008; Bolognini *et al.* 2014). Like the first-hand sensation of touch, the mirror activity of SI is somatotopically organised and it is thought to convey information about intensity and quality of the touch observed. Seeing other people being touched (compared to the control condition) also activated the Secondary Somatosensory cortex (SII). The fact that the neurons in SII have very large receptive fields (Krubitzer *et al.* 1995) suggests that vicarious activation in SII could convey a simulation of the quality of touch that one would experience if touched in a similar way, rather than the precise body location at which the touch occurred (Keysers, Kaas and Gazzola 2010). Both SI and SII have direct connection to the inferior parietal lobe and the anterior intraparietal area, which are known to be areas in which inputs from different sensory information converge (Brooks and Tracey 2005).

Emotions such as disgust (Carr *et al.* 2003; Wicker *et al.* 2003) and pain (Morrison *et al.* 2004; Singer *et al.* 2004; Jackson, Meltzoff and Decety 2005; Saarela *et al.* 2006; Keysers, Kaas and Gazzola 2010; Corradi-Dell'Acqua, Hofstetter and Vuilleumier 2011; Zaki *et al.* 2016) have been shown to also elicit common brain activity in those who experience the emotion and the observer. In this case, the network includes the Anterior Insula cortex (AI) and the Anterior and Medial Cingulate cortex (ACC and MMC).

Extended to emotional phenomena, observing an affective posture or expression can feedback from peripheral motor representations to activate associated emotional states (de Waal and Preston 2017). In this perspective, the emotional states of others are understood through personal, embodied representations that allow empathy and accuracy when perceiving other emotions to increase, based on the observer's own past experiences (Preston *et al.* 2002; Preston and De Waal 2017). Regions that

mirror property are thought to play a fundamental role in social cognition by allowing an immediate understanding of another person's actions by mean of simulation.

Pain, perception of other's pain, and empathy for pain

Pain and empathy for pain provide an interesting model for investigating different forms of empathy and their underlying neuronal circuitry (Betti and Aglioti 2016).

Pain is universal and relatively easy to genuinely recreate in the laboratory. There is a general consensus about the areas that are activated when a person receives noxious stimulation. The expression 'pain matrix' was coined to refer to these areas which encode pain sensation invoked by thermal, electrical, and mechanical stimulations. Initially derived from Melzack's 'neuromatrix' (Melzack 1999), the term departed from original conception as it became pain-specific (Iannetti and Mouraux 2010; Mouraux *et al.* 2011). The network is supposed to code for two distinct pain components: affective-motivational and sensory-discriminative (Price 2000; Mouraux *et al.* 2011). Neural pathways comprised by the AI and ACC have been regarded as 'affective-motivational' or 'affective node' because they process information related to the affective unpleasantness and motivational relevance of noxious stimuli (Rainville *et al.* 1997; Melzack 1999; Price 2000). Neural circuits in the primary and secondary somatosensory cortices have been characterised as 'sensory-discriminative' for encoding spatial localisation, duration, and intensity of noxious stimuli (Porro *et al.* 1998; Bushnell *et al.* 1999; Peyron, Laurent and García-Larrea 2000; Nickel *et al.* 2017).

There is growing evidence to support the idea that part of the network of regions active when experiencing pain is also activated in healthy participants by observing another person in pain (Singer et al. 2004; Bernhardt et al. 2013; Zaki et al. 2016), apparently allowing a direct first-person understanding of another's emotions: 'Your pain is my pain' (Gallese, Keysers and Rizzolatti 2004; Ferrari and Rizzolatti 2014). The activity induced by feeling the pain of another person is called 'vicarious pain' and the brain activation is 'vicarious brain activity'.

Intriguing is the emergence of genuine physical pain by merely observing, thinking about, or inferring that another person is in pain. This phenomenon is referred to as 'mirror pain synaesthesia' (Fitzgibbon *et al.* 2010) and it has been observed both following trauma (Bradshaw and Mattingley 2001) and in healthy subjects (Osborn and Derbyshire 2010; Grice-Jackson et al. 2017). At first glance, mirror-pain synaesthesia may be regarded as a form of empathic response—that is, a shared state between the self and the other. However, at a mechanistic level, it is far less clear how the synaesthesia should be interpreted and whether it is actually an extreme form of normal empathy or a qualitatively different form (Ward; Deroy and Spence 2013; Ward, Schnakenberg and Banissy 2018).

Recordings of AI and ACC have been made of responses to observed pain, using a wide range of different paradigms—such as the observation of pictures depicting ordinary painful situations (Jackson, Meltzoff and Decety 2005), videos depicting hands being pricked by needles (Morrison *et al.* 2004), and facial expressions of pain (Botvinick *et al.* 2005; Saarela *et al.* 2006). Additional studies have supported the idea that empathy for pain involves the brain areas mapping the affective qualities (Singer et al. 2004, 2006; Keysers and Gazzola 2009; Corradi-Dell'Acqua, Hofstetter and Vuilleumier 2011; Lamm, Decety and Singer 2011; Zaki, Ochsner and Ochsner 2012; Corradi-Dell'Acqua et al. 2016; Cui, Ma and Luo 2016). When activated by the sight of another person suffering, these areas correlate with personal distress (Singer *et al.* 2004), perceived unpleasantness (Rainville *et al.* 1997), and perceived pain intensity (Lamm, Decety and Singer 2011). They diminish after placebo analgesia pain (Rütgen *et*

al. 2015, 2017). For all these characteristics, the vicarious activation of these areas has been identified as the neural correlate of empathy for pain.

Converging studies have shown vicarious activity in the somatosensory regions (mainly primary and secondary cortices—SI and SII) and motor regions during the observation of other's pain (Avenanti *et al.* 2005; Jackson, Rainville and Decety 2006; Bufalari *et al.* 2007; Aziz-Zadeh and Damasio 2008; Valeriani *et al.* 2008; Benuzzi *et al.* 2008; Cheng *et al.* 2008; Betti *et al.* 2009; Perry, Troje and Bentin 2010; Whitmarsh *et al.* 2011; Riečanský *et al.* 2014; Hoenen, Lübke and Pause 2015), consistent with the role of these brain areas in coding the sensory qualities (i.e., the intensity or the location of the stimulus) during the direct experience of pain (Perry and Bentin 2009; Lamm, Decety and Singer 2011; Cui *et al.* 2015; Christov-Moore and Iacoboni 2016).

Although much evidence has been collected to indicate that both affective and sensorimotor pathways are called into action during empathy for pain, the way in which the other person's pain is represented seems to be of fundamental importance to the differential weight of the regions involved in the task (Lamm, Decety and Singer 2011). It is worth noting that different methodological strategies and experimental paradigms may account for the stimulus-driven activity in spatially selective regions of the brain (Betti and Aglioti 2016).

In their meta-analysis, Lamm and colleagues point to the visibility of the affected body part as a determining factor in the involvement of the sensory-motor regions (Lamm, Decety and Singer 2011). When the painfulness of the stimulation being witnessed is indicated by an abstract cue, only the affective nodes of the pain network are activated; while if the injured body part is visible, the sensory-motor nodes are also recruited.

In experiments in which participants observe specific body parts being harmed, activity in SI is consistently reported (Morrison *et al.* 2004; Jackson, Rainville and Decety 2006; Gazzola *et al.* 2007; Lamm *et al.* 2007; Lamm and Decety 2008), and EEG studies show that SI activity is modulated by the imagined painfulness of the observed actions and not merely by the exposure of the affected body parts (Perry et al. 2010; Hoenen, Lübke and Pause 2015). The involvement of the region is more consistently suggested by EEG studies (Betti and Aglioti 2016). Whenever our attention is directed to the somatic cause of the pain of others, somatosensory cortices become vicariously activated: this process could trigger a more localised, somatic sensation of pain in a particular body part.

Empathy for pain can therefore involve the somatosensory cortices, but apparently only if one attends to the localised somatic cause of the pain. Only if participants directly witness an intense, localised, harmful somatic event as the cause of another person's pain do they vicariously activate their somatosensory cortices (in addition to the AI, MCC, and ACC). Owing to the somatotopic organisation of SI, its vicarious activation is likely to add a localised, somatosensory feeling to our empathy for pain in these conditions.

An interesting case is when another person's pain is expressed by their facial expression. In this scenario, one observes a body part in movement (the face) but that is neither the source nor the location of the painful sensation. Seeing emotional motor behaviour such as emotional facial expressions vicariously activates those sectors of the inferior frontal gyrus (IFG) involved in controlling facial movements and those sectors of the SI involved in processing sensations from the face (Carr *et al.* 2003; Leslie, Johnson-Frey and Grafton 2004; Keysers, Kaas and Gazzola 2010). These observations suggest that perception of other's facial expressions is—at least partially—grounded in the same network involved in performing and sensing facial movements (Goldman and Sripada 2005; Keysers, Kaas and Gazzola

2010; Niedenthal *et al.* 2010; Wood *et al.* 2016b). Indeed, the relevance of facial mimicry is confirmed by many studies (Oberman, Winkielman and Ramachandran 2007; Hess and Fischer 2013; Wood *et al.* 2016a; Fischer and Hess 2017), and ventral somatosensory cortex causally contributes to emotion perception from facial expression (Adolphs *et al.* 2000; Paracampo *et al.* 2017), while recognising emotions from visually presented facial expressions requires ventral somatosensory-related cortices (Adolphs *et al.* 2000). In this case, one can reasonably suppose that the information the somatosensory conveys is not the physical pain itself, but the affective value of it.

While suggested by many authors, the hypothesis of a differential involvement of SI based on how the pain is depicted has not yet been directly tested.

With this goal in mind, we designed a set of video stimuli (used in the experiments described in Chapters 2 and 3) to differently engage the somatosensory cortex. In the videos, the same person was shown receiving painful stimulation of her right hand, in one case a slap by a belt on her hand and, in the second case, an electro-shock. When the stimulation was delivered by the slap, the subject's right hand visibly moved in reaction. This movement alone conveyed the intensity of the stimulation delivered and, consequently, it was possible to deduce the intensity of pain felt by her. In these videos, only the right hand, arm, and shoulder of the person were visible; thus, these stimuli were named 'hand videos'. When the stimulation was delivered by electro-shock, because of the nature of the stimulation, the hand did not show any visible sign of distress. The extent of the pain could only be deduced by the facial expression made by the person. In these videos, the entire upper body was visible, including the right hand. We therefore named these 'face videos'. The hand videos highlighted the sensory-motor representation of the hand, while the face videos highlighted the affective representation of pain and potentially also the sensory-motor representation of the face. The hand and face videos were created to show different pain intensity, from very low to high levels. On a scale of 1-7, where '1' indicates 'no pain' and '10' refers to the most intense imaginable pain, the highest level was '7'.

Direct comparison of the SI activity—specifically of the portion of SI representing the right hand—provides valuable information about the role of SI mirror activity. Based on the hypothesis that SI mirror proprieties are engaged only when the observer's attention is focused on the harmed body part, we hypothesised that SI mirror activity would rise when watching the hand videos and not—or to a lesser degree—when watching the face videos, despite the pain being localised in the hand in both stimuli. Moreover, we expected the regions known to code for the affective experience of pain (AI and ACC) to respond to both kinds of stimuli.

Relationship between vicarious activity for pain and prosocial behaviour.

The relationship between vicarious pain activity and the propensity to help a person in pain is of fundamental importance for identifying the psychological meaning of the brain response. Since empathy is hypothesised to have strong links to prosocial behaviour, if the shared activity represents empathy, it should also be related to prosocial behaviour.

Few studies had directly explored the link between shared activity and helping behaviour. In 2010, Hein and colleagues revealed that volunteers who displayed more activity in the pain matrix while witnessing the pain of another tended to engage more in costly helping (i.e., aiding the distressed person despite incurring disadvantages or suffering to themselves). In their studies, the painfulness of the stimulation

was indicated by a cue. In line with that described previously, their results highlight the involvement of the insular cortex (Hein *et al.* 2010).

In 2011, Ma and colleagues investigated the relationship between neural activity **in response** to perceived pain in others and human altruistic behaviours in real-life situations. Participants were scanned by functional MRI while observing videos of others in pain and then invited to make an anonymous monetary donation to a charitable organisation. Painful stimuli increased activity in the inferior frontal, insular, and somatosensory cortices compared to non-painful stimuli. The neural responses to perceived pain predicted the amount of monetary donations, and this relationship was mediated by the participants' social economic status (Ma, Wang and Han 2011)

In a 2015 study, FeldmanHall and colleagues observed study participants' costly helping behaviour induced by the sight of a person receiving an electro-shock to the hand.. In this case, the victim's entire body and face were visible. The study again confirmed that stronger activation of the affective nodes of the pain matrix were related to more prosocial behaviour (quantified as money donated) and identified empathic concern as the driving force underlying the mechanism (FeldmanHall *et al.* 2015).

In 2016, Christov-Moore and Iacoboni examined how neural correlates of self-other resonance relate to prosocial decision-making. Subjects performed two tasks while undergoing fMRI: observation of a human hand pierced by a needle, and observation and imitation of emotional facial expressions. Outside the scanner, subjects played the 'Dictator Game' with players of low or high income (represented by neutral-expression headshots). Subjects' offers in the Dictator Game were correlated with activity in neural systems associated with self-other resonance and anticorrelated with activity in systems implicated in the control of pain, affect, and imitation (Christov-Moore and Iacoboni 2016). The relationship between a region of the pain matrix, the MCC, and prosocial behaviour measured by the Dictator Game was confirmed in 2016 by Tomova and colleagues (Tomova *et al.* 2016).

While these studies represented an important step forward in understanding the relationship between vicarious activation of the pain matrix and prosocial behaviour, some important considerations are required.

From correlation to causation

The studies described in the section above are all neuroimaging studies. It is widely recognised that functional imaging studies reveal correlations between areas of activation and performance of a task, thus they can only reveal areas engaged in a task, rather than the areas of the brain critical for the task (Robertson *et al.* 1993; Fellows *et al.* 2005; Hillis 2014). The correlational approach of these methods cannot establish whether neural activity is necessary for a certain task. Indeed, two events may co-occur—or one may be an epiphenomenon of the other, without being necessary. Thus, to test the causal role of the mirror activity in empathy and social behaviour, it is essential to employ causal methods—in effect, by investigating the influence of altered neural activity in key nodes of the system on the ability to recognise and understand the pain of others and act prosocially.

Non-invasive brain stimulation is a very useful tool to close the gap between brain activity and behaviour. Techniques as Transcranial Magnetic Stimulation (TMS) and Transcranial Direct Current Stimulation (tDCS) can temporarily interfere with the activity of one target region, and consequently with its network, and the effects can be directly read on the behaviour.

The causal contribution of mapping the pain of others onto our own pain representations in social decision-making has remained poorly explored in neuroscience, as most scientists focus on the affective

network (AI, ACC), which has been proven to reliably correlate with prosocial behaviour but lies too deep in the brain to be selectively targeted with traditional non-invasive neuro-manipulation tools (Keysers and Gazzola 2017; Keysers, Paracampo and Gazzola 2018). In contrast, SI superficial location makes it a perfect target for brain stimulation techniques. Perturbing this region while the subject is involved in a prosocial task has the potential to shed light on whether mirror activity for pain is essential for the behaviour.

In Chapter 2, we describe a novel paradigm to induce costly helping behaviour which uses the hand and face videos, stimuli designed to differently engage the sensory-motor shared activation in our participants. First, we interrogated the face and hand representation in SI, using EEG and source reconstruction. This neuroimaging technique can assess whether these two portions of SI are differently engaged when seeing a person in pain and, therefore, whether the recruitment of SI depends on the attention given to the body part in pain. In particular, we explored the relationship between brain activity and prosocial behaviour towards the person in pain. To test whether the shared brain activity was necessary for performing the task, we leveraged that the less investigated hand representation of SI was superficial and reachable with TMS to address whether disturbing this activity (with TMS) altered prosocial decision-making.

If the somatosensory activity contributed to our decision to help, it might do so by transforming into an accurate perception the experiences of others that were witnessed. It remains unclear whether somatosensory activation levels reflect the intensity of the pain experienced by others (Lamm, Decety and Singer, 2011; Morrison et al., 2013). We therefore employed data from a third experiment in which HD-tDCS was used to alter SI activity to measure whether an SI perturbation also altered the accuracy of the pain perception.

Functional meaning of vicarious activity, towards more robust results

The finding of a network of regions coding for both observed and experience pain, which also contribute to prosocial behaviour, is extremely interesting. Nevertheless, we should be cautious before drawing any conclusions. Once the existence of such regions is established, it is fundamental to explore in depth the functional meaning of their activation (Zaki et al. 2016).

Most studies comparing experienced and observed pain use designs that do not allow for exploration of the characteristics of the brain activity. They often identify the regions by comparing low and high pain intensities in both experience and observation tasks (Phan et al. 2004). This does not guarantee that the activation closely follows—and is in tune with—the painful experience. Second, it does not allow us to disentangle the effect of processes other than pain, such as attention and salience, which participate in the elaboration of the stimuli but do not reflect affective processing (Wager et al. 2013). In addition, there is often no other ’control’ task used to systematically assess for a-specific variables. Finally, these studies often base their conclusions on only one kind of experienced and observed painful stimulation (e.g., heat, pressure, electricity), thus failing to prove generalisability of the findings.

In Chapter 3, we expand on the knowledge of shared activity and directly address some of the criticisms of classical studies. In this way, we apply more strict criteria to identify shared brain activity.

While subjects were lying inside an fMRI scanner, we asked them to observe our hand and face videos to rate the extent of the pain felt by the person in the video. Participants also underwent the same kind

of painful stimulation. This procedure allowed us to identify the network of regions linearly sensitive to both perceived and observed pain.

The hand and face videos were designed to drive attention to the body part affected by the painful stimulation in different ways, but the level of pain expressed by the person in the videos and the negative affect were alike. We would expect SI activity to discriminate between the two videos, while the regions classically defined as the affective nodes of the system would be equally engaged by the two. According to the current literature, the somatosensory cortex is the node of the shared activity network and would thus discriminate between the videos, while other nodes (AI and ACC) would be equally activated by the two kinds of stimuli.

We also presented our participants with a third stimulus, which was visually similar to the hand and face videos. In this case, no movement nor pain were depicted, but the colour saturation was manipulated so that it increased during the video. Participants were asked to rate the colour saturation changes. These 'colour videos' were used as a control task to 'clean' the fMRI results recorded while watching the hand and face videos of all those factors related to visual information and rating behaviour but not specific to pain or empathy for pain. The use of the control task is particularly important for characterising the meaning of the AI activation, whose role in shared activity has recently been challenged by new findings (Krishnan *et al.* 2016; Zaki *et al.* 2016).

Indeed, AI has been consistently found to be active for pain and perception of other's pain and involved in prosocial behaviour. This region seems to respond to a variety of unpleasant states in others, including disgust (Calder *et al.* 2000; Wicker *et al.* 2003) and social pain (Eisenberger 2012; Chang *et al.* 2015). It also responds to non-pain states, including arousal, attention, and uncertainty (Grinband, Hirsch and Ferrera 2006; Craig 2009; Singer, Critchley and Preuschoff 2009; Shackman *et al.* 2011; Lyons and Beilock 2012). Some scholars have noticed that the Insula is one of the most commonly activated brain regions across all neuroimaging studies (Yarkoni *et al.* 2011; Moayedi 2014). This ubiquitous activation aligns with the notion that this region is related to the detection of salience, and it has been suggested that it constitutes a crucial hub, connecting sensory areas to other networks involved in the processing and integration of external and internal information (Yantis 2008; Menon and Uddin 2010). Given that pain is inherently salient, it is possible that the processes encoded in the insula reflect the salience of the stimulus, not specifically pain (Moayedi 2014). Critics suggest that conclusions about the overlap between empathic and nociceptive pain rely heavily on spurious reverse inference (Poldrack 2006) and that social and nociceptive experiences might not, in fact, share pain-specific processes (Iannetti *et al.* 2013; Zaki *et al.* 2016).

Therefore, in Chapter 3, we discuss our experiment designed to assess which regions of the brain are sensitive to perceived and observed pain and are not activated by generic processes of attention and saliency, providing a better characterisation of the functional meaning of vicarious activity. Surprisingly, fMRI BOLD signal did not record activity in SI with these characteristics when the entire brain was interrogated. More specifically, tests targeting SI activity were conducted (see the Appendix to Chapter 3), together with an extensive discussion of the possible causes of the incongruence with the EEG results.

Modulation of the vicarious activity

As previously described, once the neural markers of a given behaviour are established using neuroimaging data, for scientific advancement, it is important to ensure the causal relationship between the two. If the behaviour is affected when the region is damaged, it is safe to conclude that the behaviour is not possible without that specific brain area. Causality becomes fundamental when we want to act on

a maladaptive behaviour to correct it: it is only by targeting an area responsible for the behaviour that we can hope to benefit the patients.

Non-invasive brain stimulation techniques are the only techniques currently able to assess causal relationships in healthy humans. The most popular classes are TMS and transcranial direct current stimulation (tDCS), which differ in the means they use to interact with the brain: a magnetic field in case of TMS and an electric field in case of tDCS.

TMS uses a coil to produce a focused (1-2 cm2) magnetic field. To target an area, the coil must be positioned on the scalp, over the targeted area. The magnetic field pulse causes a secondary electric field in the brain to induce neuronal firing (Rossi *et al.* 2009). The direction of the stimulation, if excitatory or inhibitory, depends on the parameters used (frequency, intensity, duration of the pulses) (Betti *et al.* 2009; Rossini *et al.* 2015; Tatti *et al.* 2016)

tDCS uses a minimum of two electrodes positioned on the participant's scalp. A direct current runs between the electrodes, across the brain. The current is not able to produce firing in the neurons per se; rather, it modifies the neurons' environment, the membrane potential at rest, so that the neural activity is facilitated or inhibited depending on the current flow direction (Nitsche and Paulus 2001; Bikson *et al.* 2004; Brunoni *et al.* 2012; Rahman *et al.* 2013). While it was traditionally thought that only the areas directly underlying the electrodes were affected, 3D simulation has shown that all areas between the electrodes—crossed by the electrical field—are affected (Datta *et al.* 2009).

TMS has the obvious advantage of causing neuronal firing and therefore having a higher potential for successfully modulating the area underlying the stimulation. Many studies have successfully used the technique to interfere with cognitive tasks. In Chapter 2, we describe the use of a protocol well known in literature to be reliable for modulating the somatosensory cortex (Harris *et al.* 2002; Balslev 2004; Merabet *et al.* 2004; Fiorio and Haggard 2005; Tegenthoff *et al.* 2005; Azañón and Haggard 2009; Jacquet and Avenanti 2015; Valchev *et al.* 2016; Paracampo *et al.* 2017). We interfered with the hand representation in SI while the participant was occupied in a costly helping task. Importantly, depending on where the coil is positioned on the scalp, TMS pulses may cause slight activations of facial muscles. The hand representation in SI is in a location that induces relatively small twitching, while the ventral SI, where the representation of the face is located, is recognised to cause much more facial muscle activation (e.g., Paracampo *et al.* 2017). This is unpleasant for the participants and particularly disruptive for the face video stimuli used in our tasks, since altering facial mimicry can impair visual recognition of expressions (Oberman, Winkielman and Ramachandran 2007; Wood *et al.* 2016b). For this reason, we were not able to test the contribution of the ventral SI to the costly helping behaviour.

tDCS electric field does not causes muscle activation and therefore more indicated to pursue our goal of discriminating between the contribution of the hand and face representation of the somatosensory cortex vicarious activity in our hand and face stimuli. tDCS has other advantages that make it a very appealing method for stimulation of the brain. The low cost, simple application, and portability make the technique easy to export from the laboratory to the clinical setting. tDCS is also considered safer than TMS. While TMS side effects may range from headaches, to painful muscle twitching, to dangerous epilepsy in people with a predisposition to this, tDCS side effects are less invasive, and no case of epilepsy has ever been reported to be induced by this kind of stimulation (Rumsey *et al.* 2017).

Unfortunately, the state-of-the-art tDCS does not reach the specificity of stimulation required to separately target the two portions of SI. The problem of specificity is very much felt in the non-invasive brain stimulation community. Delivery of direct current (DC) is usually performed using large pads,

most commonly between 25-35 cm^2, which stimulate relatively broad areas of cerebral cortex located between the anode and cathode. Therefore, focal stimulation of target cortical regions, not involving stimulation of neighbouring anatomical areas, is impossible to achieve with this technique (Datta *et al.* 2009; Dmochowski *et al.* 2011).

For research purposes, a more focal stimulation is required for a better understanding of the specific brain regions involved in the process under study (Nitsche, Bikson and Bestmann 2015). High Definition transcranial Current Stimulation (HD-tDCS) was created to increase spatial selectivity and probe the function of specific brain regions (Datta et al., 2009; Borckardt et al., 2012; Edwards et al., 2013; Caparelli-Daquer et al., 2012). HD-tDCS uses small ring electrodes of 3-5mm in diameter, with the most popular montage a 4 × 1 ring with a central electrode (anode or cathode) over the targeted area, surrounded (at 3–7.5 cm radius) by four reference electrodes (Datta et al. 2009; Edwards et al. 2013; Kuo et al. 2013; Villamar et al. 2013; Filmer, Dux and Mattingley 2014; Roy, Baxter and He 2014; Alam et al. 2016). This increases focality (Tergau et al. 2007) up to 80%, compared with standard tDCS (Datta et al. 2008; DaSilva et al. 2015; Alam et al. 2016).

We used the 4x1 HD-tDCS montage in the experiment described in Chapter 2 to assess the involvement of SI in perception of other's pain. We placed the anode over the hand knob of SI, while one of the cathodes was approximately located over the face representation. The focus of the stimulation was still the hand knob, and the intensity of the current reaching the ventral SI was a quarter of that reaching the targeted area (Villamar *et al.* 2013).

The 4x1 montage was a great improvement in focality, with respect to standard tDCS, but one advantage of using multiple electrodes is that the possible combinations are potentially infinite. As described in Chapter 4, we tested an original High Definition tDCS montage developed to be as focused as possible. Simulations using the Finite Element Method (FEM) suggested that an increment of focality can be reached by lessening the number of return electrodes: using only one cathode and one anode should support the most focused stimulation possible, albeit at the cost of depth current penetration (Alam *et al.* 2016). We aimed to leverage a 1x1 montage to focally stimulate the SI and measure modulation via Somatosensory Evoked Potentials (SEP) triggered by median nerve stimulation. In this way, we addressed the question of whether it was possible to push the focality of HD-tDCS to specifically modulate bilaterally the cortical excitability of the hand knob of the somatosensory cortices, creating the potential—if the first attempt was successful—to separately interact with the face and hand representation in SI.

We were interested in the possibility of bi-hemispheric stimulation because SI and BA2, in particular, have bilateral receptive fields (Allison *et al.* 1991; Valeriani *et al.* 1998; Iwamura *et al.* 2002; Lederman and Klatzky 2009) and it has been proven to be bi-laterally active in tasks involving witnessing others experiencing somatosensory events (Keysers *et al.* 2004; Keysers, Kaas and Gazzola 2010; Morrison *et al.* 2013). This kind of bi-hemispheric modulation that applies current in the same direction in the homologous areas had not been previously tested. With the safety of our participants in mind, we used an intensity of stimulation comparable to that traditionally used for 4x1 montages (1.5 mA) and divided it equally between the two anodes of our montage (0.75 mA each). According to the field consensus, if multiple electrodes are used, the delivered intensity is the sum of the current at all anodes (Bikson *et al.* 2017). This reduces the amount of current that can actually cross the skull (Edwards *et al.* 2013) and reach SI, exposing our experiment to a high risk of negative results (Alam *et al.* 2016).

We aim to characterise the effect of the stimulation in terms of directionality (increase vs. decrease of SEP), consistency (across participants), and reproducibility (across studies). The effectiveness of tDCS

stimulation has recently come under intense debate and the replicability of the results obtained has been challenged (Horvath, Forte and Carter 2015; Woods et al. 2015; Learmonth et al. 2017; Rumsey et al. 2017). To assess the robustness and validity of the novel montage, we tested it in two independent experiments. In the first experiment, we tested the montage on Somatosensory Evoked Potential (SEP); and in the second, we aimed to replicate the results of the first experiment by repeating the SEP recording to assess the development in time of the eventual SEP modulation.

Summary

In summary, this work explored the neurocorrelate of prosocial behaviour and empathy for pain, with specific attention to the functional relevance of the vicarious activity as a neuromarker of representation of one's own and other's pain.

In Chapter 2, we focus on role of the somatosensory cortex in the costly helping paradigm. First, using EEG, we addressed whether variation in the region activity while seeing another person in pain could predict variation in helping, measured as variation of monetary donations. Participants were presented with two kinds of stimuli, each of which differently highlighted the somatosensory component of the painful stimulation; and we assessed how each influenced the engagement of the somatosensory vicarious activity. We then applied TMS, a non-invasive brain stimulation technique that uses a focused magnetic field to temporarily interfere with brain activity over the same region to assess whether this region was necessary for the performance of the behaviour. Finally, to understand how SI activity might be related to prosocial behaviour, we interfered with our volunteers' hand representations and asked them to indicate the extent of the pain they believed the person in the video to be experiencing: if their rating was no more in line with what they were shown, SI was deemed to contributing to transforming the visual information into a more accurate understanding of the intensity of the pain.

Chapter 3 explains that, using fMRI, we aimed to deepen our understanding of the functional meaning of shared brain activity. These areas should be sensitive to both experienced and observed pain intensities. They are not active for stimuli other than pain and are generalisable between different kinds of experienced and observed pain stimulation.

In Chapter 4, we describe a novel HD-tDCS montage with which to bilaterally and focally modulate the cortical excitability of the hand knob of SI. We experimented with the validity of the montage for the modulation of SEP and the duration in time of the effects in two independent experiments. If the new montage reliably interferes with SI cortical excitability, it may be exported to the study of empathy of pain and help to disentangle the contribution of SI from that of the neighbouring areas.

Taken together, these results provide new insights into the relationship between vicarious activation and prosocial behaviour and the functional meaning of this kind of brain activity. Moreover, they provide an opportunity to reflect on how the use of different techniques affects the development of knowledge.

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Chapter 2:
The causal role of the somatosensory cortex in prosocial behaviour

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A detailed description of each author contribution can be found in the chapter *Reference list and authors' contribution*

Abstract:

Witnessing another person's suffering elicits vicarious brain activity in areas active when we ourselves are in pain. Whether this activity influences prosocial behavior remains debated. Here participants witnessed a confederate express pain via a reaction of the swatted hand or via a facial expression and could decide to reduce that pain by donating money. Participants donate more money on trials in which the confederate expressed more pain. EEG shows that activity of the SI hand region explains variance in donation; TMS shows that altering this activity interferes with the pain-donation coupling only when pain is expressed by the hand and HD-tDCS that altering SI activity also interferes with pain perception. These experiments show vicarious somatosensory activations contribute to prosocial decision-making and suggest they do so by helping transform observed reactions of affected body-parts into accurate perceptions of pain that are necessary for decision making.

Introduction

Prosocial behavior – actions intended to benefit others despite costs for the self (Batson et al., 1981) – is important in social animals but poorly understood. The role of empathy in motivating prosocial behavior is intuitive but also intensely debated (Bloom 2017; Zaki 2017). Some researchers show that people are more likely to engage in prosocial behaviour when they feel empathy for the person in distress (Batson et al., 1981) and that self-reported emphatic concern is related to prosocial behaviour (FeldmanHall et al., 2015). Others show that empathy is a poor predictor of prosociality (Vachon et al., 2014; Jordan et al., 2016), with prosocial decisions often driven by other motives (e.g. status; Bloom, 2017). Unfortunately, experiments that specifically manipulate brain activity in empathy-related regions and measure prosociality are missing, limiting our neuroscientific understanding of whether and how empathy is mechanistically linked to prosociality (Keysers & Gazzola 2017; Zaki et al. 2016). Here we use a combination of electroencephalography (EEG), transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) to explore whether altering somatosensory activity that vicariously represent the pain of others would alter the decision to donate money to alleviate their pain, and whether alterations in pain perception mediate this effect.

Witnessing somebody in pain activates two networks depending on the nature of the stimulus (Keysers et al. 2010; Lamm et al. 2011). If the pain of the other is deduced from abstract symbols or facial expressions alone, a network involving the anterior insula (AI) and anterior cingulate cortex (ACC) is activated. The AI and ACC activity correlates with personal distress (Singer et al. 2004), perceived unpleasantness (Rainville et al. 1997) and perceived pain intensity (Lamm et al. 2011), and is therefore thought to code for the unpleasantness of the pain of the other (Lamm et al. 2011). This network is often referred to as the affective path. If the injured body part is visible and in the focus of attention, the somatosensory cortices (SI and SII) are also recruited (Bufalari, Aprile, Avenanti, Russo, et al. 2007; Cheng et al. 2008; Keysers & Gazzola 2009; Lamm et al. 2011; Nummenmaa et al. 2012; Morrison et al. 2013; Ashar et al. 2017; Christov-Moore & Iacoboni 2016), and consequently included in the network of regions participating in human empathy (Keysers, Kaas and Gazzola, 2010; de Waal and Preston, 2017).

The affective and somatosensory networks are also active when experiencing pain (Melzack 2001; Iannetti & Mouraux 2010; Lockwood et al. 2013). Because of that, many interpret vicarious activation while witnessing the pain of others as the neural correlate of emotional contagion, a core component of empathy — feeling vicariously what we see someone else experience (Koban et al. 2013; Corradi-Dell'Acqua et al. 2011; Jackson et al. 2006; Lamm et al. 2011; Cui et al. 2015; Singer et al. 2004). In this perspective the emotional states of others are understood through personal, embodied

representations that allow empathy and accuracy in perceiving other emotions to increase based on the observer's past experiences (Preston and de Waal, 2002; de Waal and Preston, 2017). Pharmacological studies provide causal evidence for the involvement of our own experience of pain, the insula and anterior cingulate cortex, in perceiving the pain of other individuals (Rütgen et al. 2017; Rütgen et al. 2015), pin pointing to the opioidergic circuit, which might code representations of aversive outcomes, derived through both direct and indirect experiences (Haaker et al. 2017). Much like the original proposal of Adam Smith (Smith 1759), the pain vicariously felt because of the recruitment of these pain-related regions while viewing the pain of others is then thought to motivate prosocial behavior, which then simply serves to reduce the vicariously felt pain (Batson et al., 1981; Hein et al., 2010, 2016; Ma, Wang and Han, 2011; Tomova et al., 2016).

Testing the causal contribution of mapping the pain of others onto our own pain representations in social decision-making has remained poorly explored in neuroscience because most scientists focus on the affective network (AI, ACC), which lies too deep in the brain to selectively target with traditional non-invasive neuro-manipulation tools (Keysers & Gazzola, 2017). Here we therefore leverage that the less investigated hand representation of SI is superficial and reachable with TMS to address two questions: does activity in the somatosensory cortex (measured using EEG) explain prosocial behavior on a trial-by-trial basis and does disturbing (with TMS) this activity alter decision-making? For this aim, here we measure prosocial behavior using a costly helping paradigm (Figure 1A), in which participants make a moral decision between two conflicting motives – maximizing their financial gains and minimizing the pain of another (see FeldmanHall et al., 2015 for a similar tradeoff) – and then measure and alter brain activity in the hand region of SI to explore the impact of this activity on the decision-making.

If somatosensory activity contributes to our decision to help, does it do so by being necessary for accurately perceiving how much pain other people experience? Whether somatosensory activation levels reflect the intensity of the pain experienced by others remains unclear (Lamm, Decety and Singer, 2011; Morrison et al., 2013), and we therefore use data from a third experiment in which HD-tDCS is used to alter SI activity to measure whether an SI perturbation also alters the accuracy of pain perception.

As mentioned above, somatosensory cortices are mainly involved when the injured body parts are visible. Additionally, ventral regions of SI have been reported to be involved in emotional facial perception (e.g Adolphs et al., 2000; van der Gaag et al. 2007; Preston and de Waal., 2002, but see Rütgen et al., 2015 for absence of SI activity for perception of facial expression of pain). Given its somatotopic organization, SI could therefore be involved in pain perception in two ways. If we only see the painful reaction of a hand, the hand region of SI could reflect the intensity of the observed reaction by simulating the movements of the hand and/or the somatosensory consequences of the harm (Keysers, Kaas and Gazzola, 2010). If we know the painful stimulation originates from the hand, but the intensity of the pain has to be inferred from the facial expressions, somatosensory activation in the hand region could still reflect the intensity of the stimulation, via an indirect route in which information derived from the facial expression is referred back onto the hand region through a process akin to somatosensory imagery, or activation could instead fail to reflect pain intensity. Either way, we could expect the more ventral representation of the face in SI to be involved in representing the intensity of the facial expression (Adolphs et al. 2000; van der Gaag et al. 2007), but less involved in representing the intensity

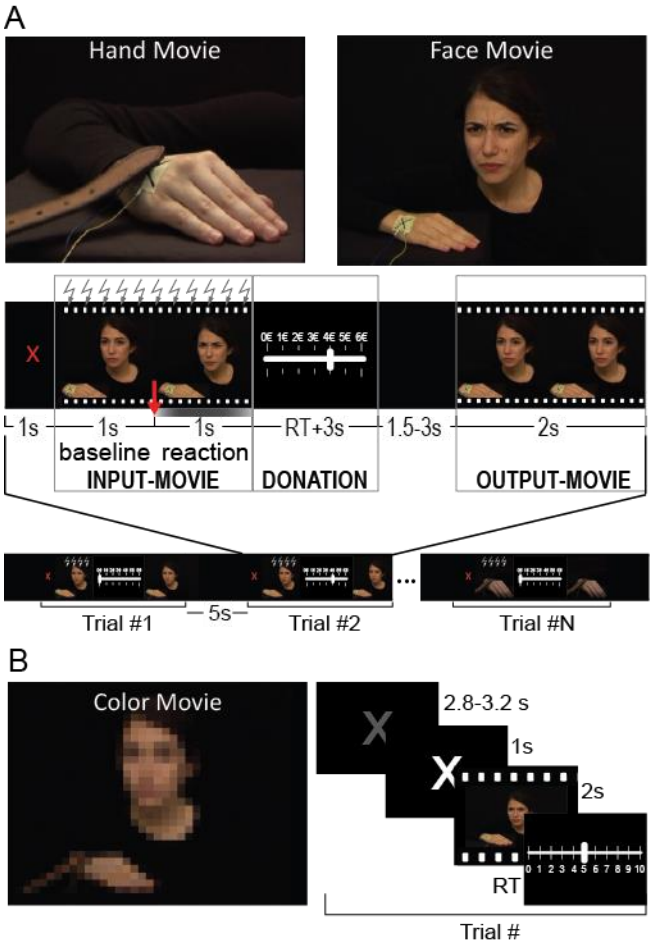

Figure 1. Paradigm. (A) *Top*: a snapshot from the Hand and Face videos (examples of each condition are presented in supplementary MovieS1-4). Middle: trial structure. The red arrow indicates the timing of the shock delivery, belt touching the hand or beginning of the color saturation changes. The gray gradient graphically illustrates the dynamic of the face reaction and color saturation changes, with stronger gray corresponding to stronger facial expression or stronger saturation. The intensity of the OutputMovie is equal to the intensity of InputMovie minus the donation. *Bottom*: run structure. The same structure was used in the EEG and TMS experiments. Gray lightning symbols indicate when TMS was applied in the TMS version of the experiment. (B) A snapshot from the Color videos (see Supplemental MovieS5-6) and the trial structure for the rating task.

of a painful hand movement when the face is not visible. To shed further light on the properties of the hand region of SI in the decision-making task presented in this study, we therefore designed two types of input stimuli that probe the above mentioned scenarios, both showing different intensity of pain (Movie S1, S2, S3, S4) in order to look at quantitative relations between brain activity, perception and behavior (Wager et al. 2013). Specifically participants witnessed a confederate receiving a noxious stimulation of randomly selected intensity (InputMovie) delivered as (a) a swat with a belt on the right hand, with only the hand reaction visible (Hand condition, Movie S1 and S2) or (b) an electroshock on the right hand with a visible facial expression and no hand movement (Face condition, Movie S3 and S4). In both kinds of videos, the confederate's right hand receiving the painful stimulation was clearly visible on the screen but in the case of the belt (Hand condition) the reaction of the hand itself was the only cue for the participants to deduce the painfulness of the stimulation. On the contrary when receiving the electroshock (Face condition), the hand did not show any reaction and the painfulness was deduced only by the confederate's facial expression. At each trial, participants then received an endowment of 6€ and could reduce the intensity of the next noxious stimulation (OutputMovie) by giving up some of that money, knowing that the remainder would be part of their compensation (Figure 1A and MovieS1-S6).

In a first experiment, we investigate whether activation of the hand region of the left SI, as measured with EEG, explains prosocial behavior. The SI hand region was identified in an independent pool of participants by correlating fMRI BOLD responses within SI with subjective experience of pain elicited by electrical stimulations on participant's right hand. We hypothesized that activation of the hand region of SI would correlate with the donation given by the participants in the Hand condition, when the intensity of the stimulation had to be deduced from the hand movement. In the Face condition we predicted that activity more ventrally in SI, where facial expressions are represented, would correlate with the donation, as the relevance of facial mimicry has been highlighted in many studies (Oberman et al. 2007; Hess & Fischer 2013; Fischer & Hess 2017; Wood, Rychlowska, et al. 2016), and ventral somatosensory cortex causally contributes to emotion perception from facial expression (Adolphs et al. 2000; Paracampo et al. 2017) and recognizing emotions from visually presented facial expressions requires ventral somatosensory-related cortices (Adolphs et al. 2000). As mentioned above, for the hand region of SI during the Face condition, we had less defined predictions: the presence or absence of correlation of SI hand region activity with the donation while perceiving facial expressions will inform whether facially deduced pain intensity is re-represented in the SI locations reflecting the inferred origin of that pain.

In a second experiment, we then perturbed the SI activity of the hand region with repetitive TMS (rTMS) to test whether disturbing SI vicarious activity altered prosocial behavior. Because disrupting SI activity using TMS or neurological lesions has been shown to alter the accuracy with which participants perceive some emotions (Adolphs et al., 2000; Paracampo et al., 2017) and hand actions (Valchev et al. 2017), and because in the nociceptive literature, SI has been associated more with perceptual than motivational processes (Keysers, Kaas and Gazzola, 2010; Lee & Tracey, 2010) we expected TMS over the hand-region of SI to disrupt the accuracy with which participants can transform the observed kinematics of the belt and hand into an accurate feeling for how painful this particular stimulation was for the other. We thus expect decision-making to become noisier, and less attuned to the level of pain experienced by the other on a trial-by-trial basis particularly when the reaction of the hand is the only source of information for the decision-making (Hand condition). This effect would be weaker or absent when information is derived from the Face, where alternative sources of information are available.

Finally, we used data from a third experiment to explore whether a disruption of the perception of pain intensity indeed mediates how disrupting SI activity alters decision-making. Brain activity in SI was altered using high-definition tDCS while participants had to rate how much pain the person in the Hand and Face movies experienced on a trial by trial basis. Because the specific montage used in this experiment was expected to facilitate brain activity under the anode placed over the SI hand region and inhibit brain activity under the return cathodes, one of which was placed over the face region of SI, we expected the accuracy of the ratings to increase in the Hand stimuli and decrease in the Face stimuli.

Results

Experiment 1: EEG study

Participants (Table 1) donated on average the same amount in the Face and Hand conditions (Face: M=2.14€ SD=1.2; Hand: M=2.16€ SD= 1.2; dependent sample t-test $t_{(28)}$=-0.2, p=0.8), but comparing the standard deviation in donation within each participant showed more variability in donation for the Face condition (Face: M_{SD}=1.47 SD_{SD}=0.44; Hand: M_{SD}=1.22 SD_{SD}=0.40; dependent sample t-test

$t_{(28)}$=4, p=0.0004). To avoid this confound in further analysis, we Z-transformed the donation of each participant separately for the two conditions.

To assess if participants' donation was driven by the intensity of the reaction shown in InputMovie, for each participant, we performed a robust linear regression (Holland & Welsch 1977), between the intensity attributed to the movies by an independent pool of participants, and the Z-donation. The analysis confirmed that participants' Z-donation closely followed the pain intensity shown in the InputMovies (Figure 2A). In the Face condition, all participants had regression slopes that were positive and significantly different from zero (Face slope: M=0.48 SD=0.6, average t value for Face slope = 21.9, SD=17, all p<0.05; group one-sample t-test on Face slopes t(28)=37.9 p=0.0006E-21). In the Hand condition one participant had a negative slope but the regression was not significant (t=-0.17, p=0.8), a second participant had a positive but not significant slope (t=1.89, p=0.06), while the remaining participants all showed positive and significant slopes (all p<0.05). We considered the former two as normal variation along the population spectrum and kept them in the analysis (Hand slope: M=0.45 SD=1.4; average t value for Hand slope =7.4, SD=5.4; group single sample t-test on Hand slopes $t_{(28)}$=16.3 p=0.0001E-11). Importantly, the average slope did not differ between the two conditions (paired t-test $t_{(28)}$=-1 p=0.3).

To interrogate the electrical activity originating from the primary somatosensory cortex we used a linear constrained minimum variance beamforming approach (Van Veen et al. 1997), in which spatial filters were designed to isolate brain electrical activity from the specified locations of interest. To identify regions of SI that reflect perceived pain intensity while participants experience pain on their own bodies, we performed an independent fMRI experiment in which participants received electrical stimulation at different intensities on their right hand and reported how painful each stimulation was. We then identified voxels in the left SI where the BOLD signal correlated with reported painfulness (see Supporting Information, Pain Localizer Experiment), and used the following clusters as ROIs for the EEG beamforming analysis: a dorsal (dSI-L; peak at $MNI_{(x,y,z)}$ = -30, -36, 62) and a ventral (vSI-L; peak at $MNI_{(x,y,z,)}$ = -54, -19, 32) somatosensory cluster (Figure 2B; Maldjian et al., 1999; Mancini et al., 2012), while the ventral cluster has a dorso-ventral extent similar to the face representation in SI but seems unusually posterior (Figure 2B; van der Gaag, Minderaa and Keysers, 2007). This suggests that the ventral cluster could originate from the posterior parietal region PF or represent the facial reaction to the pain in SI. We focused on the left hemisphere, because electrical stimulation was always delivered to the right hand of both the confederate shown in the movies, and of the participants in the pain localizer.

For each participant and ROI, beamforming returned activity time-courses along three standard dipole directions. Given the low spatial resolution of EEG, the mixed orientation of cells in the somatosensory cortex (encompassing gyri and sulci), and the fact that we use videos, for which the dipole capturing most of the variance could change over time, we included all three dipole directions in multivariate analyses.

To assess whether EEG activity explained variance in participants' donations, we used a random effect, summary statistics approach routinely used in fMRI analysis (Holmes et al. 1998). First, at the single subject level we modelled the relationship between SI ROI activity and donation by calculating a robust regression between brain activity at a given time-point and the Z-donation for all the trials of that participant (Figure 3A, left). Repeating this analysis for each time point generated a time-course of the slope separately for each participant, condition and dipole (Figure 3A, right). Second, at the group level we analyzed the group distribution of these slopes: if EEG activity in an ROI does not carry systematic information about the donation, the slopes would be randomly distributed around zero. To test this null hypothesis, we used the Hotelling's t-squared statistic, a multivariate generalization of the Student's t-

test that combines evidence from all three dipoles and all participants in a given condition and time-point. We controlled for multiple time point testing using a cluster-based randomization test

	Total N^{O} subj. (excluded)	**Age (SD)**	**Gender M (F)**	**Experimental Task**
Validation Costly Helping stimuli	40	24 (6)	23 (17)	Rating other's pain
Validation Rating Stimuli	20	24 (3.4)	8 (12)	Rating other's pain & color saturation
fMRI	25	25 (6)	11 (14)	Rating own pain
EEG	32 (4)	25 (5)	16 (16)	Decision to help
TMS	18 (3)	25 (7)	12 (6)	Decision to help
HD-tDCS	26 (1)	25 (4)	13 (13)	Rating other's pain & color saturation

Table1: Participants characteristics. The table indicates for each experiment the number of tested participants, with those excluded from the analyses within brackets; the average age and its standard deviation (SD); the gender ratio; and the experimental task. Three participants from the EEG and three from the TMS experiment were excluded because they did not sufficiently believe the cover story. One participant in the EEG was excluded because of EEG failure. One participant in the tDCS was excluded because they performed at chance level. Analyses on gender effects can be found in Supplementary File 3.

implemented in Field-Trip (Oostenveld et al. 2011), which compares the sum of the Hotelling statistics within the clusters in the real data against those in clusters obtained after switching the sign of the entire slope-time course of randomly selected participants. We repeated the procedure for each condition and ROI and accounted for those additional comparisons using a Bonferroni procedure. Only results surviving those corrections are reported in yellow in Figure 3B. Results show that variation in activity in dSI-L explained variation of Z-donation in the Hand condition in the time windows between 420 and 452 (cluster-statistic=142.2, p=0.002), and between 458 and 476 ms (cluster-statistic=91.4, p=0.006) after the belt hits the confederate's hand. Activity in dSI-L also related to Z-donation in Face condition but later in time, between 516 and 602 (cluster-statistic=602.5, p=0.0009), between 608 and 754 ms (cluster-statistic=333.8, p=0.0009), and also between 808 and 880 ms (cluster-statistic=330, p=0.0009) after the noxious stimulation was delivered. vSI-L brain activity explained Z-donation but only in the Face condition in the time window between 402 and 430 ms (cluster-statistic=102, p=0.005; Figure 3B).

To visualize this effect, we categorized the trials in low and high donation (median split per participant) and calculated grand-averages voltage time courses during the InputMovie (Figure 3C) for each dipole. The grand averages along Y and Z present a negative deflection after the InputMovie onset independently of condition and donation, which likely reflects general attentional processes. For the Hand condition the dipoles further present a positive peak some milliseconds after the slap which is sustained along Z and transient along X and Y. No clear peak is recognizable after the shock (time 0) during the Face condition. Differences between low and high donation can be observed, in particular along Y, for all ROIs and conditions (Supplementary File 1 and 2).

There is evidence for a bilateral receptive field in the Brodmann 1 and 2 sub-regions of SI (Iwamura et al. 2002), and for the involvement of the right hemisphere in the perception of emotion (not including pain) from facial expressions (Adolphs et al. 2000; bilateral activation are reported in Ashar et al. 2017; Lamm et al. 2011; Cui et al. 2015; Carr et al. 2003), and hands movement (Christov-Moore & Iacoboni 2016). Figure 3D shows the signal originating from mirroring our left ROIs to the right hemisphere, and in yellow the time points significantly explaining the donation. For the hand region of SI (d-SI), results for the two hemispheres are very similar suggesting a lack of clear hemispheric specificity

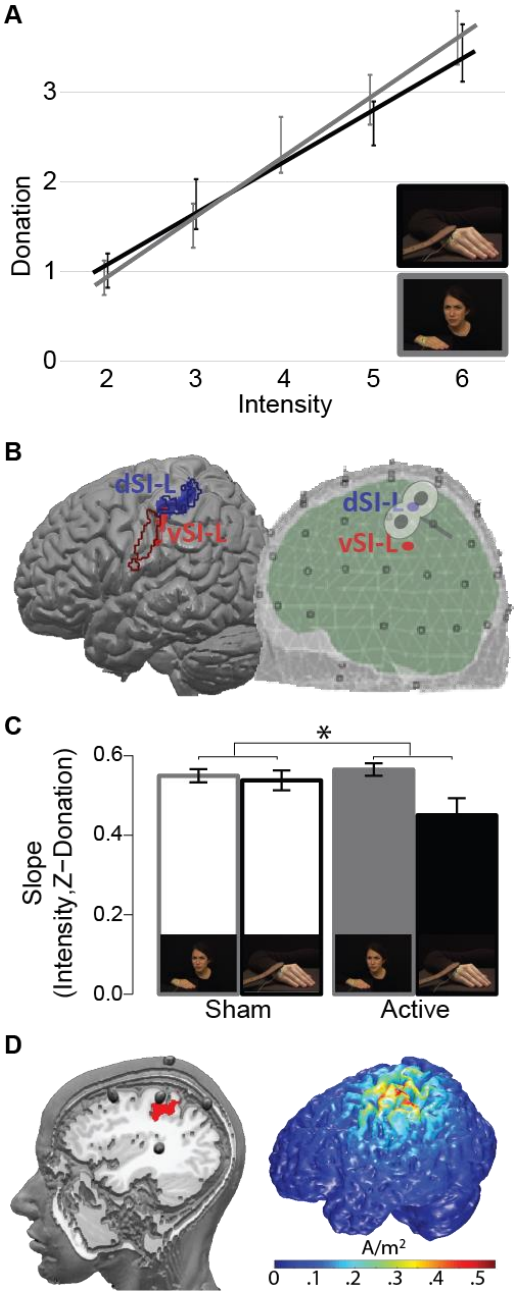

Figure 2. ROIs and TMS results. (A) The graph shows the relationship between InputMovie intensity, as assigned by an independent pool of participants during the movies validation procedure, and given donation for hand and face videos. Each point is the group average donation for the specific intensity. Error bars represent S.E.M. (B) Pain Localizer ROIs. *Left:* results of the pain localizer within the primary somatosensory cortices (Supporting Information) shown on the Colin brain together with contours of regions associated with hand (blue) and face (red) movements. These contours were generated using the meta-analyses tool Neurosynth (Yarkoni et al. 2011). Specifically, we generated reverse inference maps using the search terms "grasping" and "speech production", to probe movements of the hand and of the face respectively, and intersected each with an anatomical map of the left SI from the anatomy toolbox (as the union of BA1,2,3a and 3b). *Right:* schematic visualization of the dorsal and ventral ROIs within the EEG template space, and approximate site of the TMS stimulation. (C) Interaction Condition x TMS results. * $p<0.05$. Error bars represent S.E.M. (D) The left render shows the location of the 5 HD-tDCS electrodes on the scalp and where the central anode is positioned relative to our d-SIL ROI (red). The image was created by inserting fish oil omega 3 pills in place of the HD-tDCS electrodes inside the electrodes holders. A participant was wearing the montage while a T1-weighted anatomical image was acquired (TR = 8.2 ms, TE = 3.8 ms, flip angle = 8°, FOV = 240 mm × 240mm, 1 x 1 x 1 mm isotropic voxels). The right render shows the 3D simulation of current density changes expected from our tDCS montage, obtained using the electrostatic finite element method (FEM) offered by the Matlab toolbox COMETS 2 (Jung et al. 2013).

(Figure 3D and Supplementary Information). For the more ventral, putative face region of SI (v-SI), responses appear stronger on the right hemisphere, in line with previous findings (Adolphs et al. 2000).

Our EEG findings therefore suggest that while witnessing the pain of another person, the magnitude of brain activity in the hand region of SI (d-SI) could inform decision making. To examine its causal contribution to decision making, in a second experiment we use TMS to disturb the activity of the SI hand region. We target the left hemisphere because it is contralateral to the hand that is stimulated in the confederate.

Experiment 2: TMS study

A separate group of participants (Table 1) performed the costly helping paradigm for the Face and Hand conditions under both active and sham rTMS over the left SI hand region.

The within subject ANOVA with 2 factors, Condition (Face and Hand) and TMS (Active and Sham), on the standard deviation calculated across the donation of each participant reveals that also in this second experiment participants varied their donations from trial-to-trial more in the Face than Hand condition (main effect of condition $F_{(1,14)}$=98, p=0.001E-4), but there was no variance difference between the two TMS protocols (main effect of TMS $F_{(1,14)}$=0.2, p=0.65, interaction between Condition and TMS $F_{(1,14)}$=0.2, p=0.7). Therefore, the same Z-transformation of the donation was used as in Experiment 1, standardizing separately all the donations of the Hand and Face condition of each participant (but without separating Active and Sham to preserve TMS effects).

TMS had no effect on the average Z-donation in either tasks, as indicated by the repeated ANOVA with factor Condition (Face and Hand) and TMS (Active and Sham): TMS $F_{(1,14)}$=2.3, p=0.15, TMS x Condition $F_{(1,14)}$=0.5, p=0.56. To test whether TMS interferes with the relationship between the intensity of the movie and the donation, for each participant we calculated the slope between intensity of the movie and Z-donation by means of a robust regression, separately for the Condition (Face and Hand) and TMS protocol (Sham and Active).

We then performed a 2 factor repeated ANOVA on these slopes, using the above mentioned two level summary statistic approach. The analysis showed that the slopes for the Face condition are steeper than for the Hand (Face: M=0.56 SD=0.06, Hand: M=0.49 SD=0.14, Main effect of Condition: $F_{(1,14)}$=7, p=0.02). TMS did not have a general effect common to the two conditions (Main effect of TMS: $F_{(1,14)}$=1.9, p=0.19). Interestingly the ANOVA showed a significant interaction effect ($F_{(1,14)}$=4.8, p=0.04, partial η^2=0.25; Figure 2C) with a larger TMS effect in the Hand compared to the Face condition. A Newman-Keuls post-hoc test indicated that active rTMS on SI significantly flattened the relationship between intensity and donation only for the Hand condition (Hand Sham M=0.54 DS=0.1; Hand TMS M=0.45, DS=0.2, p=0.02), while there is no evidence for such and effect in the Face condition (Face Sham M=0.55 SD=0.06; Face TMS M=0.56, SD=0.06, p=0.6). To test if the lack of effect in the Face condition was due to limited statistical power or provides evidence for the null hypothesis (H_0) of no (sizable) effect of TMS, we used Bayesian statistics. We calculated an index of TMS effect for each participant in the Face condition as follows: slope in the Active session - slope in the Sham condition. We then performed a Bayesian one sample t-test using JASP with default priors (https://jasp-stats.org) that showed the null hypothesis is 5.7 times more likely than the alternative H_1 (Bayes factor $p(H_0{:}index\geq 0|data)/p(H_1{:}index<0|data)=5.7$) providing positive evidence for the absence of a sizable effect in the Face condition (Kass & Raftery 1995).

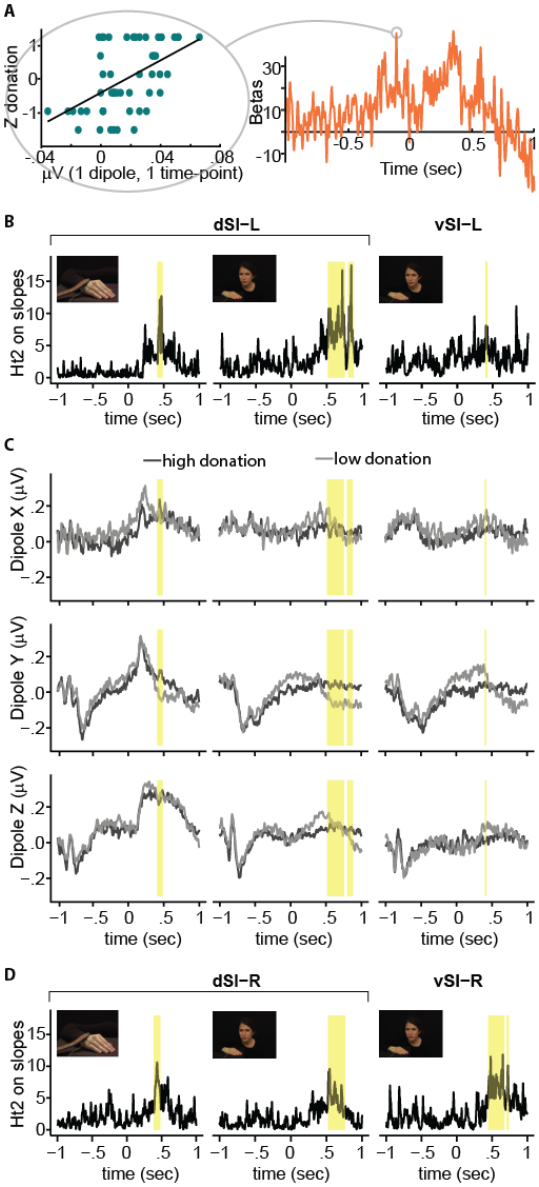

Figure 3. Regression between SI activity and donation. (A) *Left:* Relationship between brain activity of one example participant at a given time-point and the Z-donation for all the trials of that participant. The linear trend represents the slope of the robust regression performed on these values. *Right:* time-course of the robust regression slopes (betas) for the same example participant. (B) Time-course of the Hotelling's t-squared (Ht2) test on the slopes for the significant ROI and condition. Because the two significant Hand clusters are very close in time, for illustrative purposes only, they have been evidenced by a single yellow band. (C) Grand averages for high (darker lines) and low (lighter lines) donation for each dipole, SI-ROI and condition. (D) Right hemisphere results. Significant clusters based on Ht2 are shown in yellow.

The effect on the Hand condition did not correlate with any of the TMS side effects perceived by the participant while performing the experiment (measured by questionnaire, all $p>0.05$). This suggests that TMS on the SI hand representation interferes with the process that normally couples a person's donation to the needs of the other person (i.e. the observed pain intensity) when this need is perceived through the movements of the affected body part (Hand condition), but this is less the case when the need is perceived through facial expressions. To test whether the impact of TMS is mediated by an effect on pain perception we used data from a third experiment.

Experiment 3: HD-tDCS study

In this experiment, participants (Table 1) had to rate how much pain they perceived while watching the Face and Hand videos under the effect of tDCS centered over left SI. The high density 4x1 electrodes

tDCS montage we used would be expected to have a facilitatory effect on the hand region of SI under the anode (Figure 2D), and a weaker inhibitory effect on ventral SI, including the face representation, under one of the four return cathodes. To control for unspecific effects on intensity rating processes unrelated to pain, in this experiment we introduced a new type of video in which participants needed to rate color saturation intensity (Color condition, Figure 1B).

A 2 Stimulation (tDCS and Sham) x 3 Condition (Face, Hand and Color) repeated measures ANOVA on the standard deviation calculated for the participants' rating reveals that participants did not use the rating scale differently in the three conditions (main effect of condition $F_{(1,24)}=0.4$, $p=0.5$), nor between the two tDCS sessions (main effect of Stimulation $F_{(1,48)}=2$, $p=0.1$, interaction between Condition and Stimulation $F_{(1,48)}=0.6$, $p=0.5$). To be consistent between our studies we applied Z-transformation of the ratings separately for the three conditions but pooling the two tDCS conditions. A Stimulation (tDCS and Sham) x Condition (Face, Hand and Color) ANOVA on the average rating revealed that average rating remained stable across conditions (Stimulation $F_{(1,24)}=0.5$, $p=0.5$, Condition $F_{(1,48)}=0.8$, $p=0.5$, Interaction $F_{(1,48)}=0.3$, $p=0.7$). For each condition and session, we then correlated the intensity assigned to the movies during the validation process and the Z-rating given by the participant. The correlation values were normalized using the Fisher z-transformation. We then performed a 2 factor repeated ANOVA on the correlation coefficients obtained. The analysis showed that the correlation coefficients differed between conditions (Face: M=1.3 SD=0.2, Hand: M=1 SD=0.2, Color: M=1.2 SD=0.3, Main effect of Condition: $F_{(2,48)}=17$, $p<0.002E-3$). Post-Hoc Newman-Keuls test revealed that the Hand condition had a lower correlation coefficient than both Face and Color, while the latter did not differ from each other. TDCS had no main effect on the correlation coefficients (main effect of tDCS $F_{(2,48)}=0.5$, $p=0.5$). Interestingly tDCS had a different effect depending on the conditions (Interaction: $F_{(2,48)}=3.4$, $p=0.04$, $\eta^2=0.12$). Planned paired t-test comparison between sham and tDCS session for each condition indicated that the tDCS on SI significantly improved the relationship between intensity and rating for the Hand condition (Hand Sham: M=1 DS=0.2; Hand tDCS: M=1.1, DS=0.2, $t_{(24)}=-2$, $p=0.04$), while it showed a trend for reduction in the Face condition (Face Sham: M=1.3 DS=0.2; Face tDCS: M=1.2, DS=0.2, $t_{(24)}=1.8$ $p=0.07$), and no appreciable change in the Color condition (Color Sham: M=1.3 DS=0.2; Color tDCS: M=1.2, DS=0.3, $t_{(24)}=1$, $p=0.3$). Again, to test if the lack of effect in the Color condition supports the null hypothesis, we calculated an index of the stimulation effect by subtracting the z-transformed correlation score calculated in sham from the one calculated in the real tDCS session. Since Hand and Face conditions showed opposite effect, we performed on the Color condition a 2-tails one sample Bayesian t-test. The Bayes factor indicated that the H_0 was 3.1 times more likely than the H_1, confirming that the Color condition does not change after HD-tDCS. The Hand and Face effects did not correlate with any of the tDCS side effects perceived by participants while performing the experiment (all $p>0.05$).

Discussion

We used a helping task in which brain activity can be related to prosocial behavior on a trial-by-trial basis and concentrated on measuring and altering activity in the hand region of SI to shed light on the contribution of this region to prosocial decision-making.

Specifically, we localized regions in the left SI that encode the intensity of pain experienced by a group of participants using fMRI. This evidenced two clusters in the left SI, a dorsal cluster corresponding to the hand representation of SI and a ventral cluster that had a dorso-ventral extent similar to the face representation of SI. These ROIs served as ROIs for our EEG experiment that shows that the magnitude

of brain activity originating from the dorsal cluster has a significant relationship with helping. This was true whether victims expressed their reaction through the afflicted body-part or the face. Figure 3 shows how the timing of that activity followed the timing of the information in the movies: while the hand immediately retracts at the moment the belt hits the hand, and SI activity had a sharp and sudden peak in explanatory power, the facial expression develops more slowly after the shock is delivered, and SI activity showed a more progressive and sustained explanatory power. Figure 3 also shows that the ventral and dorsal sector of our functionally localized SI nociceptive representation behave differently in our task, with the dorsal (hand) ROI explaining donation for either source of information (Face or Hand) and the ventral (face) ROI being explanatory only for facial expressions.

The choice to interrogate the signal during pain observation within regions coding the intensity of pain during self-pain experience was, as mentioned in the introduction, dictated by the theoretical framework of emotional contagion and vicarious activity. In this framework the activation of cells involved in experiencing pain during the observation of other people's pain would help "feel" what the other person is experiencing by inducing a psychological state similar to that which these neurons contribute to during the experience of pain. FMRI overlaps between the experience and observation of pain have been widely documented, and taken as support for such framework (Keysers, Kaas and Gazzola, 2010; Lamm, Decety and Singer, 2011). This notion was recently in the focus of debate because of mixed results from multi-voxels pattern analyses (Zaki et al. 2016; Corradi-Dell'Acqua et al. 2016; Krishnan et al. 2016). The logic of these analyses is to identify a pattern across voxels that discriminates different intensities of experienced pain from fMRI signals. If pain observation triggers a neuronal representation of felt pain, the logic goes, the same pattern should discriminate different intensities of observed pain – so called above chance cross-modal classification. Some scientists find this to be true (Corradi-Dell'Acqua et al. 2016) others not (Krishnan et al. 2016). However, it is important to realize that a region may have neurons involved in experiencing and observing pain, as our framework predicts, without significant cross-modal classification of fMRI signals (Zaki et al. 2016): decades of work on mirror neurons for actions show only 10% of neurons involved in performing an action become recruited while observing that action (Gallese et al. 1996; Keysers et al. 2003; Mukamel et al. 2010). If the same is true for pain, only 10% of neurons involved in pain experience may also be recruited during pain observation. This low percentage means that a pattern classifier trained on pain experience would be dominated by the signals originating from the 90% of neurons that are not involved in pain observation, and would then fail to reliably interrogate the 10% involved in observation when tested with pain observation data. Until we have systematic single cell data during the experience and observation of pain (Hutchison et al. 1999), whether neurons represent felt and observed pain reliably remains unclear (Zaki et al. 2016). Accordingly, that we find signals from a region of SI involved in actual pain experience to explain variance in helping in our paradigm is compatible with the notion that this signal originates from neurons also involved in pain experience, but this signal could originate from neurons not involved in pain experience that are simply spatially intertwined with those involved in pain experience (Zaki et al. 2016; Keysers & Gazzola 2009). In this manuscript, when we speak of vicarious pain activations, we therefore mean activations of regions involved in pain experience during the observation of the pain of others, without having the means to assess whether this activation originates from the same neurons involved in pain experience.

When pain is expressed by the reaction of the hand, the dorsal SI hand region activation correlates with decision making (EEG). Additionally, altering this activity changes (TMS) decision making, suggesting that the SI hand region activation feeds into the decision making process. When pain is expressed by the face, the SI hand region activation correlates with decision making (EEG) but altering this activity influences decision making less. This latter finding is compatible with three interpretations. (i) Activity

in the hand-region of SI is an epiphenomenon, representing an imagination of what the painful stimulation would have felt on the hand (Fairhurst et al. 2012), that is not used in decision making. (ii) The SI hand region activity is used for decision making in the sham condition, but can be substituted by alternative sources of information derived from the face elsewhere in the brain in the active TMS condition. Both these interpretations are reminiscent of the notion that pain information takes different paths based on the stimulus it is derived from (Keysers, Kaas and Gazzola, 2010; Lamm, Decety and Singer, 2011). (iii) Information in the SI hand region has a higher signal to noise ratio in the Face condition compared to the Hand condition, making it less susceptible to TMS interference.

Our results show that trial-by-trial amplitude of the EEG activity from ventral SI significantly explains changes in donation in the Face condition only. This effect could be driven by a covert internal simulation of the other's facial expression or by overt facial mimicry. Interfering with facial mimicry has been shown to impair visual recognition of expressions (Oberman et al. 2007; Wood, Lupyan, et al. 2016) and interfering with activity in ventral somatosensory cortex alters emotion recognition from faces (Adolphs et al., 2000; Paracampo et al., 2017). Future research should neuro-modulate brain activity in ventral SI in addition to the hand representation we targeted here while measuring the willingness to help in order to further investigate the dissociation suggested by our results. The emergence of focused ultrasounds as a focal neuro-modulation method (Mueller et al. 2014; Lee et al. 2015; Lee et al. 2016), could enable such studies without the muscle artefacts inevitable with TMS. HD-tDCS, as used in our third experiment, also has the advantage not to cause muscle twitches, but lacks the focality to argue with confidence that one can disentangle the contribution of the face and hand region located only 2cm away.

In the Hand movie, movements are displayed both in the first half of the video by the agent wielding the belt and in the second half by the victim's hand being compressed by and reacting to the swat. Activity in SI has been shown to potentially encode all of these (Keysers, Kaas and Gazzola, 2010). Interestingly, SI activity significantly predicted the donation only during the second half, in which the victim's hand is compressed by the belt and reacts to it. This suggests that it is SI's ability to represent the impact of the belt on the hand or the reaction of the victim to the stimulation on the hand that induced the prosocial decision making.

Furthermore we addressed the issue of how SI contributes to decision making, leveraging a third HD-tDCS experiment that allowed us to discriminate between perceptual or motivational contributions. Our results suggest that SI activation in the hand region contributes to prosocial decision-making by transforming the sight of hand-movements caused by a swat into a perception of pain-intensity, which then serves as an input to a decision making process elsewhere. If this trial-by-trial perception is perturbed, our decision to help no longer optimally follows the trial-by-trial variance in pain experienced by others. This function is similar to the function that SI has during the observation of actions. For instance, disturbing the activity of the SI hand region with TMS makes ratings of the weight of an object seen lifted noisier compared to a sham condition, suggesting that the region is necessary for transforming observed hand kinematics into an estimate of the forces that have been acting on the hand (Valchev et al. 2017) similar kinematic analysis may underpin the transformation of the observed hand kinematics following the swat into a painfulness estimate in our Hand condition. Affective social reactions, be they personal distress or empathic concern, would be informed by this kinematic analysis in SI, but require additional processes that the pain experience literature would ascribe to the anterior insula and cingulate (Lee & Tracey 2010). In this interpretation, a neural network including SI informs the participant on how intense the swat was on a given trial, and determines the ability of the participant to adjust the donation to the circumstances of a specific trial. In contrast, the mean donation could reflect trait differences in empathic concern (measured by the Interpersonal Reactive Index, Davis 1983) and

money attitude (Yamauchi & Templer 1982) (Supplemental analysis Correlation with self-reported questionnaires). A more in depth understanding of what emotional feelings (pain-like personal distress vs. more positively valenced empathic concern) accompany the motivational effect of SI activation on high pain trials remains unclear from our data, and could be studied in future research by asking participants to provide specific ratings of their own affect on a trial-by-trial basis.

In summary, we hereby provide evidence that activity in the hand region of SI while witnessing the bodily reactions of a victim to a painful stimulation is not only correlated with the willingness to help but significantly influences prosocial decision making. Our data further constrain the mechanisms through which SI influences prosocial decision-making by showing that altering its activity also influences the perception of other people's pain intensity. This suggests that the role of SI is to help us transform the kinematics of affected body-parts into a perception of pain, which is then a significant input to a decision-making process that occurs elsewhere in the brain. If pain is expressed not through the affected body part but communicated through facial expressions, SI activity in the somatotopic representation of the initially affected body-part no longer seems a necessary input to this decision making. These neuromodulation findings support the notion derived from neuroimaging literature that multiple networks can be recruited during the perception of the pain of others depending on the nature of the stimulus (Keysers, Kaas and Gazzola, 2010; Lamm, Decety and Singer, 2011). Future studies will be needed to isolate and characterize the causal contribution and interaction across the nodes of these networks, and further characterize the conditions under which each network is necessary. That SI vicarious activations directly influence prosociality provides empirical foundation for the intuitively attractive and often suggested causal links between the ability to represent what other people feel and prosocial actions.

Methods and material

Participants

A total of 169 healthy, right-handed volunteers, with normal or corrected-to-normal vision, (mean age= 25 +/- 5 SD) were recruited for our studies (Table 1). Because previous studies reported racial biases to modulate empathy (Xu et al. 2009; Avenanti et al. 2010; Cikara et al. 2014) and our videos showed a Caucasian confederate, only Caucasian individuals were recruited. All participants received monetary compensation and gave their informed consent for participation in the study. None of the participants reported neurological, psychiatric, or other medical problems or any contraindication to fMRI, TMS or tDCS (Rossini et al. 2015; Rossi et al. 2009). No discomfort or adverse TMS effects were reported by participants or noticed by the experimenter. Table1 summarizes the number and characteristics of the participants for each study.

All studies have been approved by the Ethics Committee of the University of Amsterdam, the Netherlands (project identifiers: 2016-BC-7394, 2016-BC-7130, 2017-EXT-8467, 2016-PSY-6485, 2014-EXT-3476, 2014-EXT-3432). All participants received monetary compensation and gave their informed consent for participation in the study. Consent authorization for images publication has been obtained.

Costly Helping Experimental Set-up

Central to our task was the aim of inducing an effective, naturalistic moral dilemma, in which the state induced by witnessing the distress of another individual is pitched against financial rewards. The other

person's distress was elicited by delivering electroshocks or slaps on the right-hand dorsum. To limit the total number of shocks/slaps delivered throughout the experiments and to avoid uncontrollable variance in the reactions of the victim, we developed a cover story. Each participant was made to believe she/he will be paired to another participant, with whom she/he will draw lots to decide who would play the role of the observer and who of the pain-taker. The observer and the pain-taker will be allocated to separate adjacent rooms, connected through a video camera. While the pain-taker would receive the electroshocks/slaps, the observer would witness the reaction of the pain-taker to the stimulations while

Perceived intensity	**EEG exp**		**TMS exp/per session**	
	Hand	Face	Hand	Face
2	13	23	4	10
3	2	3	10	4
4	0	4	4	4
5	27	10	10	6
6	1	11	2	6
7		1		
Average intensity	4 ± 1.4	3.7 ± 1.7	3.9 ± 1.2	3.8 ± 1.6

Table2: Number of videos for each intensity presented as InputMovie in the EEG and TMS experiment. Last line calculates the average movie intensity and its standard deviation presented for each condition and experiment.

EEG was recorded (Experiment 1) or brain stimulation was delivered over SI (Experiment 2). In reality, the lots were manipulated in such a way that the confederate would always be selected as the pain-taker and the participant as the observer. Additionally, participants were misled to think that the noxious stimulations were delivered to the confederate in real-time, and that what participants saw on the monitor was live fed from the pain-taker's room. In reality, we presented prerecorded videos of face and hand reactions to noxious stimuli previously delivered to the confederate (Movies S1, S2, S3, S4). The confederate's appearance during the experiment was carefully matched to the pre-recorded videos. The exact setup shown in the videos was recreated at every session and shown to the participants, including the belt and electric stimulator used. Face and Hand videos were shown in separate sessions (order randomized across participants), with a long break in between. During the break participants could move, leave the experimental room and, importantly, briefly interact with the confederate. This short break helped maintain the cover story.

The choice to use a cover story was dictated by (i) pilot data showed that relaxing the cover-story, for instance by acknowledging that SG was in fact an experimenter, led to a notable reduction in donation, and (ii) the effort to keep the variance introduced by having different victims at minimum, facilitating group analyses. Additionally, our initial piloting also revealed that testing participants over multiple days led to increased skepticism, which was why we decided to use different pools of participants for each experiment, and had to limit the number of trials in the TMS study to what could fit a within subject design.

At the end of the costly helping paradigm, participants answered the question "Do you think the experimental setup was realistic enough to believe it" on a scale from 1 (strongly disagree) to 7 (strongly agree). Five was used as cut off to discriminate between participants who believed in the cover story from those who did not, and participant's reporting 4 or less were excluded from the analyses. The credibility values for the whole sample of participants are shown in Figure 1-Supplementary Figure 1.

Costly Helping Visual Stimuli

Two types of 2s long videos were generated (Figure 1A). The Hand-videos depicted the confederate's right hand reactions to a slap delivered by a brown leather belt (procedure adapted from Meffert et al., 2013). The hand, right arm and shoulder are the confederate's only visible body parts. While the belt was visible, the hand holding it was only at times marginally entering the field of view, and was covered by a black glove to blend in with the black background. The videos started with the belt laying on the hand dorsum. The slap occurred at the end of the first second, during which the belt would be lifted and prepared to hit. Videos ended one second after the slap, after showing the hand and shoulder reaction. A total of 200 Hand-videos were recorded by varying the intensity of the slap at every trial, with 30 seconds between each trial. The Face-videos showed the actor's facial expressions in response to an electroshock delivered to the right-hand dorsum. The upper part of her body was clearly visible on a black background. Even though the stimulation was given to her hand and the hand was visible, the hand did not move in response to the shock making the face the main source of information about the stimulation intensity. The videos started with the face in a neutral expression. During the first second the expression was kept neutral until the stimulation occurred.

Both the hand and face movies were centered not on the moment in which the noxious stimulation was delivered but on the reaction of the actress to them. In the Hand videos the central frame was the one in which the belt hit the hand with consequent immediate reaction of the hand. In the Face Videos the central frame was the one in which the face started changing facial expression. (i.e. at +1s from the beginning of the movie).

A total of 392 Face-videos were recorded. The electrical stimulation was a 100 Hz train of electrical pulses of 2ms pulse duration (square pulse waveform) delivered via a bipolar concentric surface electrode (stimulation areas: 16 mm2). The electrodes were attached to the skin with tape, which was left in place during the Hand-video recording as well. A black 'X' was drawn on the tape to clearly show the electrodes position. Each stimulation lasted 1000ms and varied in current intensity, which ranged from 0.2 mA to 8.0 mA. Thirty seconds were left between stimulations. Current intensity was determined prior to video recording, by following a procedure well established in the literature (de Vignemont & Singer 2006; Cui et al. 2015): starting from a 0.1 mA the current was gradually increased until maximally 8.0 mA in increments of 0.2 mA. The actor was instructed to evaluate how painful each stimulation was on a 10-point scale, and current intensity to be used during video recording were chosen according to a maximum perceived intensity of 8. The actor was the same Caucasian woman throughout videos and experiments: author SG who played the confederate role. The videos were recorded using Sony DSR-PDX10P Camcorder (Sony, Minato, Tokyo, Japan), and edited using Adobe Premiere Pro CS6 (Adobe, San Jose, CA, USA).

The generated 392 videos were validated by an independent group of 40 participants who did not participate in the other experiments (Table 1). Hand and Face videos were presented (EventIDE; OkazoLab Ltd., 2012) in separate blocks, counterbalanced across participants. Participants were instructed to observe them and to report the perceived pain intensity the person in the video felt, using the same 10-point scale employed in the pain threshold assessment. Average ratings were computed and rounded off. The results yielded 6 different movie categories with average pain intensity perceived as 2, 3, 4, 5, 6, and 7 out of 10. Of those, we selected different subsamples for each experiment based on the following two criteria: (a) a low standard deviation in rating across participants to reliably communicate a specific pain intensity, and (b) maximize the statistical power of the regression (i.e. privileging movies at the extremes of the intensity range). For the EEG task, we privileged the criterion (b) and included a larger number of trials to reduce neural habituation (95 trials in total). For the TMS

experiment we privileged criterion (a) to maximize the sensitivity to small changes in perceptual accuracy, and only used those movies in which ratings were most concordant. In both the EEG and TMS experiments, the Face and Hand condition did not differ in average perceived intensity (EEG: M_{Face}=3.7, SD=1.7, M_{Hand} =4 SD=1.4, t(93)=0.88, p=0.4; TMS: M_{Face}=3.8 SD=1.6; M_{Hand}=3.9 SD=1.2, t-test $t_{(118)}$= -0.26, p=0.9) and standard deviation (EEG: $F_{(42,51)}$=1.49, p=0.09; TMS: $F_{(14,14)}$=1.67, p=1.17).

Because the intensity of the OutputMovie depended on the participant's donation it was impossible to precisely predict the number of videos needed for each intensity and participant. This means that in some cases, the number of recorded videos was lower than the number of actual presentation of a particular intensity, and few videos had to be shown more than once. Care was taken to maximize the distance between repetitions of the same stimulus. The number of repeated videos is low and it was never the case that a participant saw all the movies twice. During debriefing, we additionally asked our participants to indicate on a 7 step scale (from 1=strongly disagree to 7=strongly agree) how well the following statement applied to them: "You think you saw twice the same movie." In average, participants of the EEG experiment reported a value of 6.1 (Figure 1-Supplementary Figure 1), and those of the TMS one of 6.3, suggesting seeing the movies twice was not easily recognized by our participants

Costly Helping Paradigm

For the participant, each trial of the costly helping paradigm started with a 1s red fixation cross, followed by a video showing the actor's reaction to the first stimulation (InputMovie, Figure 1A), the intensity of which was randomly determined by the computer. Participants then received an endowment of 6€, and had to decide whether to donate all or part of them to reduce the intensity of the following stimulation. Participants knew, that every 1€ donated reduced the intensity by one unit, and that money was not donated directly to the confederate, who did not have monetary benefit from the donation. Instead, 10% of the money not donated was added as a 'bonus' to participants' compensation for taking part in the study. InputMovie intensity varied from trial to trial to cover the entire range from 2 to 7, and was randomly chosen. Participants' implicit task was to infer the intensity of the stimulation from the confederate's reaction. Participants selected the donation amount by moving a rectangle along a bar with possible donations (0 to 6€). The starting position of the cursor was randomized to avoid motor preparation of the response. Participant were instructed to use a two-pedal controller with their right foot to select the donation (USB Double Foot Switch II, Scythe Co., Ltd., Tokyo, Japan). We used a foot-pedal rather than a traditional button-box because SI activity in the hand region, critical for this experiment, may otherwise have been contaminated by the planning/ performance of the button-presses. After three seconds without pressing any pedal, the software would select the current position of the rectangle as the chosen donation and move to the following black screen, which had a random duration from 1.5 to 3 s. Finally, depending on the donation, 2 sec feedback video (OutputMovie) showed the confederate's response to the second stimulation. OutputMovie would represent the end of a trial. A 5s black screen separated consecutive trials.

EEG study

For the entire duration of the costly helping paradigm, electrophysiological brain signals were recorded from 64 active channels (10-20 positioning) by an ActiCHamp Brain Vision system. The ground electrode was placed on Fz. Electrode impedances were kept below 5 kΩ, all signals digitized (rate of 500 Hz) and stored for off-line processing. All data analyses were performed using the FieldTrip Toolbox (Oostenveld et al. 2011) and customized MATLAB (Mathworks Inc., Natick, MA, USA) scripts. The signals were low-pass filtered at 60 Hz and band-stop filtered within the range of 49.5-50.5

Hz and harmonics to eliminate the electrical line noise. The data were re-referenced to common average and segmented in epochs of 7s containing 5s before InputMovie onset, and lasting until InputMovie end. The segmented signals were visually inspected across all channels. Trials containing muscular and other non-ocular movement artifacts were discarded. The artifact rejection procedure resulted in 41.9±1.7 artifact-free trials in the Hand task and 50.6 ±1.9 in the Face task. Blinks and eye movements were corrected using Independent Component Analysis (Jung et al. 2000). Each trial was then baseline corrected using the average of the signal from 400 to 200 ms before the appearance of the fixation cross.

We used a linear constrained minimum variance beam former approach (Van Veen et al. 1997). We used a 3-layer BEM volume conductance model of 1cm³ resolution as the forward model (Figure 2B, Oostenveld et al. 2003). For each participant, we used the BEM model together with the covariate matrix of the ERP (entire trial length, obtained by averaging all the videos belonging to each conditions) to create an adaptive spatial filter such that its inverse applied to the sensor level representation would reconstruct the source power with maximum strength at the location of the source of interest and suppress the output from the sources of no-interest. To derive the complex source estimates, the time-course of each trial was multiplied with the real-valued filters. The procedure resulted in three orthogonal dipoles within each ROI, oriented in the three spatial dimensions. We constructed our filters separately for the Face and Hand sessions to account for the possibility that the noise structure changed during the long pause (~20 minutes) separating the two sessions. This maximizes the ability to compare different trials of the same session to establish whether donation and brain activity are related within a condition, but reduces the ability to compare the two conditions directly.

To test if brain activity in the ROIs while watching the videos could explain the Z-donation, we first conducted a mass-univariate robust regression analysis (within subjects) with the brain activity as the predictor variable and Z-donation as observed variable. This was done separately for each time point, dipole, task, subject and ROI. Robust regression was chosen to be less sensitive to outliers in the data (Wager et al. 2005). The regression slopes of this subject level analysis were then subjected to Hotelling's T-Squared test (Ht2) at the group level, a multivariate test examining whether the average slope for the three dipoles are all zero. This test was repeated for each time point, condition and ROI. The family-wise error rate arising from multiple comparison of time-points was dealt with a cluster based non-parametric Monte-Carlo correction. Neighboring values exceeding the cluster-cutting threshold (corresponding to Ht2>4.675 and punc<0.01) were combined into a single cluster. Cluster-level statistics were computed by comparing the summed Ht2 values of each cluster against a permutation distribution. The permutation distribution was constructed by randomly flipping the sign of all the sloped of randomly selected participants (1000 iterations) and calculating the maximum group cluster statistic on each iteration. The null distribution of the cluster-based test statistic was obtained by taking the most extreme value of the statistic in each permutation. The cluster-based test statistics in the observed data was then associated a corrected pseudo-p value based on its percentile in the null distribution for each cluster. Furthermore we corrected for number 4 ROIs (2 left and 2 right mirror ROIs) and 2 conditions tested using a Bonferroni correction of 6 (Figure 3), leading to a pseudo-p-value of 0.0063 as the cut-off for the cluster-based test statistic.

TMS study

Sample size for the TMS experiment was determined though a power analysis conducted using G*Power 3 (Faul et al. 2007), with power (1 – β) set at 0.95 and α = 0.05. The effect size was chosen based on the work of Paracampo and colleagues (Paracampo et al., 2017; Cohen's d = 0.94), because it was conducted by the same experimenter that was responsible for the TMS part in the current manuscript (RP); had a task that made similar cognitive demands; used an equivalent rTMS protocol,

and was targeting the same brain region (SI). Comparable effect size (Cohen's d = 0.89) was found when taking into account TMS studies in which participants are required to observe others and understand their behavior (Paracampo et al. 2017; Valchev et al. 2017; Paracampo et al. 2018; Tidoni et al. 2013). Because in our within subjects study we hypothesed a perturbation of the activity in SI, and therefore a reduction in performance as a consequence of the perturbation, we conducted a power analysis for the comparison between performance in active stimulation versus sham stimulation using a matched paired one-tailed t-test at the second (group) level. This analysis yielded a required sample size of 15 participants.

TMS was administered using a figure-of-eight coil (diameter: 70 mm) connected to a Magstim Rapid2 stimulator (Magstim, Whitland, Dyfed, U.K.). To set rTMS intensity and determine coil location, the resting motor threshold (rMT) was estimated for all participants in a preliminary phase of the experiment using standard procedures (Rossi et al. 2009). Motor-evoked potentials (MEPs) induced by stimulation of the left motor cortex were recorded from the right first dorsal interosseous (FDI) by means of a Biopac MP-35. EMG signals were band-pass filtered (30-500 Hz) and digitized (sampling rate: 5 kHz). Pairs of Ag-AgCl surface electrodes (Ø35mm) were placed in a belly-tendon montage with a ground electrode on the wrist. The intersection of the coil was placed tangentially to the scalp with the handle pointing backward and laterally at a 45° angle away from the midline. The rMT was defined as the minimal intensity of stimulator output that produces MEPs with an amplitude of at least 50 μV in the FDI with 50% probability (Rossini et al. 2015). After the rMT procedure, the optimal scalp location for the hand representation in the left motor cortex was marked.

A large body of evidence shows that the hand area in the somatosensory cortex can be successfully targeted positioning the coil 1–4cm posterior to the motor hotspot (Harris et al. 2002; Balslev 2004; Merabet et al. 2004; Fiorio & Haggard 2005; Tegenthoff et al. 2005; Bufalari, Aprile, Avenanti, Di Russo, et al. 2007; Azañón & Haggard 2009; Jacquet & Avenanti 2015; Valchev et al. 2017). This approach is based on the close correspondence between the motor and somatic homunculi (Buccino et al. 2001; Yang et al. 1994; Schulz et al. 2004; Nakamura et al. 1998; Amunts & Zilles 2015; Kuehn et al. 2014). In line with this, we identified our region of interest using a two-step procedure. First, we localized the hand region in the primary motor cortex, corresponding to the optimal scalp position (OSP) for evoking MEPs in the FDI muscle. After that, we moved the coil 2cm backward following a parasagittal plane assuming that this displacement would not produce effects on M1. We tested this assumption directly by checking that TMS pulses applied at 105% rMT with the coil in the final target position did not elicit any detectable MEPs. To rule out any possible interference with the primary motor cortex, intensity for the rTMS was set at 90% of the resting motor threshold. Moreover, before the rTMS session, the position of the coil over the SI-L was verified by applying single pulses of TMS to ensure that no muscle activity was associated with our repetitive stimulations. While performing the costly helping paradigm, a time-locked single train of subthreshold 6Hz rTMS (12 pulses, 2 seconds) was delivered (Tidoni et al. 2013; Paracampo et al. 2017), starting at the onset of the movie and thus covering its entire duration. During active rTMS blocks, the intersection of the coil was placed tangentially to the scalp directly above the scalp location of the target region with the handle pointing backward and laterally at a 45° angle away from the midline. Sham rTMS blocks were performed by tilting the coil at 90° over the same target region, to provide some scalp sensations and TMS sounds comparable to active stimulation but without inducing a current in the brain.

The general procedure of the experiment was kept the same as in Experiment 1, except for changes in number of trials and video presented necessary to adapt the task to the TMS (sham vs active) set-up. Participants underwent a total of 60 trials of Face Condition and 60 trials for Hand condition. The conditions were presented in four blocks (two blocks for the Face and two for the Hand condition),

separated by a long break in the middle in which participants further interacted with the confederate. Each block was equally divided in two parts, which were assigned to active and sham TMS in a pseudo-randomized order (e.g. for one participant: ActiveTMSFaceBlock1part1, ShamTMSFaceBlock1part2, ShamHandBlock1part2, ActiveHandBlock1part2, long break, ActiveHandBlock2part1, ShamHandBlock2part2, ActiveFaceBlock2part2, ShamFaceBlock2part1). While some videos might have been shown twice within block1 or block2, video in block1 were different from videos in block2. At the end of the experimental session, participants had to rate from 1 to 4 how much headache, neck stiffness, itching on the skin, pain on the skin below the stimulation site, sleepiness and mood-swing they experienced, and whether it was difficult to concentrate. Answers were then compared with the TMS effect in the two tasks by calculating the difference in slopes between Active and Sham Condition and correlating this difference with the answer to these questions across participants. Results were corrected for multiple comparison and found to be non-significant.

HD-tDCS study

For this experiment, in addition to the Face and Hand movies, a new set of videos was created, in which no pain was depicted but the color saturation changed over time (Figure 1B, and Movie S5-6). To match the temporal dynamic of Hand and Face videos, the saturation change started after 1 sec from the beginning of the videos and reached its peak 0.5 sec after. Videos were created with 3 different levels of saturation changes. An independent group of twenty participants (Table 1) watched Color, Face and Hand videos (presented using EventIDE; OkazoLab Ltd., 2012), and rated from 1 to 10 how painful the stimulation was for the person in the Face and Hand videos, and how much the saturation changed in the Color videos. Using their left hand they moved a rectangle along a bar with possible ratings, from 0 to 10. The starting position of the cursor was randomized to avoid motor preparation of the response. The validation procedure resulted in a total of 32 videos per category matched for average rating ($F_{(2,93)}$=0.2, p=0.8) and accuracy, calculated as the square of the difference from the expected value ($F_{(2,93)=}$0.4, p=0.6).

Sample size for the tDCS experiment was determined though a power analysis conducted using G*Power 3 (Faul et al. 2007), with power ($1 - \beta$) set at 0.95 and $\alpha = 0.05$. We expected a small effect size based on recent transcranial electrical stimulation experiments (Bolognini et al. 2013; Avenanti et al. 2017). In these studies, the somatosensory cortices were targeted, and similar design and task requirements were used. This analysis yielded a required sample size of 26 participants (Table 1).

1.5 mA was delivered to the left primary somatosensory cortex for 18 minutes through a 4x1 ring-electrode set up consisted of a central active anode, and 4 surrounding return electrodes (Kuo et al. 2013; Figure 2D), which were connected to a battery-operated tDCS MXN-9 High-Definition (HD) Stimulator (Soterix Medical Inc., USA). The HD-tDCS electrodes were fixed to a cap by means of HD-tDCS electrode holders, with the central anode placed over the primary somatosensory cortex, between the EEG electrode sites C3 and CP3. The HD-tDCS electrodes' impedance was kept below 10 kΩ. Throughout the stimulation, the participant was comfortably sat in an arm chair.

Participants received both real and sham stimulation in two different days separated by an average of 7.8 days (SD=3). After sham and real stimulation participants performed the rating task as in the validation procedure. Each trial started with the presentation of a white fixation cross (1s), which was followed by the video clip (2s), followed by the presentation of the visual analogue scale. Using their left hand, participants moved a rectangle along the scale using two keys (one for moving the rectangle to the right, one to the left). A third key was pressed to confirm the intensity selection. A variable interval of between 2800 and 3200 ms separated the trials. Videos were presented in 6 blocks (2 per

tasks) containing multiple intensities. Blocks presentation order was randomized between participants and between sessions.

Both after real and sham sessions, participants rated from 1 to 4 how much headache, neck stiffness, itching on the skin, pain on the skin below the stimulation site, sleepiness and mood-swing they experienced, and whether it was difficult to concentrate.

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Supplementary Information

Pain Localizer and Region Of Interest Definition

An fMRI experiment (Pain Localizer) was performed to identify areas of the somatosensory cortex that encode the subjective intensity of pain stimulation. These regions were then interrogated via beamforming during the costly helping paradigm. 25 Participants (age M=24, SD=5.6, 9 males) with no reported neurological, psychiatric, or other medical problems or any contraindication to fMRI, participated in the experiment. No discomfort was reported by participants or noticed by the experimenter. All participants were paid for their participation. The study has been approved by the Ethics Committee of the University of Amsterdam, the Netherlands. Participants underwent a total of 40 electrical and 40 mechanical stimulations, split in 8 runs (4 electrical and 4 mechanical) of 10 (5 high intensity and 5 low intensity) stimulations each, while BOLD changes were recorded. High and low stimulation were delivered in a pseudo-randomized order (no more than two consequential stimulation of the same intensity) on the participant's right hand using a MRI-compatible stimulation systems (DS7A stimulator - Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK). After a random interval ranging from 2 to 5 s, a question mark appeared on the screen and participants were asked to report their pain intensity by selecting, with their left hand, 1 of 4 available buttons. Each button corresponded to a double step on a scale from 1 to 10, in which 1 corresponded to a non-painful stimulation and 10 the most intense imaginable pain. Because we were not allowed to deliver intensity higher than a reported value of 8, the fifth button (9-10) was not given as a possible option (1-2 index finger, 3-4 middle finger, 5-6 ring finger, 7-8 little finger). The question mark disappeared as soon as a button was pressed. A variable interval of 8-12 s separated the rating period from the next stimulation. Electrical stimulations lasted 0.5 s and current intensity was delivered and chosen for each participant as described in the main text for the video recording. We selected current intensity consistently rated as 2 and 6 prior to scanning, and used them as low and high stimulation during data collection. The mechanical stimulation was delivered through a rubber band mounted on a plastic tube cut longitudinally: participant’s hand was resting inside the tube and the experimenter would snap the rubber band on the participant’s hand following auditory instructions. Also in this case participants underwent a threshold-like procedure similar to the one described for the electrical stimulation.

A Phillips Achieva 3.0 T MRI scanner was used to acquire T2*-weighted echo-planar (32 interleaved 3.5 mm thick axial slices, 0.35 mm gap, TR = 1700 ms, TE = 27.6 ms, flip angle = 73°, FOV = 240 mm × 240mm, 80 × 80 matrix of 3.5 mm isotropic voxels), and whole brain T1-weighted anatomical (1×1×1 mm) images. Each stimulation block lasted a minimum of 3.8 min to a maximum of 6.5 min. FMRI data was pre-processed using SPM12 (www.fil.ion.ucl.ac.uk). All echo planar images (EPIs) were slice-time corrected and realigned to the participant's mean EPI. The T1 image was co-registered to the mean EPI, segmented, and the estimated normalization parameters applied to all EPIs. Normalized (2×2×2mm) EPIs were smoothed with a 6 mm isotropic FWHM Gaussian kernel. A general linear model (GLM) was applied at the single subject level. Predictors were modelled using a standard boxcar function convolved with the hemodynamic response function (HRF). For each of the eight runs of the pain localizer we included: a 5s-length predictor modelling the instruction screen at the beginning of each run, a 500ms predictor aligned to the electrical and a 200ms aligned to the mechanical stimulations, a predictor aligned to the appearance of the question mark until the participant’s button response. For both of the predictors aligned to the stimulation participant’s rating was included as linear parametric modulator. If a participant pressed two or more different buttons after the stimulation, the impossibility to assign a subjective painfulness to the event excluded it from the main predictor, and the event was included in a separated predictor of no interest, together with button presses outside the rating period.

Six additional regressors of no interest were included to account for head movements (none of the included participants had frame-wise displacement along x y or z exceeding the acquired voxel-size). The modulators of the mechanical and electrical stimulation were added and this sum was tested against zero at the second level by means of a t-test. The contrast identified a number of clusters surviving voxelwise FDR at $q<0.05$ ($t>2.23$). Because the uncorrected threshold at $p<0.001$ leaded to more conservative t values ($t>3.5$), we used this threshold to draw our ROIs. ROIs were defined by calculating (ImCalc) the voxels common to (i.e. logical &) the cytoarchitectonic maximum probability maps of SI Left (BA 3a, 3b, 1 and 2) from the Anatomy toolbox for SPM (Eickhoff et al. 2005) & modulated by perceived pain intensity ($t>3.5$). This analysis revealed a dorsal (dSI-L) and a ventral (vSI-L) cluster. Given the spatial resolution of the forward model used in the EEG beam forming (1cm), we rounded the MNI coordinates of the activation peak of each cluster (dSI-L X,Y,Z = -30, -40, 60 and vSI-L X,Y, Z = -50, -20, 30) to guide the EEG source reconstruction procedure (Figure 2 main text). Mirror ROIs have been create flipping sign of the coordinate of the Y axe (dSI-R X,Y,Z = -30, 40, 60 and vSI-R X,Y, Z = -50, 20, 30).

EEG Experiment: mirror ROIs results

We replicated the procedure described in the main test to test whether brain activity originating from the mirror ROIs (dSI-R and vSI-R) also predicts donation. Results show that activity in dSI-R predicts Z-donation in the Hand condition in the time windows between 402 and 484 ms (cluster-statistic=318.5, $p<0.0001$) after the belt hits the confederate's hand and predicts Z-donation in Face between 522 and 562 ms (cluster-statistic=161.0, $p<0.005$). vSI-R brain activity predicted Z-donation but only in the Face condition in the time window between 444 and 676 ms (cluster-statistic=804.3, $p<0.0001$), and between 706 and 738 ms (cluster-statistic=804.3, $p<0.0063$; Figure 3C). Results survive multiple comparisons.

Correlation with self-reported questionnaires

At debriefing, participants completed the Interpersonal Reactivity Index questionnaire (IRI, Davis 1983) and Money Attitude Scale (MAS, Yamauchi & Templer 1982), to respectively measure empathy related traits and attitude towards money.

To assess if this personality traits explained participants' differences in average donation we performed, in the costly helping experiment with the highest power (EEG, 28 participants) a multiple regression with the 4 IRI subscales (Empathic Concern, Perspective Taking, Fantasy Scale and Personal distress), and the MAS score as predictor of the average donation. In line with the notion that average donation reflects a trait, individual that gave high average donations in the Face condition also gave high average donations in the Hand condition (r(Hand,Face)=0.85). We therefore used the grand average donation across both conditions as the dependent measure in the regression. The regression resulted significant ($F(5,22)=3.5$, $p=0.02$, $r2=0.45$). In particular, Empathic Concern and the MAS significantly explained the average donation: the more participants rated themselves as having high empathic concern towards other and the less they affirmed to value money, the more they donated during the Costly Helping Paradigm (EC: $b=0.4$, $p=0.02$; MAS: $b=-0.5$, $p=0.02$; all others $p>0.4$).

Examining individual differences in the slope that links the intensity of pain in the InputMovie (as judged by an independent sample) with the trial-by-trial donation, lead to very different results. First, the slope in the Face and Hand condition were not strongly correlated (r(Hand,Face)=0.23), and multiple regressions performed separately for the Face and Hand slopes revealed that none of the scales significantly explained variance in the slopes (all $p>0.1$).

The above results are not replicated within the smaller group of participants for the TMS study (N=15) and should therefore be considered tentative.

Exploring gender differences

There is some evidence in the literature for gender differences in empathic behavior in a broad range of measures, not only in humans but also in other animals (see Christov-Moore et al., 2014 for a recent overview). Particularly relevant for the present studies are the difference regarding face processing. Women are more accurate and/or efficient in processing facial expressions of emotions in general (Hoffman 1977; Hall & Matsumoto 2004; Korb et al. 2015; Proverbio 2017), and pain in particular (Keogh 2014). Women show more facial mimicry than men (Dimberg & Lundquist 1990), and are better than men in recognize pain also when it is expressed by the body posture (Walsh et al. 2017). In this section we therefore explored the effect of gender in our data. Given the low number of participants in each gender, these analyses should be interpreted in the light of their low statistical power. Detailed analyses are presented below, but in summary, we found no significant evidence for gender effects in our EEG study, and for the neuromodulation studies, we found no significant interactions between gender and neuromodulation.

Experiment 1: EEG study (15 males, 13 females)

Behaviorally there was no evidence for gender differences in average donation or slopes: males average donation Hand Condition: M=2 SD=1.2, females average donation Hand Condition: M=2.3 SD=1.2, independent groups t-test t(26)=0.7 p=0.4; males average donation Face Condition: M=2 SD=1.3, females average donation Face Condition: M=2.2 SD=1.2, independent groups t-test t(26)=0.4 p=0.7; males average slope Hand Condition: M=0.5 SD=0.1, females average slope Hand Condition: M=0.4 SD=0.2, independent groups t-test t(26)=-1.2 p=0.2; males average slope Face Condition: M=0.5 SD=0.05, females average slope Face Condition: M=0.5 SD=0.07, independent groups t-test t(26)=-1.7 p=0.1.

To explore potential gender effects in the relationship between given donation and brain activity recorded in dSI-L and vSI-L, we first averaged together the slopes belonging to all the significant time-points in each task. Then using a t-test for independent groups for each cluster, we compared the average in the genders. Supplementary File 3 summarized the results. There is no evidence for gender difference in any of the clusters average.

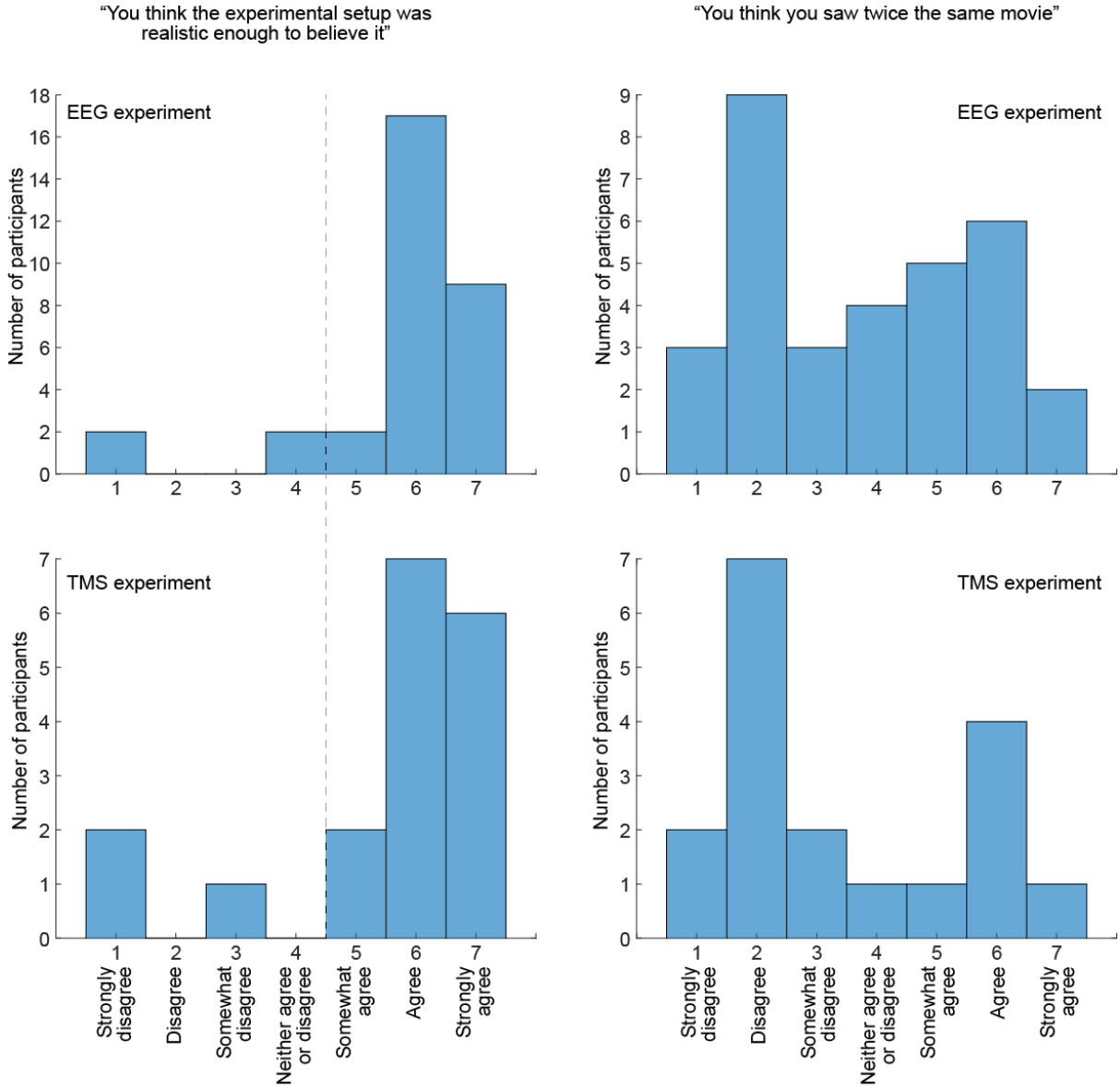
Experiment 2: TMS study (9 males, 6 females)

We performed the same analysis on the slopes described in the main text adding ‘gender’ as a group factor. This new mixed ANOVA with 2 repeated factors (Condition and TMS) and a between participants factor (Gender) did not reveal any gender effect: main effect of Gender F(1,13)=0.008, p=0.9; Condition X Gender interaction F(1,13)=0.02, p=0.9; TMS X Gender interaction F(1,13)= 0.9, p=0.3; Condition X TMS X Gender interaction F(1,13)= 0.06, p=0.8.

Experiment 3: HD-tDCS study (13 males, 13 females)

As in experiment 2, we repeated the ANOVA described in the main text adding ‘Gender’ as a group factor. This new mixed ANOVA with 2 repeated factors (Stimulation and Condition) and a between participants factor (Gender) did not show any significant interaction with between Gender and Condition or Stimulation factors (Interaction Stimulation X Gender F(1,23)=0.3, p=0.6; Condition X Gender F(1,23)=1.5, p=0.2; Stimulation X Condition X Gender F(1,23)=0.03, p=1), suggesting that our the effect of our experimental manipulation does not depend on gender. However the ANOVA shows

a main effect of Gender (F(1,23)=10, p=0.004): females had on average a steeper slope then males in all the tasks, independently of Condition and Stimulation. This result does not seems to be related to differences in empathic ability, since also in the control task females showed a steeper slope. It might be a general and unspecific difference in involvement in the task.



Supplementary Figure 1: histograms of participants' responses for the credibility and movie repetition detection for the experiment with the Costly Helping paradigm. The dotted grey line indicates the cut-off used as exclusion criteria.

Correlation with self-reported questionnaires - Experiment 1: EEG study

Participants from Experiment 1: EEG study completed the Interpersonal Reactivity Index questionnaire (IRI, Davis 1983) and Money Attitude Scale (MAS, Yamauchi & Templer 1982), to respectively measure empathy related traits and attitude towards money. To assess if this personality traits explained participants' differences in performance in the Costly Helping Paradigm we performed a multiple regression with the 4 IRI subscales (Empathic Concern, Perspective Taking, Fantasy Scale and Personal distress) and the MAS score as predictor of the average donation given by the participants on average to the Face and Hand conditions. For both condition the regression resulted significant (Face: F(5,22)=3.8, p=0.01, r2=0.5; Hand: F(5,22)=2.6, p=0.05, r2=0.4). In particular, Empathic Concern and the MAS were in both condition significant predictors of the average donation both in the Face and in

the Hand condition: the more participants rated themselves as having high empathic concern towards other and the less they affirmed to value money, the more they donated during the Costly Helping Paradigm (Face-EC: b=0.4, p=0.03; Face-MAS: b=-0.5, p=0.02; Hand-EC: b=0.4, p=0.3; Hand-MAS: b=-0.4, p=0.03).

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Chapter 3:
Shared brain activity in rating own and others' pain

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A detailed description of each author contribution can be found in the chapter *Reference list and authors' contribution*

Abstract

There is some evidence that areas active when we are in pain are active also when we witness someone else in pain. These areas are referred to as share activity system and are thought to be the neuro-correlate of empathy. Recently the role of these regions has been challenged by new results and analyses techniques. Between other reasons, detractors criticized the designs employed by the classical experiments for not being able to discriminate between brain activity strictly related to perceived/witnessed pain and confounding variables (e.g. a-specific cognitive process, attention and saliency). Another criticism highlights that experiments that induce pain by only one type of noxious stimulation allows for the low level quality of the sensation to impact the results and therefore undermine the generalizability of the findings. Here we aimed to deepen the knowledge on shared brain activity, defined as voxels in the brain linearly modulated by variation in perceived intensity of both experienced and observed pain. We ensured that the results obtained are not attributable to a-specific cognitive process involved in evaluation of intensities and assessed the generalizability of the results to different kind of noxious stimulations. The results revealed that only a region in the AI/ fronto-insular cortex was able to satisfy the criteria, and therefore only a portion of what traditionally called shared activity systems survives a stricter test. Important limitations and weakness of the study are discussed.

Introduction

Pain is a multidimensional experience characterized by sensory, emotional, cognitive and social components, and both when lived in first hand and when witnessed in others, powerfully triggers actions with the goal to make the pain stop. Given its importance in our lives, the extent to which this emotion is studied by researchers of various field is of little surprise. The last two decades have seen an explosion of studies with the goal of investigating how we understand others' people suffering.

A very intriguing framework was given by the discovery of the mirror proprieties of some regions in the brain. A network of key regions seems to be active both when we experience pain and when we witness other people in pain, apparently allowing a direct first-person understanding of others' emotion: 'Your pain is my pain' (Gallese, Keysers and Rizzolatti 2004; Ferrari and Rizzolatti 2014). Brain structures as the anterior insula (AI) and parts of the cingulate cortex (CC) have been recorded while responding for both experience and observed pain (Singer *et al.* 2004a, 2006; Keysers and Gazzola 2009; Corradi-Dell'Acqua, Hofstetter and Vuilleumier 2011a; Lamm, Decety and Singer 2011a; Zaki, Ochsner and Ochsner 2012; Corradi-Dell'Acqua *et al.* 2016; Cui, Ma and Luo 2016). In some cases, also the somatosensory cortex responds in the same fashion. These activations correlate with personal distress (Singer *et al.* 2004b), perceived unpleasantness (Rainville *et al.* 1997) and perceived pain intensity (Lamm, Decety and Singer 2011b). They diminish after placebo analgesia pain (Rütgen *et al.* 2015, 2017) and are predictive of prosocial behaviour (Hein et al., 2010; FeldmanHall et al., 2015, Gallo et al., 2018).

Even though these results are extremely interesting and suggestive, the functional characteristics of these bran activities in not yet extensively explored and recently, because of contradictory new results, many of the findings have been challenged (Woo and Wager, 2015 in Krishnan et al., 2016, Zaki *et al.* 2016). In the presented study, we aimed to expand on the knowledge about shared activity and directly address few of the criticisms moved to classical studies, therefore applying more strict criteria to identify shared brain activity.

Most of the studies comparing experienced and observed pain use designs that do not allow to explore the characteristics of the brain activity. Often they identified the regions by comparing low and high

pain intensities in both experience and observation tasks (Phan *et al.* 2004). This does not guarantee that the activation closely follow and is in tune with the painful experience. Secondly it does not allow to disentangle the effect of processes other than pain, for example attention and salience, that even though participate in the elaboration of the stimuli do not reflect affective processing (Wager *et al.* 2013). In addition, often no other "control" task is used to systematically assess for a-specific variables. Finally, often these studies based their conclusion using only one kind of experienced and observed painful stimulation (i.e. heat, pressure, electricity) failing to prove generalizability of the findings.

We designed a study that aimed to identify brain areas that are sensitive to both experienced and observed pain perceived intensities. We developed stimuli representing different levels of pain intensity and asked the participants to judge their painfulness on a 4 steps-scale. This allowed to identify regions linearly tuned to experienced and observed pain. To control for sensitivity to a-specific cognitive processes that could be involved in the task but not selectively for pain (e.g., attention), we introduced a control condition in which pain was not depicted. To determine that the brain activation was generalizable between different kind of experienced and observed pain stimulations, and not drive by low sensory aspects of it, we compared two type of noxious stimulations. While laying inside the scanner, we exposed our participants to two kind of painful stimulations: whips by a rubber band (mechanical stimulation) and electro-shocks (electric stimulation) on their right hands. Participants also witnessed videos of another person receiving similar kind of stimulations in varying intensities (Pain videos, Figure 1A). In one case the person in the video received an electro-shock on her right hand, and her facial expression in response to the stimulation was shown (Face video). In the other case the person had her hand swatted with a belt, only the reaction of the hand was shown visible to the witness (Hand video). In both the experience and the observation of pain tasks, participants had to rate how painful the stimulation was for them or for the person in the videos respectively. Importantly, the participants observed a third kind of video created by overlapping two frames of the Pain videos. These videos were visually similar to the other two but no pain was depicted. Instead the color saturation increased in various intensities (Color videos, Figure 1A). Participants' task was to rate the saturation intensification using the same scale. We use this as control condition. Here participants were performing the same process, namely rating intensity, but the content of the process was different: in one case it was pain and in the other was color saturation. Subtracting the activity modulated by the perceived change in saturation from the activity while watching the Pain videos allows to clean our results from unwanted task-related variables that did not appertain to pain or affective stimuli processes and from eventual a-specific activity related to the process of rating, such as evaluation of intensity.

We performed a series of analyses focused on independently locate voxels whose activity correlated with subjective perception of own pain and with subjective rating of others' pain and identify the ones that both tasks share.

We compared the brain activation induced by the experience and the observation of two different types of painful stimulations (mechanical and electrical) to test the generalization between kinds of pain experiences. This allowed to assess if the shared activity is tight to the specific somatosensory sensation of pain induce by one of the stimulations, or if it was instead coding for the negative experience induced by the pain independently by how the pain is induced.

Thanks to our design we aimed to identify brain areas that are modulated by both experienced and observed pain perceived intensities, but not to processes regarding general decision making and saliency, and are generalizable between different kinds of experienced and observed pain stimulations.

Methods

Participants and general procedure

26 healthy subjects (age 25 ± 5.6 SD, 15 females, all right handed) participated in the experiment. One participant did not complete the experience task for failure of the fMRI machine, and this specific data were therefore excluded. Because previous studies reported racial biases to modulate empathy (Xu *et al.* 2009; Avenanti, Sirigu and Aglioti 2010; Cikara *et al.* 2014) and our videos showed a Caucasian actress, only Caucasian individuals were recruited. All participants received monetary compensation and gave their informed consent for participation in the study. None of the participants reported neurological, psychiatric, or other medical problems or any contraindication to fMRI. The study has been approved by the Ethics Committee of the University of Amsterdam, the Netherlands.

Participants came to the MRI facility in two different days (sessions). In both days, the experiment started with an explanation (or a reminder for the second day) of the procedures after which the participant underwent a pain threshold procedure outside the scanner to identify level of stimulation consistently associated with perceived pain intensities. Doing so, we allowed the objective intensity of the stimulation to be different in the two sessions, privileging the consistency of the subjective intensity over the objective stimulation intensity. After the pain threshold procedure, the participant underwent a training to familiarize with the visual stimuli and the rating procedure. Author SG was depicted in the video stimuli and she herself explained the stimuli preparation, highlighting that during the recording she received painful stimulation of perceived intensity varying from 1 (not painful) to 8 (painful but bearable) on a scale up to 10 (most imaginable pain). Finally, the participant was placed in to the MRI scanner, where she/he completed the observation task followed by the experience task, in this order in both sessions for all the participants. An anatomical T1 image was recorded between the two condition of the Experience task.

Pain threshold procedure

Participants received painful stimulation on the right hand and observed a person receiving painful stimulation on the right hand. To determinate the appropriate intensity of the stimulation, participants underwent, outside the scanner, a pain threshold procedure well established in the literature (Singer et al., 2004a; Cui et al., 2015, Gallo et al, 2018). Two bipolar MRI compatible Ag-AgCl surface electrodes (Ø35mm) were place on the participants' dorsum of the hand, approximately 5 cm apart.

Current was delivered using DS7A stimulator (Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK) for 0.5 in pulses of 100Hz. We started from a 0.2 mA current that was gradually increased until maximally 8.0 mA in increments of 0.2 mA. The participant was instructed to verbally evaluate how painful the stimulation was on the 10- point scale, where 1 meant no painful and 10 the most imaginable pain. Mechanical stimulation was delivered by a house-made device: a rubber band was mounted on a plastic tube cut longitudinally, the participant's hand was resting inside the half tube and the experimenter would pinch the rubber band on the participant's hand (see Figure 1B for an illustration of the device). The mechanical stimulation was adapted from a previous study conducted in the laboratory (Meffert *et al.* 2013). The maximum reported intensity reached was 7. Intensities consistently rated as 2 and 6 were noted to be used inside the MRI scanner in the Experience task.

Experience task

Participants were laying inside the MRI scanner. Before starting the task, the intensities of the stimulations were verified to ensure their consistency with those collected during the pain threshold procedure. For each of the two fMRI sessions, participants underwent to a total of 20 electrical and 20 mechanical stimulations, split into four fMRI runs, each containing either 10 electrical or 10 mechanical stimulations. Each run contained five high intensity stimulation (i.e. perceived as 6 in the pain threshold procedure), and five low intensity stimulation (i.e. perceived as 2). Within each run, the intensities were delivered in a pseudo-randomized order (no more than two consequential stimulations of the same intensity). The order of the kind of stimulation was randomized between participants. A grey cross was

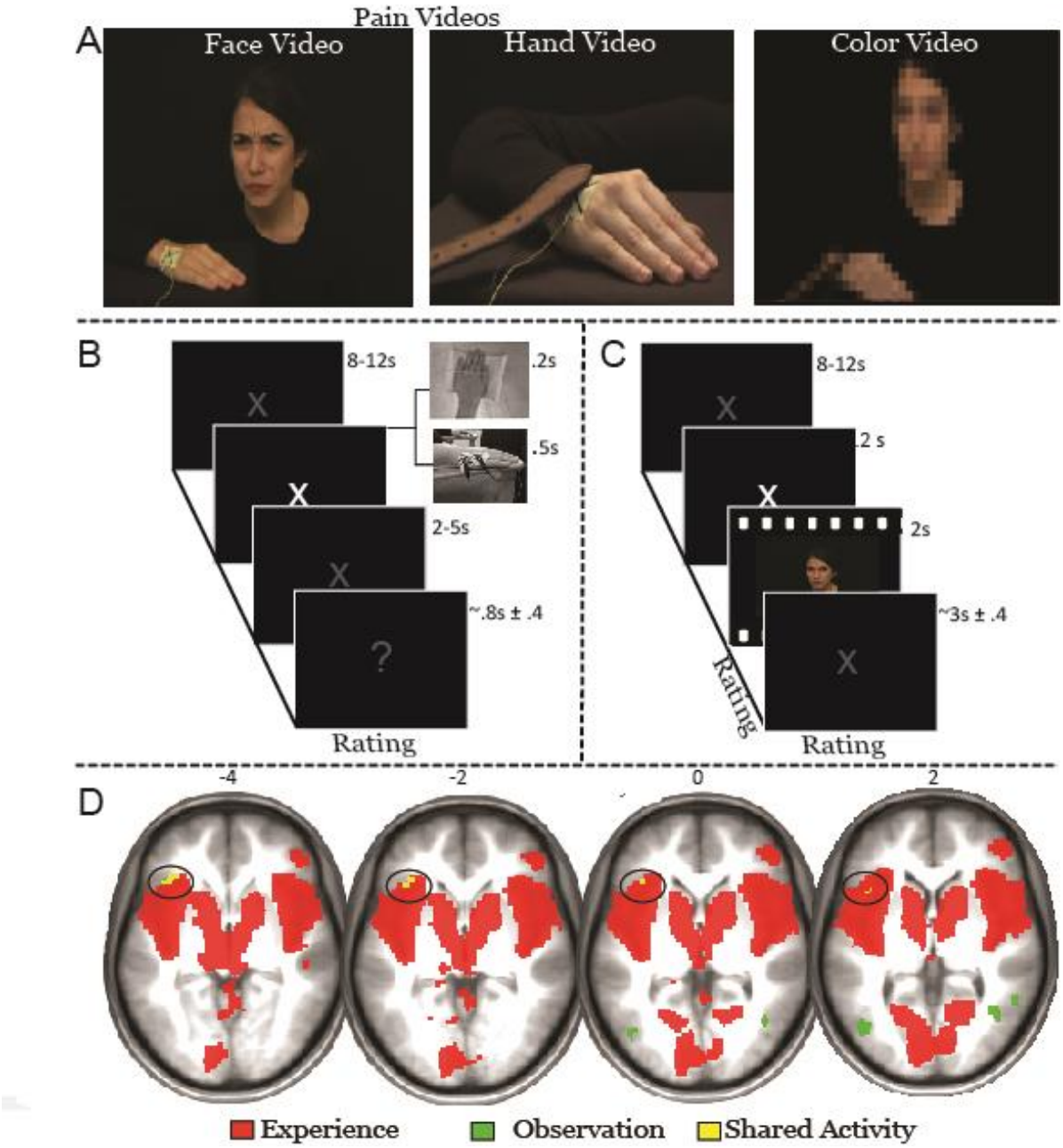

Figure 1. A) A snapshot from the Face, Hand and Color videos. B) A schematic representation of a trial from the Experience task. C) A schematic representation of a trial of the Observation task. D) All brain results of the Experience task contrast *ExpPainModulator* (red) and Observation task contrast *ObsPainModulator* (green). Each voxel has a p_{unc}<0.001 and survived correction for multiple comparison (qFDR=0.05, k=10). In yellow is the shared activity between the two tasks. Slice number is reported on top of each slice.

presented as visual baseline throughout the fMRI run. During the stimulation, the cross color changed into white. The change in color was important for the experimenter in the MRI control room to signal that the stimulation happened and as a cue to manually prepare the device to deliver the next one. After a 2-5 s random interval, a question mark appeared, and participants reported the intensity of the received stimulation by selecting, with their left hand, 1 of 4 available buttons on an fMRI-compatible button-box. Each button corresponded to a double step on a scale from 1 to 10, in which 1 corresponded to no pain and 10 to the most intense imaginable pain. Note that the participants were aware that they would never receive a stimulation higher than a reported value of 7, so the fifth button (9-10) was not given as a possible option (1-2 index finger, 3-4 middle finger, 5-6 ring finger, 7-8 little finger). When the participant pressed the button, the question mark disappeared from the screen. A variable interval of 8-12 s separated the rating period from the next stimulation (Figure 1B). Electrical stimulations were delivered on the participant's right hand using the MRI-compatible version of the electrical stimulation system used to determine the pain threshold (DS7A constant current stimulator, Digitimer and Biopac System Inc filter cables), while the mechanical stimulation was delivered by the same device used during the pain threshold procedure.

Data acquired during this task have been reported also in Gallo at al. 2018.

Observation Task

A total of 168 unique videos (repeated twice and divided in four runs of 84 videos each) were presented during the Others' Pain task. All videos lasted 2s and depicted three scenarios, which defined to three experimental conditions: the Face, Hand and Color condition. The same stimuli are used in Gallo at al. 2018 (please refer to that study in Chapter 2 for more details about the videos making and their validation).

The first scenario (Face condition, Figure 1A) showed a person seated at a table with the right hand leaning on it. The face, torso and the right harm were visible, and an electrode was attached to the dorsum of the hand and covered with a tape marked with a black cross. The first half of the videos (0 to 1s) captured the static image of the actor SG, the second half the reaction to the electrical stimulation which was manifest through a change in facial expression (1 to 2s). Videos were created so that the face expression started changing exactly after 1s from beginning. The actress's hand did not move during the stimulation, therefore the manifestation of distress was only observable from the face. The intensity of the electrical stimulation was varied during the recording in order to induce difference distress reactions. The second scenario showed a hand receiving a mechanical stimulation, more specifically, being whipped by a belt (Hand condition, Figure 1A). Only the actress's right hand, her arm and shoulder were visible. The same tape described above was attached to the hand dorsum. The video started with the belt resting on the hand. In the first half of it (0 to 1s) the belt rises to hit the hand. Videos were created so that the belt hit the hand exactly 1s after beginning. The second half showed the hand/arm/shoulder reaction to the stimulation. Face and Hand conditions are referred together as Pain condition. The third scenario showed videos composed by superimposition of a blurred version of the first still frame of the previous videos (Color condition, Figure 1A). The frame remained still for the first half of the video (from 0 to 1s), after which the saturation of the color gradually increases, reaching its peak after 1.5s from the beginning of the video and remaining stable until the end. The intensity of the color saturation in the Color video varied across stimuli and was used as a control condition (no pain depicted). All the videos were previously validated by independent pools of 40 participants, who rated them on a scale from 1 (no pain/no saturation change) to 10 (most imaginable pain/maximum saturation change). Average ratings were computed and rounded off. The three conditions did not differ

in average ratings (One-way Anova $F_{(2,165)}$=.51084, p=.60094, Face mean=3.6, sd =1.6; Hand mean=3.7, sd=1.5; Color mean=4, sd=1.6).

In each fMRI session, the following number of unique videos were presented: 20 Face videos of intensity 2, 20 Hand videos of intensity 2, 20 Color videos of intensity 2, 20 Face videos of intensity 4, 20 Hand video of intensity 4, 40 Color videos of intensity 4, 16 Face videos of intensity 6, 12 Hand video of intensity 6, 20 Color videos of intensity 6. Videos presentation was pseudo-randomized: the same condition and intensity were not repeated more than 3 times in a row. Videos were equally divided in two runs and each participant was presented with the same set of videos in both sessions, so that each participant saw every video twice. The software EventIDE (v1.3.10, Okazolab Ltd, UK) was used for all the videos' presentation.

Each trial consisted of a two-second video, preceded (1s) by a white fixation cross in a black background and followed by a grey fixation cross (6-10s). Participants could give their rating anytime during or after movie presentation (Figure 1C). In either cases, the video played its entire duration (2s) and was replaced by the grey cross once it ended. Participants were asked to report the chosen rating using the same procedure described above for the experience task.

MRI data acquisition

A Phillips Achieva 3.0 MRI scanner was used for image acquisition. We used a T2*-weighted echo-planar sequence with 32 interleaved 3.5 mm thick axial slices and a 0.35 mm gap for functional imaging (TR = 1700 ms, TE = 27.6 ms, flip angle = 73°, FOV = 240 mm × 240mm, 80 × 80matrix of 3.5mm isotropic voxels). For both sessions, between two runs of Experience task, a T1-weighted anatomical image (TR = 8.2 ms, TE = 3.8 ms, flip angle = 8°, FOV = 240 mm × 240mm, 1 x 1 x 1 mm isotropic voxels) covering the whole brain was acquired.

Image pre-processing

FMRI data was pre-processed using SPM12 (www.fil.ion.ucl.ac.uk) under MATLAB environment (version R2014b, The MathWorks Inc, Natick, Mass). All echo planar images (EPIs) were slice-time corrected and realigned to the participant's mean EPI. The two T1 images were realigned between themselves and the resulting mean-T1 was then co-registered to the mean EPI, segmented, and the grey matter was used to estimate the normalization parameters which were then applied to all EPIs. Normalized (2×2×2) EPIs were smoothed with a 6 mm isotropic FWHM Gaussian kernel.

Data Analyses

Behavioural Analyses

We tested participants' consistency in rating style by correlating the average ratings given in the Experience and Observation tasks.

In both Experience and Observation tasks, repeated measure ANOVAs were used to test behavioural difference in average rating between the different types of stimulation and the effect of repeating the tasks in two different days including 'Sessions' as factor.

We assessed the consistency of the rating given to the video shown in the Observation task with the ratings given to the same videos by the independent poll of participants who participated in the video validation procedure by correlating the average rating for each condition (Face, Hand, and Color).

In the Observation task participants indicated the rating they attributed to each videos by pressing a button as soon as they assigned it. Reaction time to rating the videos intensities during Observation task were locked to stimulus presentation and reported in seconds. Reaction time shorter than 2s would indicate that the participants responded during video presentation, while after 2s meant they responded after the video ended. Eventual average differences in reaction times between conditions might have an influence when analysing the fMRI data. To test if there was a different in reaction time, we run a repeated measure ANOVAs between the three conditions (Face, Hand, and Color).

Results from both frequentist and Bayesian approaches are reported. Theoretical differences between frequentist and Bayesian approaches have been amply described in dedicated literature (Wagenmakers *et al.* 2017, 2018). In particular, Bayesian approach is a very useful complement to the more common frequentist approach in analysis in which the alternative hypothesis is rejected, since it allows to make statistical statement about the null hypothesis. This is of particular relevance when we hypothesized a lack of difference between conditions as we do when testing the generalization of the brain activity to different kind of painful stimulations. This also allows to display how much of our results depend on the statistical method employed.

fMRI analyses

Two separate general linear models (GLM) were applied at the single subject level for the Experience and Observation Task. Predictors were modelled using a standard boxcar function convolved with the hemodynamic response function (HRF).

Pain Experience Task GLM

For each of the runs of the Pain Experience task, we included the following predictors. Two predictors captured the time of the electrical (duration=500ms) and mechanical (duration=200ms) stimulations. Stimulation duration have been decided a-priori based on the actual duration of the stimulations and not on the subjective perception of the stimulation duration. For both kind of stimulation the rating given by the participant was included as linear parametric modulator. Another predictor contained the rating period from the appearance of the question mark until the participant's button press. Because each run started with a screen of instruction, one predictor collected this initial visual stimuli. The instruction screen showed a schematic representation of the respond box and the rating values associated to each button, together with the text "When "?" appears, press the button that corresponds to the value of pain you feel. Remember: 1= no pain, only tactile sensation; 10= the most intense pain you can imagine". The predictor was aligned with the presentation of the initial screen and lasted for 5s.Two more predictors were additionally present in the model depending on the participant's behaviour. If any button was pressed outside the rating period, a predictor of duration zero collected it. If a participant pressed two or more different buttons after the stimulation, the impossibility to assign a subjective painfulness excluded the stimulation from the main predictor and were included in a separated one. Six additional regressors of no interest, resulting from the realignment procedure, were entered for each of the eight runs to account for translations and rotations of the head (none of the included participants had head motions parameters exceeding the acquired voxel-size).

Statistical analysis was performed in a two-level procedure. First, for each participant a contrast identifying brain activations parametrically modulated by perception of pain independently by its source was calculated by combining (i.e. summing) the BOLD signal that correlated with the subjective rating of intensity of electrical and mechanical stimulation. The resulting β-maps of the rating of painfulness of the stimulation (contrast *Shock Modulator* + *Mechanical Modulator*, called *ExpPainModulator*) were analysed using a one-sample t-test. Results were thresholded at p_{unc}=0.001 (uncorrected) with a

minimum cluster size of 10. All results presented also survived qFDR=0.05 (false discovery rate). This procedure allowed to correct for false discovering rate at the cluster level and also insure significance of as the significance of individual voxels within that cluster (Cui *et al.* 2015).

Observation Task GLM

For each of the Observation task, the following predictors were included. Three separated predictors collected the video of each condition: one regressor for Face video, one for Hand video and one for Color video. Since each video was preceded by 1s fixation cross, the regressor was aligned with the appearance of the fixation cross and lasted for the entire duration of the movie for a total of 3s duration (cross + movie duration). The rating given to the video was included as linear parametric modulator. One regressor collected the instruction screen (5s). As for the Experience task, the instruction screen showed a schematic representation of the response box and the rating values associated to each button. The text said: "Press the button that corresponds to the intensity of pain or color you see". Two more predictors were additionally present in the model depending on the participant's behaviour. In case a participant did not press any button or pressed two or more different buttons after the videos, the impossibility to assign a subjective painfulness excluded the video from the main predictor. Six additional regressors of no interest, resulting from the realignment procedure, were entered for each of the eight runs to account for translations and rotations of the head (none of the included participants had motions parameters exceeding the acquired voxel-size). Button presses were not modelled. The activity related to planning and pressing the buttons was common between conditions and therefore cancelled out when the conditions were compared.

To identify the brain activity that is modulated by the pain rating independently from how the pain is depicted, for each participant, the main contrast of interest representing the hemodynamic response specifically modulated by pain rating was calculated as Face Modulator + Hand Modulator -2*Color Modulator. Throughout the manuscript we will refer to this contrast as *ObsPain modulator*. We also calculated the contrasts representing the BOLD signal parametrically modulated by the subjective rating of the Face minus Color videos and Hand minus Color videos. These two contrasts would identify the brain activity specifically modulated by the two conditions (Face or Hand video) independently from each other but corrected for the control task activity. Finally to assess if there are difference in the brain activity modulated by the two conditions we calculated the contrast representing brain activity modulated by the rating of the Face videos more than by the Hand videos and vice versa. Brain activity related to the presentation of the Pain videos not modulated by the rating are described in "Supplementary Information - Face videos vs Hand videos: average brain activity".

To obtain all brain group results, a random effect test was performed on all contrasts (single sample t-test) to find voxel consistently greater or lesser than zero between participants (typically referred as second level analysis). As in the Experience task, results were thresholded at p_{unc}=0.001 with a minimum cluster size of 10 and all presented results also survived qFDR=0.05.

Please note that since the brain activity representing the button presses is not modelled and it might correlate with the rating itself, it is not advisable to calculate the activation related only to the videos and not contrasted with other tasks. To assess the brain activity related to each kind of video separately and modulated by rating of intensity, we design another GLM. There we categorized each trial as high or low rating and for each condition we contrast the High-Rating trials against the Low-Rating trials. Details of the GLM design and results are described in "Supplementary Information: High vs Low Pain design."

Pain shared activation

β-map of each main contrasts of interest, *ExpPain modulator* and *ObsPain modulator* was analyzed using a one-sample t-test. We first identified cluster of voxel surviving a threshold of FDR q>0.05 and uncorrected p>0.001 for each task and then we selected the ones active in both tasks (using ImCalc, logic operation "&"). This analysis resulted in clusters of voxels responding to both subjective ratings of perceived painfulness in others and self, irrespectively from the type of pain inflicted.

Pain shared activation – Generalization between pain

To assess the results are not pull by one of the two kinds of stimulation and that the cluster codes for the two stimulation in the same fashion, we extracted from these clusters, using the Marsbar toolbox for SPM (http://marsbar.sourceforge.net), the contrasts corresponding to the difference between hemodynamic response modulated by rating of Face Videos minus the rating of Hand Videos (first level analysis) to explore if this clusters differentiate between the two king of stimuli (single sample t-test) in the observation tasks. For the experience task we extracted the contrasts corresponding to the difference between hemodynamic responses modulated by rating of *Shock Modulator minus Mechanical Modulator*.

Results

Behavioural results

Participants were consistent in their rating style between Experience and Observation tasks, meaning that participants who gave on average high ratings in the Experience task (average between conditions) also give high rating in the Observation task (average between conditions; Pearson's r=0.63, p<0.001, $B_{10=53.4)}$

In the Experience task, the frequentist ANOVA with Sessions (first and second) and Conditions (shock and mechanical) as repeated factor did not show a difference in average rating between the two sessions (First Session mean= 3.8, sd=0.4; Second Session mean= 3.9, sd=0.4; main effect of Session: $F_{(1,24)}$=1.9, p=0.2), nor a difference in average rating between conditions (Shock mean=3.9 sd=0.4; Mechanical mean=3.8 sd=0.3; main effect of Condition: $F_{(1,24)}$=2.3, p=0.1), nor an interaction between the factors Sessions and Condition (First Session-Shock: mean=3.8 sd=0.5; First Session-Mechanical: mean=3.8 sd=0.3; Second Session-Shock: mean=3.4 sd=0.5; Second Session-Mechanical: mean=3.8 sd=0.3; Interaction: : $F_{(1,24)}$=0.25, p=0.6). Bayesian repeated measure ANOVA (using JASP default priors: r scale random effect prior=0.5, r scale fix effect prior=1, r scale covariate=0.354, Wagenmakers et al., 2017a) failed to support either of the hypothesis for a difference in Session (B_{10}=0.5) and for a difference between type of stimulations (B_{10}=0.5), but confirmed that there was no interaction of Session and Condition on average rating (BF_{10}=0.07).

Rating given to the videos shown in the Observation task were consistent with the ratings given to the same videos by the independent poll of participants who participated in the video validation procedure (all correlation p-val<0.005; Face Video average Pearson's r =0.85, minimum r=0.68, maximum r=0.94; Hand Video average Pearson's r =0.59, minimum r=0.29, maximum r=0.74; Color Video average r=0.79, minimum r=0.50, maximum r=0.88).

Frequentist repeated measure ANOVA did not find difference in the average rating of the two sessions (First Session mean= 4.3, sd=0.7; Second Session mean= 4.2, sd=0.7; main effect of Session: $F_{(1,24)}$=1.7,

p=0.2). As expected from the video validation, the analyses did not find difference in the average rating of the different video conditions (Face Video: mean= 4.5, sd=0.5, Hand Video: mean= 4.3, sd=0.8, Color Video: mean=4,2, sd=0.6; $F_{(2,24)}$=0.83, p=0.4, Greenhouse-Geisser correction for violated sphericity applied). The analysis did not reveal difference in average rating between conditions depending on the session order (First Session-Face video: mean=4.2 sd=0.6; First Session-Hand video: mean=4.4 sd=0.8; First Session-Color video: mean=4.2 sd=0.6; Second Session-Face video: mean=4.1 sd=0.5; Second Session-Hand video: mean=4.2 sd=1.0; Second Session-Color video: mean=4.2 sd=0.7; F $F_{(2,48)}$=1.1, p=0.3). Bayesian approach (using JASP default priors: r scale random effect prior=0.5, r scale fix effect prior=1 (Wagenmakers *et al.* 2017) confirms that there was no difference in average rating between sessions (BF_{10}=0.3), nor condition (BF_{10}=0.07), nor interaction between the two factors (BF_{10}=0.01)

Frequentist repeated measure ANOVA showed that average reaction times between the three conditions of the Observation task are different ((Face Video: mean= 3.3, sd=0.3, Hand Video: mean= 3.2, sd=0.4, Color Video: mean=3.3, sd=0.3), $F_{(2)}$=5, p=0.01). Bayesian approach (using JASP default priors as described above) supported the hypothesis that the averages differed (B_{10}=4). To explore the difference in reaction time, we conducted frequentist post-hoc t-tests between the conditions and corrected the results for multiple comparison using Holm procedure. These analyses highlight a difference in average reaction time between Hand and Color condition ($t_{(24)}$=-2.7, p_{holm}=0.03) but not between Face and Color ($t_{(24)}$=-0.8, p_{holm}=0.4) nor Face and Hand condition ($t_{(24)}$=2.1, p_{holm}=0.09). Bayesian dependent sample t-test confirmed the difference on average reaction times between Hand and Color condition (B_{10}=4.1). The test showed there were no difference between average reaction time between Face and Color (B_{10}=0.28). Finally, there were no evidence to support either difference or lack of difference between Face and Hand (B_{10}=1.4). Given the design of our experiment, difference in reaction time between conditions could have had an impact on fMRI results. The results of this analyses were later compared to the fMRI results and the implication commented in the discussion session.

fMRI results

Brain regions modulated by subjective perception of pain (Experience task)

In the Experience Task, all brain single sample t-test on the contrast *ExpPainModulator* identified a number of clusters surviving voxel wise FDR at q<0.05 (t>2.23) and uncorrected p<0.001 (t>3.47), including the areas traditionally associated with pain, bilateral ACC and MCC, bilateral anterior and posterior insula and the bilateral primary and secondary somatosensory cortex (Table1 and Figure1D). Our results are in line and replicated the brain regions obtained by automated meta-analysis toolbox Neurosynth (www.neurosynth.org Yarkoni et al., 2011) for reverse inference of the term 'pain'.

ExpPainModulator							
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**	**Assign to**
1	26453	8	24	28	10.86	R ACC	
		-40	12	-8	10	L Insula Lobe	
		-6	28	20	9.97	L ACC	Area 33
		-4	-6	48	9.77	L MCC	
		-4	28	34	9.6	L MCC	
		4	18	38	9.49	R MCC	
		38	12	-4	9.29	R Insula Lobe	
		42	22	-4	9.27	R Insula Lobe	

ExpPainModulator							
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**	**Assign to**
		34	18	-6	9.19	R Insula Lobe	
		-44	-22	10	9.16	L Heschls Gyrus	Area TE 1.0
		-34	-24	64	9.1	L Precentral Gyrus	
2	3570	22	-48	-24	8.86	R Cerebelum (IV-V)	Lobule VI (Hem)
		0	-66	-16	6.74	Cerebellar Vermis (6)	Lobule VI (Verm)
		30	-66	-28	6.3	R Cerebelum (VI)	Lobule VI (Hem)
		4	-62	-22	6.22	Cerebellar Vermis (6)	Lobule VI (Verm)
		42	-56	-30	6.21	R Cerebelum (Crus 1)	Lobule VIIa crusI (Hem)
		-6	-88	2	6.21	L Calcarine Gyrus	Area hOc1 [V1]
		14	-62	4	5.94	R Lingual Gyrus	Area hOc1 [V1]
		16	-68	6	5.79	R Calcarine Gyrus	Area hOc1 [V1]
		-8	-76	8	5.52	L Calcarine Gyrus	Area hOc1 [V1]
		24	-60	2	5.41	R Lingual Gyrus	Area hOc1 [V1]
		12	-80	4	5.32	R Calcarine Gyrus	Area hOc1 [V1]
3	551	-30	-48	-30	6.44	L Cerebelum (VI)	Lobule VI (Hem)
		-28	-62	-24	5.88	L Cerebelum (VI)	Lobule VI (Hem)
		-18	-74	-26	5.01	L Cerebelum (Crus 1)	Lobule VIIa crusI (Hem)
		-36	-56	-28	4.98	L Cerebelum (VI)	Lobule VI (Hem)
4	350	-30	46	22	6.9	L Middle Frontal Gyrus	
		-30	40	32	4.57	L Middle Frontal Gyrus	
		-28	42	30	4.57	L Middle Frontal Gyrus	
		-44	50	12	4.43	L Middle Frontal Gyrus	
		-46	48	10	4.38	L IFG (p. Triangularis)	
		-38	52	16	4.11	L Middle Frontal Gyrus	
		-34	36	18	4.06	L Middle Frontal Gyrus	
5	319	50	40	0	4.88	R IFG (p. Triangularis)	
		46	48	8	4.66	R Middle Frontal Gyrus	
		48	48	2	4.6	R Middle Frontal Gyrus	
6	287	12	-66	50	5.85	R Precuneus	
		16	-56	58	4.41	R Superior Parietal Lobule	
7	225	28	52	20	5.58	R Middle Frontal Gyrus	
		38	42	24	4.08	R Middle Frontal Gyrus	
		34	42	28	4.08	R Middle Frontal Gyrus	
		32	44	30	4.04	R Middle Frontal Gyrus	
		48	42	20	4.01	R Middle Frontal Gyrus	
8	54	-46	0	38	4.41	L Precentral Gyrus	
		-52	10	38	4.34	L Precentral Gyrus	Area 44
9	19	50	-26	-6	4.86	R Middle Temporal Gyrus	
10	14	40	-42	40	3.76	R Inferior Parietal Lobule	Area hIP1 (IPS)

Table1. Activation table of the contrast *ExpPainModulator*. Coordinate and t-values of the cluster peaks are reported. The results in each voxel are individually thresholded at p_{unc} =0.001, k=10, and survived qFDR =0.05.

Brain regions modulated by subjective rating of videos (Observation task)

In the Observation Task, all brain single sample t-test (positive tails) on the contrast *ObsPainModulator* identified a number of clusters surviving voxel wise FDR at q<0.05 (t>4.42) and uncorrected p<0.001 (t>3.47) in which the hemodynamic response was linearly modulated by the pain videos rating more then by the Color videos rating. See Table2 for the full description of the results.

The contrast *Face modulator minus Color modulator* identified a number of areas in which the hemodynamic response was linearly modulated by the Face videos rating more then by the Color videos rating. This included cluster peaks in regions traditionally associated with mirror system activity such as Insula and Secondary somatosensory cortex. See Table3 for the full description of the results.

The contrast Hand minus Color videos did not results in any voxels surviving the threshold. Contrasting brain activity modulated by the rating of the Face videos and the Hand videos did not results in any voxels surviving the threshold neither.

ObsPainModulator							
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**	**Assigned to**
1	143	44	-66	10	6.15	R Middle Temporal Gyrus	
		54	-56	4	5.94	R Middle Temporal Gyrus	
		40	-64	2	5.68	R Middle Occipital Gyrus	
		50	-60	10	5.34	R Middle Temporal Gyrus	
2	129	-44	-66	8	6.1	L Middle Temporal Gyrus	
		-44	-74	2	5.9	L Middle Occipital Gyrus	Area hOc5 [V5/MT]
3	28	-36	30	-4	5.09	L Inferior Frontal Gyrus (p. Orbitalis)	
4	20	-30	-64	40	5.17	L Inferior Parietal Lobule	Area hIP3 (IPS)
5	19	-32	-70	14	5	N/A	
		-28	-70	18	4.68	N/A	

Table 2. Activation table of the contrast *ObsPainModulator*. Coordinate and t-values of the cluster peaks are reported. The results are individually thresholded at p_{unc} = 0.001, k = 10, and survived qFDR=0.05.

Face modulator minus Color modulator							
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**	**Assigned to**
1	850	40	-64	2	7.55	R Middle Occipital Gyrus	
		52	-56	6	6.71	R Middle Temporal Gyrus	
		42	-62	8	6.66	N/A	
		52	-50	6	6.44	R Middle Temporal Gyrus	
		52	-44	8	5.75	R Middle Temporal Gyrus	
		60	-24	18	4.98	R SupraMarginal Gyrus	Area OP1 [SII]
		60	-42	14	4.71	R Superior Temporal Gyrus	Area PFm (IPL)
		44	-78	0	4.37	R Middle Occipital Gyrus	Area hOc4la
		66	-18	20	4.33	R SupraMarginal Gyrus	Area PFop (IPL)

Face modulator minus Color modulator							
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**	**Assigned to**
		56	-18	14	4.3	R Rolandic Operculum	Area OP1 [SII]
		64	-36	16	4.22	R Superior Temporal Gyrus	Area PF (IPL)
2	779	-44	-66	6	7.65	L Middle Temporal Gyrus	
		-40	-68	6	7.45	L Middle Occipital Gyrus	
		-32	-70	12	6.16	N/A	
		-48	-56	10	6.08	L Middle Temporal Gyrus	
		-60	-46	10	5.61	L Middle Temporal Gyrus	
		-24	-72	16	4.85	N/A	
		-18	-80	18	4.57	N/A	
		-30	-78	8	3.84	N/A	
3	73	-12	26	36	5.11	N/A	
4	66	-28	-62	40	5.81	L Inferior Parietal Lobule	Area hIP3 (IPS)
5	59	-24	-90	-8	6.13	L Inferior Occipital Gyrus	Area hOc3v [V3v]
		-28	-88	-8	5.6	L Inferior Occipital Gyrus	Area hOc3v [V3v]
6	52	10	-4	50	4.6	R Posterior-Medial Frontal	
7	51	14	-20	-8	4.68	N/A	
		20	-22	-6	4.07	N/A	Thal: Parietal
		24	-24	-6	4.06	N/A	Thal: Temporal
		6	-10	0	3.96	R Thalamus	Thal: Prefrontal
		10	-14	-2	3.7	R Thalamus	Thal: Prefrontal
8	49	-38	30	-4	4.56	L IFG (p. Orbitalis)	
9	37	36	6	10	4.07	R Insula Lobe	
10	35	-10	-12	0	5.12	L Thalamus	Thal: Prefrontal
11	32	50	6	24	4.94	R IFG (p. Opercularis)	
12	28	-4	-8	64	4.62	L Posterior-Medial Frontal	
13	26	6	16	62	4.57	R Posterior-Medial Frontal	
14	26	38	-16	14	4.32	R Insula Lobe	Area Ig2
15	23	18	-60	46	4.36	N/A	
		22	-62	52	4.24	R Superior Parietal Lobule	Area 7A (SPL)
16	20	26	-70	6	4.28	R Calcarine Gyrus	Area hOc1 [V1]
17	18	6	-12	72	4.63	R Posterior-Medial Frontal	
		6	-6	74	4.3	R Posterior-Medial Frontal	
18	17	-42	-28	12	4.67	L Superior Temporal Gyrus	Area TE 1.1
19	17	-38	18	2	4	L Insula Lobe	
20	15	10	-6	62	4.42	R Posterior-Medial Frontal	
21	10	-8	-76	42	4.13	L Precuneus	
22	10	36	-78	14	4.25	R Middle Occipital Gyrus	
23	10	-36	2	8	4.15	L Insula Lobe	
24	10	30	-90	-2	3.93	R Inferior Occipital Gyrus	Area hOc4lp
25	10	-56	8	-16	4.6	L Temporal Pole	

Table 3. Activation table of the contrast *Face modulator minus Color modulator*. Coordinate and t-values of the cluster peaks are reported. The results are individually thresholded at p_{unc} = 0.001, k = 10, and survived qFDR = 0.05.

Pain shared activation

Figure1D shows the overlap between the Experience and Observation tasks. It consists in one clusters with centre of mass -38 28 -3 x/y/z, situated in a portion of the par Orbitalis, crossing AI and adjacent inferior frontal gyrus/ventral frontal operculum, cytoarchitectonic Area44.

Pain shared activation – Generalization of pain

To ensure that all the tasks contributed equally to this result and therefore that the results is generalizable to different kind of observed and experience pain, using Marsbar we extracted the average hemodynamics signal from the cluster discovered above and run the appropriate contrasts.

Regarding the Experience task, to assure that both condition contributed equally to the results we extracted from the cluster the contrasts Shock Modulator, Mechanical Modulator and Shock Modulator minus Mechanical Modulator. On each of them a Student single sample t-test and a Bayesian single sample t-test was performed. Both the first and second contrasts results significantly greater than zero, meaning that both kind of stimulation contribute to the brain activity recorded in the voxels (Shock Modulator: student t-test $t(24)=4.5$ $p<0.001$ and $BF10=195$; Mechanical Modulator: student t-test $t(24)=4.6$ $p<0.001$ and $BF10=224$). The single t-test on the contrast Shock Modulator minus Mechanical Modulator suggests that the level of activation was the same for two conditions (Student t-test $t(24)=-1$ $p=0.3$ and $BF10=0.3$)

Regarding the observation task, to assure that both Face video and Hand video conditions equally contributed to the result, for each participant we calculate on the cluster average signal the contrasts Face Modulator minus Color Modulator and Hand Modulator minus Color Modulator. We also calculated the contrast Face Modulator minus Hand Modulator to assess difference between the two tasks. On each of them a Student single sample t-test and a Bayesian single sample t-test was performed. The contrasts Face Modulator minus Color Modulator (mean=0.4, sd=0.7) and Hand Modulator minus Color Modulator (mean=0.4, sd=0.5) resulted positively different from zero for both kind of statistical analysis (respectively $t(25)=3.3$, $p=0.003$, $BF10=13$ and $t(25)=3.6$, $p=0.001$, $BF10=28$). The contrast Face Modulator minus Hand Modulator was not different from zero according to the Student T-test ($t(25)=0.2$, $p=0.8$), while Bayesian statistic confirmed the two task are not different ($BF10=0.2$).

Discussion

Many efforts have been successfully carried on in the last decades to identify regions in the brain that potentially allow us to understand others' pain through a simulation mechanism. This notion has been recently at the centre of a debate because of contrasting results, and the design of the studies supporting it has been challenged. The study here described aimed to investigate brain regions that are active both when experience and observing someone in pain, when particular attention is placed into correcting for a-specific cognitive task related processed, and communality between different kinds of pain, allowing for a stricter definition of shared brain activity. We interrogated independently all the voxels recorded in the brain and we were able to identify a cluster of them crossing the AI/ fronto-insular cortex, which activation was common to experience and observation of pain. The results are partially in line with current literature, but some clear discrepancy appears. Below we discuss differences between our results and previous literature and the potential causes of the discrepancy. Finally, we highlight specific limitation of our study.

The brain activation modulated by experience of pain well represented the typical neural network described in literature for such tasks (Melzack, 1999; Iannetti and Mouraux, 2010; Wager et al., 2013,

Figure 1D). The brain activation parametrically modulated more by the perceived intensity of the Pain Videos than by the perceived intensity of the saturation of the Color Videos included visual related regions, not shared with the experience task, and a cluster crossing the left pars orbitalis and the most frontal portion of the Insula, shared with the experience task. Interestingly it is enough to slightly relax the threshold of the observation task results to appreciate that the cluster is a peak of wider one expanding in the rest of the AI (and not in the Inferior Frontal Gyrus, see Supplementary Figure S1). Moreover, another small and symmetric cluster appears in the right hemisphere. The lateralization of our results might be a direct consequence of the lateralization of our Pain Videos, in which the actor always receive the painful stimulation on her right hand (Figure 1A).

In literature, the shared brain activity between experienced and observed pain is said to encompass a larger network of areas including the Insula, MCC, and ACC (Singer *et al.* 2004, 2006; Keysers and Gazzola 2009; Corradi-Dell'Acqua, Hofstetter and Vuilleumier 2011; Lamm, Decety and Singer 2011; Zaki, Ochsner and Ochsner 2012; Corradi-Dell'Acqua *et al.* 2016; Cui, Ma and Luo 2016). In the Supplementary Information section "Pain shared activations: regions of interest approach", we reported results obtained by interrogating directly those regions of interest: we identified voxels modulated by variation of intensity in experience of pain and we then interrogate the average of those selected voxels in variation of intensity of observed pain. AI, ACC and MCC showed a pattern of activity modulated by perceived intensity of experienced pain but not by rating of observed pain when the control task was used to clean the brain activity of a-specific factors (See Supplementary Information "Pain shared activations: regions of interest approach"). These regions are indeed modulated by both experience and observed intensities of pain, but the brain activity seems related to a-specifc factors (See Supplementary Information: High vs low rating design)

In Gallo *et al.* 2018 we proved using EEG and brain stimulation that the Somatosensory Cortex is sensitive and necessary to correctly understand the intensity of other's pain, but only when this is represented by movement of the affected body part (Hand video). In the appendix "The role of the dorsal Somatosensory Cortex in understanding observed pain" we interrogated the activity of this area in the rating task and the results are further expanded and discussed.

Our study differs in many aspect of the design from other studies investigating the phenomenon of empathy for pain. Unlikely many precedent studies, our participants rated the painfulness of the stimuli inside the scanner. We explicitly ask our participants to report the intensity of pain and used this online individual judgment of painfulness to identify the voxels of interest. Therefore the resulting cluster was not tested to be responding to the objective intensity of the stimulation given to the participants or to the person in the videos, but to the participants' subjective perception. This is a fundamental difference with other studies and in general it might be the cause of contrasting results in the literature. Authors hypothesized that this kind of instruction highlighting the intensity might resolved in brain activity that is dissociable from the one resulting from the instruction of report on the affective unpleasantness of pain (Zaki *et al.* 2016). Indeed recent studies support the idea that the anterior insula activation does not follow directly variation in objective pain intensity (Geuter *et al.* 2017) but integrates physiological arousal and conscious appraisal (Critchley and Harrison 2013; Uddin 2015). The left AI/ fronto-insular cortex in our study does not discriminate between how pain is inflicted (electrically or mechanically, both experienced and observed), suggesting it might be the affective/aversive component of pain, presents in equal measure in the two videos driving its activation. Bilateral AI has been often being associate to the sense of aversion for pain and to negative affected in general. In the current design it is not possible to pin point to one specific characteristic. To be able to discriminate between general negative affect and specific aversion for pain it would be necessary to contrast the results with a task in which negative affect is present without physical harm. If this region responds also to not-physical pain,

we could conclude that it is the affective valence to be represented, if it doesn't it would be specific for pain cause by physical harm. The first idea is support by many study in the literature but also matter of controversy (Iannetti *et al.* 2013; Eisenberger 2015).

An alternative framework would put the insula as an important hub of the saliency network, having a central role in the detection of behaviourally relevant stimuli and the coordination of neural resources (Uddin 2015) Salience processing is cognitive processes involved in detecting, orienting toward, or reacting to salient stimuli and can be dependent on top-down attention and cognitive control processes that are focused on the execution of goal-directed behaviours (Corbetta, Patel and Shulman 2008). All our visual stimuli start with a baseline of one second after which the video proceeded showing various degree of painful reaction or change in colour saturation. In both cases the detection of a change from baseline was the trigger of the rating behaviour. The control task was used to detect a potential effect of saliency for the behavioural task, independent from pain. Given the type of correction we applied on our results it is possible to affirm that each single voxel included in the results is significantly parametrically modulated by the rating judgment of the tasks involving pain but not (or less) by the control task. It would be interesting to test if the region does not respond to the control task or respond significantly less but this is not possible due to the experiment design: being the rating highly correlated with the respond press of the buttons, it is unlikely to successfully disentangle the two using a model, it is necessary to contrast another condition that included the same motor button press.

In the last few years, many authors approached shared activation for pain using Multi Voxel Patter Analysis (MVPA). Usually this technique is used first to identify a spatial patter of voxel able to discriminate different intensities of experienced pain and then applies the patter to observed intensity. This approach is commonly referred to as above chance cross-modal classification and at logical level is similar to our ROIs approach (first find a region responsible for pain execution and then test the same area on the observation tasks). Some studies have successfully identified such patterns (Corradi-Dell'Acqua, Hofstetter and Vuilleumier 2011b) while others didn't, claiming dissociability of the mechanisms (Krishnan *et al.* 2016). MVPA is in general more sensitive to high frequency spatial difference between conditions than a univariate general linear model (GLM) approach but it is important to note that the classification algorithms used in this kind of analysis tend to focus on discriminative features and ignore features that are shared across the categories (Haynes 2015). Decades of work on mirror neurons for actions show only 10% of neurons involved in performing an action become recruited while observing that action (Gallese *et al.* 1996; Keysers *et al.* 2003; Mukamel *et al.* 2010). A classification algorithm will less likely focus on this common neural substrate to discriminate between intensities and will more likely relies on signal from the other 90%. A region could contain neurons that code for both variation in intensity of experience and observation of pain but the MVPA will not detect them. Until we have systematic single cell data during the experience and observation of pain (Hutchison *et al.* 1999), whether neurons represent felt and observed pain reliably remains unclear (Zaki *et al.* 2016).

Subjective ratings of experience of pain activated a wide network of brain regions and this is in line with similar studies (around 31500 voxels). Only 0.001% of them were shared with the observation of pain (28 voxels). On the contrary, the contrast *ObsPainModulator* activated a small amount of voxels (around 340) and 10% of them were shared with experience of pain. Both tasks have been corrected with FDR method at p=0.05. This means that there is 5% of voxels that might still be due to false discovery. In the specific case of the Experience task that sums to 1550 voxels. In the Observation task sums to 17 voxels: a number dangerously close to the one in our cluster. Considered the MNI brain has 902629 total voxels, it is not impossible to exclude that the overlap between the two tasks results by chance.

Indeed the brain activation during our Observation task is unusually limited. This might be due to various reasons. One might be that the control task we have chosen elicits very similar activation, very difficult to disentangle given the temporal and spatial fMRI resolution and using with GLM analyses. Another possible explanation lays in how we design our Observation experiment. The three conditions were presented mixed in the same block. This might have had created an environment in which pain was dominant in the participants' mind, as it is known that context has a strong influence in how a stimuli is perceived (Oosterwijk *et al.* 2015). Another important factor that might had interfered with the representation of cerebral activity while watching the movies is the instruction to rate the intensity by pressing a button as soon as they assigned one. On average participants responded after one second from the end of the videos and the rating was correlated to the buttons presses. Given the time resolution of our data collection, it is not unlikely that the brain activity caused by the motor response for all the conditions had covered the brain activity linked to watching the movies and that our results underestimate the cerebral areas that correlates with judging pain intensity. The fact that when we apply to the data a design that does not contain a linear regressor but tests stimuli rated as high pain contrasted with stimuli indicated as low pain, the Face videos results in the network usually described by the literature, while the Hand and the Color videos failed to show any voxel surviving the correction for multiple comparison (see chapter 2 Supplementary High vs Low Rating Design) supports this hypothesis. It is important to notice that the possible issue would speak in favour of a potentially larger overlap between experience and observation of pain than the one detected in our current study. To be able to disentangle these possible methodological issues, we are currently running the Observation task in an independent pool of participants. To facilitate segregation of signal between the conditions, we are presenting them in separated blocks. To isolate the brain activity correlated to rating the videos from the one elicitated by pressing a button, we added a jitter time between the movie end and the presentation of a rating scale. To avoid that button presses and subjective rating are correlated, we now present the entire scale from 1 to 10 and participants have to move a curse to the chosen rating by pressing two buttons.

The cluster of activation common between subjective rating of experience and observation of pain did not differentiate between different kinds of infliction of pain. Even though there is a difference in average reaction time between the conditions, it is unlikely that this result was confounded by it, since the difference are not in the same direction: for example Face and Color condition do not have difference in reaction time but they do have difference in brain activity.

In this study we identify a portion of the AI/fronto-insula that is sensitive to modulation in perceived intensity of an experienced painful stimulation on the right hand and to modulation in perceived intensity of similar painful stimulation observed on another person's the right hand. The region equally responds to pain when this is provided by an electrical shock or a mechanical stimulation. Finally, this region is also specific to pain, at least to the extent that it is not/less active for stimuli salient for the behavioural task but not involving pain. Even though with the limitation described above, this results are in line with the current literature, adding to it a fundamental step of solidity and robustness that was, at our knowledge, otherwise missing.

Supplementary Information

The uncorrected results

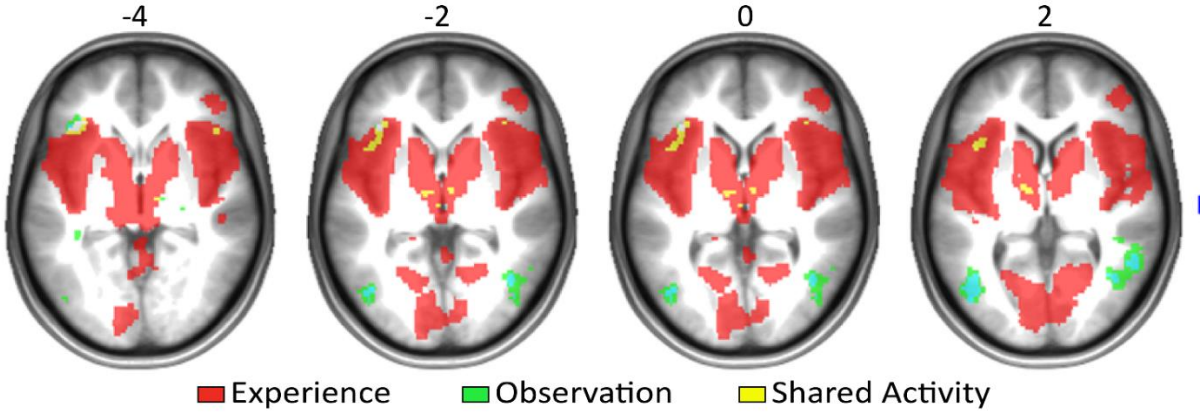

Figure S1. All brain results of the Experience task contrast *ExpPainModulator* (red) and Observation task contrast *ObsPainModulator* (green). *ExpPainModulator* voxels are corrected for multiple comparison (qFDR=0.05, k=10) and each voxel also has a p_{unc}<0.001. *ObsPainModulator* results are not corrected for multiple comparison and have a p_{unc}<0.001. In yellow is the shared activity between the two tasks.

Pain shared activations: regions of interest approach

The Anterior Insula (AI) and Anterior and Middle Cingulate cortex (ACC, MCC) have repeatedly been implicated in both first-person pain perception (Melzack 2001; Iannetti and Mouraux 2010; Wager *et al.* 2013) and in the perception of other individual's pain (vicarious pain; Singer et al., 2004; Zaki et al., 2016). The somatosensory cortex is known to be recruited when the body part of the person in pain and is known, as in our stimuli (Keysers, Kaas and Gazzola 2010; Lamm, Decety and Singer 2011a; Christov-Moore and Iacoboni 2016). We also demonstrated that the same stimuli involve the somatosensory cortex (SI) and that this region is functionally involved in coding the intensity of observed pain and in prosocial behaviour (Gallo *et al.* 2018). In this section of the manuscript we will focus on AI, ACC and MCC. The analyses related to SI are described and discussed in the dedicated Appendix: "The role of the dorsal Somatosensory Cortex in understanding observed pain".

To assess whether the areas modulated by the pain experience are the same ones modulated by pain observation, we first identified portions of regions known to be involved in pain experience that are modulated by the stimulations in our task. Then we extracted signal from these regions of interest and tested if they are also involved in pain observation. The regions of interest were the bilateral AI, ACC and MCC. AI left and right (AI-L and AI-R) were identified using in vivo probabilistic white-matter tractography and Laplacian eigenmaps (Cerliani *et al.* 2012). ACC and MCC were defined using the Automated were identified using a Macroscopic Anatomical Parcellation of the MNI MRI Single-Subject Brain (AAL Atlas, WFU Pick, Maldjian *et al.* 2003),

To identify sub-region of the anatomical ROIs that are modulated by the rating in the Experience task, we used SPM imagecalc logic function '&' between the anatomical ROIs and the significant voxels of the contrast *Shock Modulator* + *Mechanical Modulator* (t>3.5), from now on referred as *ExpPainModulator*.

This procedure leaded to 6 ROIs: AI-R, AI-L, ACC-R, ACC-L, MCC-R, MCC-L. For each participant, parameter estimates of the Observation Task contrast of interest, *ObsPain modulator*, were extracted from each ROIs using the Marsbar toolbox for SPM (http://marsbar.sourceforge.net).

To identify the brain activity specifically modulated by the two conditions (Face or Hand video) we extracted the contrasts representing the BOLD signal parametrically modulated by the subjective rating of the Face minus Color videos, Hand minus Color videos.

To assess if there were difference in the brain activity modulated by the two conditions we extracted the contrast representing brain activity modulated by the rating of the Face videos more than by the Hand videos and vicevesa.

The extract data were then analyzed using single sample ttest. Analysis were performed in both a frequentistic and a bayesian framework using JASP (https://jasp-stats.org).

***ObsPainModulator* extracted from ROIs**							
	com x/y/z	**Vol**	**Mean**	**St.D.**	**T**	**pval**	**BF10**
AI-L	-34/16/5	2088	0.33	0.87	1.92	0.07	1.01
AI-R	36/17/-3	1936	0.25	1.05	1.22	0.24	0.40
ACC-L	-4/29/23	4880	0.24	1.02	1.20	0.24	0.40
ACC-R	7/29/22	3528	0.09	0.88	0.51	0.62	0.23*
MCC-L	-5/-1/40	7400	0.34	1.08	1.62	0.12	0.65
MCC-R	7/7/39	7680	0.30	0.99	1.54	0.14	0.59

Table S1. Centre of mass (com) and volume in mm3 (vol) of the ROIs. Descriptive statistic of the contrast *ObsPainModulator* extracted from the ROIs, results from single sample Student t-test (T and pval) and Bayesian single sample t-test (BF10). Student t-test degree of freedom are equal to 25 for all test. For the Bayesian single sample t-test we used JASP default prior Cauchy scale=0.707. *signals BF10 indicating support for the null hypothesis.

For each ROI (AI-L, AI-R, ACC-L, ACC-R, MCC-L, MCC-R) we performed a single sample t-test on the contrast of interest *ObsPainModulator*. TableS1 illustrated the descriptive statistic and t-tests results. None of the Student t-test confirmed a difference from zeros in the hemodynamic response. A Bayesian factor included between 0.3 and 3, indicated a lack of information to support neither of the hypothesis for AI-L, AI-R, MCC-L, MCC-R. Bayesian single sample t-test of the ACC-R indicated a support for the null hypothesis meaning that in these regions the hemodynamic response is either modulate in the same fashion by the rating of the pain videos and the control videos or not modulated at all for none of the videos. In either cases if it possible to exclude that the areas are specifically modulated by pain observation (Table S1).

High vs Low Rating Design

In the design of the GLM riported in the main text, the button presses are not modelled and therefore we cannot look at the activity induced by each observation conditions alone. To be able to observe this activity and to replicate the design common in literature, we performed a different GLM on the data acquired for the Experience and the Observation task.

In this second GLM we did not use the rating given by the participants as a linear regressor in the model. Instead, we used the rating given to categorize the trial as low-rating and high-rating. For each participants, we calculated the average rating given in each conditions and categorized the trial as Low Rating and High Rating, if the rating given was lower or higher than the average ratings. Average,

standard deviation, minimum number of trials and maximum number of trial per conditions are illustrated in Table S2.

Fmri pre-processing was carried out as described in the main body of the manuscript. Two separated GLM were performed independently for Experience and Observation task.

Task	Condition	Average number of trials (sd)	Min number of trials	Max number of trials
Experience	Shock Low-Rating	40.1 (5)	30	49
	Shock High Rating	39.6 (5)	31	50
	Mech Low-Rating	41.8 (6)	34	53
	Mech High Rating	37.2 (6)	26	46
Observation	Face Low-Rating	56.5 (12)	33	76
	Face High-Rating	51.8 (11)	30	69
	Hand Low-Rating	48.3 (14)	23	74
	Hand High-Rating	53.3 (14)	29	79
	Color Low-Rating	60.0 (16)	27	84
	Color High-Rating	57.3 (15)	34	83

Table S2. Average, maximum and minimum number of trials per conditions and tasks.

Pain Experience Task GLM

For each of the runs of the Pain Experience task, we included predictors that captured the time of the electrical stimulation rated as below average (Shock Low-Rating), the electrical stimulation rated above average (Shock High-Rating), the mechanical stimulation rated as below average (Mech Low-Rating), the mechanical stimulation rated above average (Mech High-Rating). As in the main GLM duration of the electrical stimulation had duration=500ms and mechanical stimulations duration=200ms. The other predictors were left identical to the one in the GLM described in the main text: one predictor contained the rating period from the appearance of the question mark until the participant's button press, one predictor collected the instruction screen (5s), two predictors were additionally present if any button was pressed outside the rating period (duration=0). Finally, if a participant pressed two or more different buttons after the stimulation, the impossibility to assign a subjective painfulness excluded the stimulation from the main predictor and were included in a separated one. Six additional regressors of no interest, resulting from the realignment procedure, were entered for each of the eight runs to account for translations and rotations of the head (none of the included participants had head motions parameters exceeding the acquired voxel-size).

Statistical analysis was performed in a two-level procedure. First, for each participant we identified brain activity related to pain and not to low intensity stimulation or general decision making procedure by subtracting the activity correlated to the Low-Rating trials from the High-Rating trials, combining together (summing) the electrical and mechanical stimulations (contrast *ExpHighPain)*. The resulting β-maps were analyzed at the group level using a one-sample t-test. Results were corrected for false discovery rate (qFDR=0.05 with a minimum cluster size of 10).

Observation Task GLM

For each of the Observation task, the following predictors were included. Two separated predictors collected the video rated below average and the video rated above of each condition, resulting in a total of six predictors: Face Low-Rating, Face High-Rating, Hand Low-Rating, Hand High-Rating, Color Low-Rating, Color High-Rating. As in the GLM described in the main text, the regressors were aligned with the appearance of the fixation cross and lasted for the entire duration of the movie for a total of 3s duration (cross + movie duration). The other regressors were kept identical to the ones describe in the

main text: one regressor collected the instruction screen (5s), one regressor collected eventual videos for which was not possible assign a rating, six additional regressors of no interest, resulting from the realignment procedure, were entered for each of the eight runs to account for translations and rotations of the head (none of the included participants had motions parameters exceeding the acquired voxel-size). Button presses were not modelled. The activity related to planning and pressing the buttons was common between conditions and therefore cancelled out when the conditions were compared.

For each participant the contrast of interest representing the hemodynamic response modulated by high pain rating was calculated as (Face High-Rating minus Face Low-Rating) plus (Hand High-Rating minus Hand low-Rating) (*ObsHighPain* contrast).

We also calculated the contrasts:

- Face High Pain minus Face Low Pain;
- Hand High Pain minus Hand Low Pain;
- Color High minus Color Low;
- (Face High Pain minus Face Low) minus (Color High minus Color Low);
- (Hand High Pain minus Hand Low) minus (Face High Pain minus Face Low);
- (Hand High Pain minus Hand Low) minus (Color High minus Color Low).

To obtain all brain group results, a random effect test was performed on each contrasts (single sample t-test) to find voxel consistently greater or lesser than zero between participants (typically referred as second level analysis). As for the Experience task, results were corrected for false discovery rate (qFDR=0.05 with a minimum cluster size of 10).

Results

Experience task

In the Experience Task, all brain single sample t-test on the contrast *ExpHighPain* identified a number of clusters surviving voxel wise FDR at q<0.05 (t>2.21). In Table S2 the number of clusters and cluster peaks are reported (Table S2). Our results are in line and replicated the brain regions obtained by automated meta-analysis toolbox Neurosynth (www.neurosynth.org Yarkoni et al., 2011) for reverse inference of the term 'pain' and the results obtained in the GLM described in the main text. (Table S3 and Figure S2).

ExpHighPain						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
1	57390	8	24	28	10.78	R ACC
		-2	34	24	10.42	L ACC
		-4	30	26	10.19	L ACC
		-6	28	22	9.82	L ACC
		-4	-8	48	9.65	L MCC
		-36	6	-8	9.58	L Insula Lobe
		4	26	32	9.52	R MCC
		-40	-18	16	9.49	L Rolandic Operculum
		-2	26	34	9.39	L MCC

ExpHighPain						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
		-32	26	-4	9.36	L Insula Lobe
		-36	-4	-12	9.22	L Insula Lobe
2	1563	-32	44	22	4.93	L Middle Frontal Gyrus
		-26	48	22	4.68	L Superior Frontal Gyrus
		-44	50	12	3.96	L Middle Frontal Gyrus
		-32	58	18	3.85	L Middle Frontal Gyrus
		-28	40	32	3.75	L Middle Frontal Gyrus
		-38	44	8	3.71	L Middle Frontal Gyrus
		-46	30	32	3.55	L Middle Frontal Gyrus
		-40	36	22	3.43	L Middle Frontal Gyrus
		-32	46	-2	3.22	L Middle Orbital Gyrus
		-42	54	0	2.93	L Middle Frontal Gyrus
3	141	-50	-74	10	3.76	L Middle Occipital Gyrus
		-56	-66	8	3.05	L Middle Temporal Gyrus
4	77	50	4	42	3.14	RPrecentral Gyrus
5	11	30	-8	-30	2.65	CA1 (Hippocampus)
		30	-12	-26	2.59	CA1 (Hippocampus)
		30	-16	-24	2.53	Subiculum
6	10	-48	38	-16	2.54	L IFG (p. Orbitalis)
		-44	38	-18	2.49	L IFG (p. Orbitalis)

Table S3. Activation table of the contrast *ExpHighPain*. Coordinate and t-values of the cluster peaks are reported. The results are corrected for multiple comparison (qFDR b 0.05, k=10).

Brain regions modulated by high rating of pain videos (Observation task)

In the Observation Task, all brain single sample t-test (positive tails) on the contrast *ObsHighPain* identified a number of clusters surviving voxel wise FDR at q<0.05 (t>2.59) in which the hemodynamic response was greater for stimulation rated as high pain then stimulations rated as low pain. See TableS3 for the list of clusters and cluster peaks, Figure S2 for the graphic representation.

The contrast *Face High Pain minus Face Low Pain* identified a number of clusters described in Table S5. The contrast *Hand High Pain minus Hand Low Pain* and the contrast *Color High Pain minus Color Low* did not results in any clusters surviving the FDR correction. The contrast *(Face High Pain minus Face Low) minus (Hand High Pain minus Hand Low)* identified a number of clusters described in Table S6.The contrast *(Face High Pain minus Face Low) minus (Color High Pain minus Color Low)* identified a number of clusters described in Table S7.The contrast *(Hand High Pain minus Hand Low) minus (Face High Pain minus Face Low)* identified a number of clusters described in Table S8.The contrast *(Hand High Pain minus Hand Low) minus (Color High Pain minus Color Low)* did not results in any clusters surviving the FDR correction.

ObsHighPain						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
1	10817	-4	-20	0	7.01	L Thalamus
		-4	-12	-2	6.48	L Thalamus
		10	8	12	6.21	R Caudate Nucleus

ObsHighPain						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
		-34	6	-4	5.98	N/A
		-44	16	0	5.9	L Insula Lobe
		-12	-16	12	5.79	L Thalamus
		-30	16	-2	5.76	N/A
		10	4	-2	5.72	N/A
		-8	-12	-6	5.71	N/A
		-14	-10	8	5.71	L Thalamus
		32	8	-8	5.7	R Putamen
2	9766	-6	10	52	6.55	L Posterior-Medial Frontal
		26	-4	52	6.53	R Middle Frontal Gyrus
		-4	20	38	6.29	L MCC
		-6	22	36	6.18	L MCC
		-4	28	34	5.74	L MCC
		14	-58	56	5.65	R Precuneus
		-30	2	56	5.48	L Middle Frontal Gyrus
		-2	32	30	5.3	L ACC
		26	6	62	5.27	R Superior Frontal Gyrus
		28	6	58	5.25	R Superior Frontal Gyrus
		34	-22	58	5.23	RPrecentral Gyrus
3	1362	-32	-56	44	4.98	L Inferior Parietal Lobule
		-44	-38	42	4.09	L Inferior Parietal Lobule
		-8	-66	44	4.02	L Precuneus
		-18	-60	50	3.89	L Superior Parietal Lobule
		-26	-66	50	3.79	L Superior Parietal Lobule
4	993	-50	-68	8	5.53	L Middle Temporal Gyrus
		-46	-80	-2	5.03	L Middle Occipital Gyrus
		-56	-52	8	4.78	L Middle Temporal Gyrus
		-52	-60	4	4.37	L Middle Temporal Gyrus
		-46	-48	12	3.84	L Middle Temporal Gyrus
5	655	-38	4	28	5.63	L IFG (p. Opercularis)
		-52	8	40	5.16	L Precentral Gyrus
		-38	4	38	3.98	L Precentral Gyrus
		-40	16	24	3.34	L IFG (p. Triangularis)
6	308	44	6	20	4.45	N/A
		50	6	24	4.3	R IFG (p. Opercularis)
		34	8	28	2.95	R IFG (p. Opercularis)
		48	10	10	2.78	R IFG (p. Opercularis)
7		26	-50	-26	3.62	R Cerebelum (VI)
		40	-56	-12	3.6	R Fusiform Gyrus
		32	-52	-30	3.43	R Cerebelum (VI)
		42	-54	-22	3.41	R Fusiform Gyrus
		42	-52	-18	3.39	R Fusiform Gyrus

ObsHighPain						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
		40	-46	-32	3.19	R Cerebelum (Crus 1)
		26	-60	-28	3.09	R Cerebelum (VI)
		38	-68	-28	3.01	R Cerebelum (Crus 1)
		40	-62	-30	2.98	R Cerebelum (Crus 1)
		38	-42	-16	2.92	R Fusiform Gyrus
		40	-44	-14	2.71	R Fusiform Gyrus
8	228	50	12	-22	4.01	R Temporal Pole
		48	-2	-14	3.99	R Superior Temporal Gyrus
		40	8	-22	3.63	R Temporal Pole
9	212	-42	-58	-26	4.12	L Cerebelum (Crus 1)
		-38	-56	-16	3.75	L Fusiform Gyrus
		-40	-50	-22	3.34	L Fusiform Gyrus
		-32	-50	-30	2.65	L Cerebelum (VI)
10	197	58	-16	30	4.03	R Postcentral Gyrus (Area 3b)
		58	-16	24	3.78	R SupraMarginal Gyrus
		50	-22	20	3.17	R Rolandic Operculum
11	97	-30	12	-20	4.35	L Insula Lobe
12	95	16	-68	8	3.25	R Calcarine Gyrus
		12	-70	10	3.22	R Calcarine Gyrus
13	94	-24	-92	-10	4.91	L Inferior Occipital Gyrus
		-14	-92	-2	2.86	L Lingual Gyrus
14	25	-2	-30	28	2.95	N/A
15	20	44	32	24	2.89	R IFG (p. Triangularis)
		46	32	28	2.75	R Middle Frontal Gyrus
		44	38	28	2.69	R Middle Frontal Gyrus
16		-20	-48	-22	3.02	L Cerebelum (IV-V)

Table S4. Activation table of the contrast ObsHighPain. Coordinate and t-values of the cluster peaks are reported. The results are corrected for multiple comparison (qFDR b 0.05, k=10).

Face High Pain minus Face Low Pain						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
1	14430	-6	22	36	T = 7.10	L MCC
		22	-2	52	T = 6.59	N/A
		26	-4	60	T = 6.53	R Superior Frontal Gyrus
		-4	8	54	T = 6.47	L Posterior-Medial Frontal
		2	2	64	T = 6.16	R Posterior-Medial Frontal
		24	2	60	T = 6.09	R Superior Frontal Gyrus
		16	-58	56	T = 6.09	R Superior Parietal Lobule
		36	-36	48	T = 5.93	R Postcentral Gyrus
		32	-48	48	T = 5.65	R Inferior Parietal Lobule
		42	-52	-18	T = 5.63	R Fusiform Gyrus
		34	-22	58	T = 5.60	RPrecentral Gyrus

Face High Pain minus Face Low Pain						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
2	11549	-16	12	0	T = 6.43	L Putamen
		-6	-10	-2	T = 6.20	L Thalamus
		44	2	-16	T = 6.13	R Temporal Pole
		-10	8	0	T = 6.03	N/A
		-54	10	-20	T = 6.01	L Temporal Pole
		-30	20	4	T = 5.98	L Insula Lobe
		-8	4	2	T = 5.89	N/A
		-40	2	30	T = 5.85	L Precentral Gyrus
		52	8	24	T = 5.66	R IFG (p. Opercularis)
		-52	8	40	T = 5.64	L Precentral Gyrus
		12	10	6	T = 5.62	R Caudate Nucleus
3	1785	-18	-60	50	T = 5.02	L Superior Parietal Lobule
		-38	-38	40	T = 4.84	L Inferior Parietal Lobule
		-48	-36	46	T = 4.82	L Inferior Parietal Lobule
		-30	-54	44	T = 4.76	L Inferior Parietal Lobule
		-60	-18	34	T = 3.88	L Postcentral Gyrus
		-36	-44	50	T = 3.78	L Inferior Parietal Lobule
		-60	-18	20	T = 3.73	L Postcentral Gyrus
		-30	-64	40	T = 3.70	L Inferior Parietal Lobule
		-44	-44	50	T = 3.70	L Inferior Parietal Lobule
		-56	-22	16	T = 3.18	L SupraMarginal Gyrus
		-64	-20	12	T = 2.93	L Superior Temporal Gyrus
4	1049	-52	-70	6	T = 6.04	L Middle Temporal Gyrus
		-42	-66	6	T = 5.56	L Middle Temporal Gyrus
		-58	-52	8	T = 5.39	L Middle Temporal Gyrus
		-48	-60	4	T = 4.98	L Middle Temporal Gyrus
		-48	-76	-6	T = 4.69	L Inferior Occipital Gyrus
		-48	-50	10	T = 4.64	L Middle Temporal Gyrus
		-32	-72	12	T = 2.93	N/A
5	616	-38	-56	-14	T = 4.78	L Fusiform Gyrus
		-40	-58	-26	T = 4.74	L Cerebelum (VI)
		-34	-48	-26	T = 4.36	L Cerebelum (VI)
		-38	-50	-24	T = 4.35	L Fusiform Gyrus
		-46	-52	-10	T = 3.36	L Inferior Temporal Gyrus
		-42	-36	-18	T = 3.07	L Inferior Temporal Gyrus
		-24	-62	-28	T = 2.79	L Cerebelum (VI)
		-30	-64	-24	T = 2.62	L Cerebelum (VI)
6	233	-16	-58	0	T = 3.67	L Lingual Gyrus
		-12	-66	10	T = 3.33	L Calcarine Gyrus
		-22	-64	2	T = 3.27	L Lingual Gyrus
		-22	-74	6	T = 2.84	L Calcarine Gyrus
7	173	-22	-90	-10	T = 5.16	L Inferior Occipital Gyrus
		-24	-86	-12	T = 4.89	L Inferior Occipital Gyrus

Face High Pain minus Face Low Pain						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
		-10	-90	-12	T = 3.05	L Lingual Gyrus
8	107	44	34	24	T = 3.77	R IFG (p. Triangularis)
9	28	10	-70	-28	T = 3.25	R Cerebelum (VI)
10	18	12	48	34	T = 3.62	R Superior Medial Gyrus
11	16	66	-16	8	T = 3.17	R Superior Temporal Gyrus
12	15	-46	48	-8	T = 2.97	L Middle Orbital Gyrus

Table S5. Activation table of the contrast Face High Pain minus Face Low Pain. Coordinate and t-values of the cluster peaks are reported. The results are corrected for multiple comparison (qFDR = 0.05, k=10).

(Face High Pain minus Face Low) minus (Hand High Pain minus Hand Low)						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
1	10917	-4	-12	-2	6.48	L Thalamus
		10	8	12	6.21	R Caudate Nucleus
		-34	6	-4	5.98	N/A
		-44	16	0	5.9	L Insula Lobe
		-12	-16	12	5.79	L Thalamus
		-30	16	-2	5.76	N/A
		10	4	-2	5.72	N/A
		-8	-12	-6	5.71	N/A
		-14	-10	8	5.71	L Thalamus
		32	8	-8	5.7	R Putamen
2	9766	-6	10	52	6.55	L Posterior-Medial Frontal
		26	-4	52	6.53	R Middle Frontal Gyrus
		-4	20	38	6.29	L MCC
		-6	22	36	6.18	L MCC
		-4	28	34	5.74	L MCC
		14	-58	56	5.65	R Precuneus
		-30	2	56	5.48	L Middle Frontal Gyrus
		-2	32	30	5.3	L ACC
		26	6	62	5.27	R Superior Frontal Gyrus
		28	6	58	5.25	R Superior Frontal Gyrus
		34	-22	58	5.23	RPrecentral Gyrus
3	1362	-32	-56	44	4.98	L Inferior Parietal Lobule
		-44	-38	42	4.09	L Inferior Parietal Lobule
		-8	-66	44	4.02	L Precuneus
		-18	-60	50	3.89	L Superior Parietal Lobule
		-26	-66	50	3.79	L Superior Parietal Lobule
		-14	-64	48	3.75	L Superior Parietal Lobule
		-32	-46	38	3.7	L Inferior Parietal Lobule
		-6	-74	34	3.56	L Cuneus

(Face High Pain minus Face Low) minus (Hand High Pain minus Hand Low)						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
		-12	-68	32	3.29	L Precuneus
4	993	-50	-68	8	5.53	L Middle Temporal Gyrus
		-46	-80	-2	5.03	L Middle Occipital Gyrus
		-56	-52	8	4.78	L Middle Temporal Gyrus
		-52	-60	4	4.37	L Middle Temporal Gyrus
		-46	-48	12	3.84	L Middle Temporal Gyrus
5	655	-38	4	28	5.63	L IFG (p. Opercularis)
		-52	8	40	5.16	L Precentral Gyrus
		-38	4	38	3.98	L Precentral Gyrus
		-40	16	24	3.34	L IFG (p. Triangularis)
6	308	44	6	20	4.45	N/A
		50	6	24	4.3	R IFG (p. Opercularis)
		34	8	28	2.95	R IFG (p. Opercularis)
		48	10	10	2.78	R IFG (p. Opercularis)
7	273	26	-50	-26	3.62	R Cerebelum (VI)
		40	-56	-12	3.6	R Fusiform Gyrus
		32	-52	-30	3.43	R Cerebelum (VI)
		42	-54	-22	3.41	R Fusiform Gyrus
		42	-52	-18	3.39	R Fusiform Gyrus
		40	-46	-32	3.19	R Cerebelum (Crus 1)
		26	-60	-28	3.09	R Cerebelum (VI)
		38	-68	-28	3.01	R Cerebelum (Crus 1)
		40	-62	-30	2.98	R Cerebelum (Crus 1)
		38	-42	-16	2.92	R Fusiform Gyrus
		40	-44	-14	2.71	R Fusiform Gyrus
8	228	50	12	-22	4.01	R Temporal Pole
		48	-2	-14	3.99	R Superior Temporal Gyrus
		40	8	-22	3.63	R Temporal Pole
9	212	-42	-58	-26	4.12	L Cerebelum (Crus 1)
		-38	-56	-16	3.75	L Fusiform Gyrus
		-40	-50	-22	3.34	L Fusiform Gyrus
		-32	-50	-30	2.65	L Cerebelum (VI)
10	197	58	-16	30	4.03	R Postcentral Gyrus
		58	-16	24	3.78	R SupraMarginal Gyrus
		50	-22	20	3.17	R Rolandic Operculum
11	97	-30	12	-20	4.35	L Insula Lobe
12	95	16	-68	8	3.25	R Calcarine Gyrus
		12	-70	10	3.22	R Calcarine Gyrus
13	94	-24	-92	-10	4.91	L Inferior Occipital Gyrus
		-14	-92	-2	2.86	L Lingual Gyrus

(Face High Pain minus Face Low) minus (Hand High Pain minus Hand Low)						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
14	25	-2	-30	28	2.95	N/A
15	20	44	32	24	2.89	R IFG (p. Triangularis)
		46	32	28	2.75	R Middle Frontal Gyrus
		44	38	28	2.69	R Middle Frontal Gyrus
16	19	-20	-48	-22	3.02	L Cerebelum (IV-V)
17	13	-16	6	74	3.17	N/A
		-14	10	72	2.96	N/A
18	13	22	-58	4	2.94	R Calcarine Gyrus
19	12	30	4	-32	3.55	R ParaHippocampal Gyrus

Table S6. Activation table of the contrast (Face High Pain minus Face Low) minus (Hand High Pain minus Hand Low). Coordinate and t-values of the cluster peaks are reported. The results are corrected for multiple comparison (qFDR =0.05, k=10).

(Face High Pain minus Face Low) minus (Color High minus Color Low)						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
1	480	40	-62	10	6.53	N/A
		54	-52	6	5.93	R Middle Temporal Gyrus
		48	-60	10	5.44	R Middle Temporal Gyrus
		40	-62	4	5.42	R Middle Temporal Gyrus
		50	-42	8	5.31	R Middle Temporal Gyrus
		60	-42	14	4.26	R Superior Temporal Gyrus
2	327	-40	-68	6	5.53	L Middle Occipital Gyrus
		-46	-72	4	5.27	L Middle Occipital Gyrus
		-48	-70	6	5.21	L Middle Temporal Gyrus
		-58	-54	8	5.18	L Middle Temporal Gyrus
		-46	-78	-2	4.48	L Middle Occipital Gyrus
		-56	-66	10	4.29	L Middle Temporal Gyrus
		-60	-46	10	4.25	L Middle Temporal Gyrus
3	85	-10	12	30	5.19	N/A
		-10	20	34	5.02	L MCC
		-24	-72	16	5.42	N/A
		-30	-70	14	5.34	N/A
5	32	50	6	22	4.59	R IFG (p. Opercularis)
		42	8	20	4.22	N/A
		52	10	18	3.86	R IFG (p. Opercularis)
6	30	10	-2	18	5.65	N/A
7	26	-2	-8	66	4.22	L Posterior-Medial Frontal
		-6	-6	68	4.19	L Posterior-Medial Frontal
8	26	-48	-2	-14	6.06	L Superior Temporal Gyrus

(Face High Pain minus Face Low) minus (Color High minus Color Low)						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
9	24	6	22	58	4.64	R Posterior-Medial Frontal
		6	16	62	4.11	R Posterior-Medial Frontal
10	24	-40	30	-4	4.64	L IFG (p. Orbitalis)
11	23	18	-4	48	4.24	N/A
		12	-2	46	4.11	R Posterior-Medial Frontal
12	23	42	-2	-16	4.56	N/A
13	16	-24	-92	-8	4.67	L Inferior Occipital Gyrus
14	16	42	-52	-16	4.58	R Fusiform Gyrus
15	14	12	2	60	4.25	R Posterior-Medial Frontal
		6	0	58	3.88	R Posterior-Medial Frontal
16	13	-2	-34	-8	4.61	N/A
17	12	-50	4	42	4.36	L Precentral Gyrus
		-52	8	40	4.24	L Precentral Gyrus
18	10	-36	20	14	4.62	L IFG (p. Triangularis)

Table S7. Activation table of the contrast *(Face High Pain minus Face Low) minus (Color High minus Color Low)*. Coordinate and t-values of the cluster peaks are reported. The results are corrected for multiple comparison (qFDR =0.05, k=10).

(Hand High Pain minus Hand Low) minus (Face High Pain minus Face Low)						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
1	27	52	-62	28	6.77	R Angular Gyrus
2	15	56	-52	32	6.27	R Angular Gyrus

Table S8. Activation table of the contrast *(Hand High Pain minus Hand Low) minus (Face High Pain minus Face Low)*. Coordinate and t-values of the cluster peaks are reported. The results are corrected for multiple comparison (qFDR b 0.05, k=10).

Shared Activation

Figure S2 illustrates the overlap of the brain activity that responded more to high pain experience than low pain experience and high pain observation more than low pain observation. Table S5 shows a list of areas encompasses by the overlap. The common activity between experience and observation of pain is in line with previous results from our lab and from the literature and included areas traditionally associated with mirror activity like the bilateral Insula, MCC, ACC and the somatosensory cortices.

Face videos vs Hand videos: average brain activity

In this section, we report the results of the contrast between brain activity induced by the Face and the Hand videos, when the subjective rating is not taken into the equation.

Fmri preprocessing and GLM were carried out as described in the main body of the manuscript. We calculated the contrasts *Face videos minus Hand Videos* and *Hand videos minus Face Videos.*

To obtain all brain group results, a random effect test was performed on the contrasts (single sample t-test) to find voxel consistently greater or lesser than zero between. Results were corrected for false discovery rate (qFDR=0.05 with a minimum cluster size of 10) and are reported respectively in Table S9 and Table S10. In Figure S3 is possible to appreciate the illustration of the results.

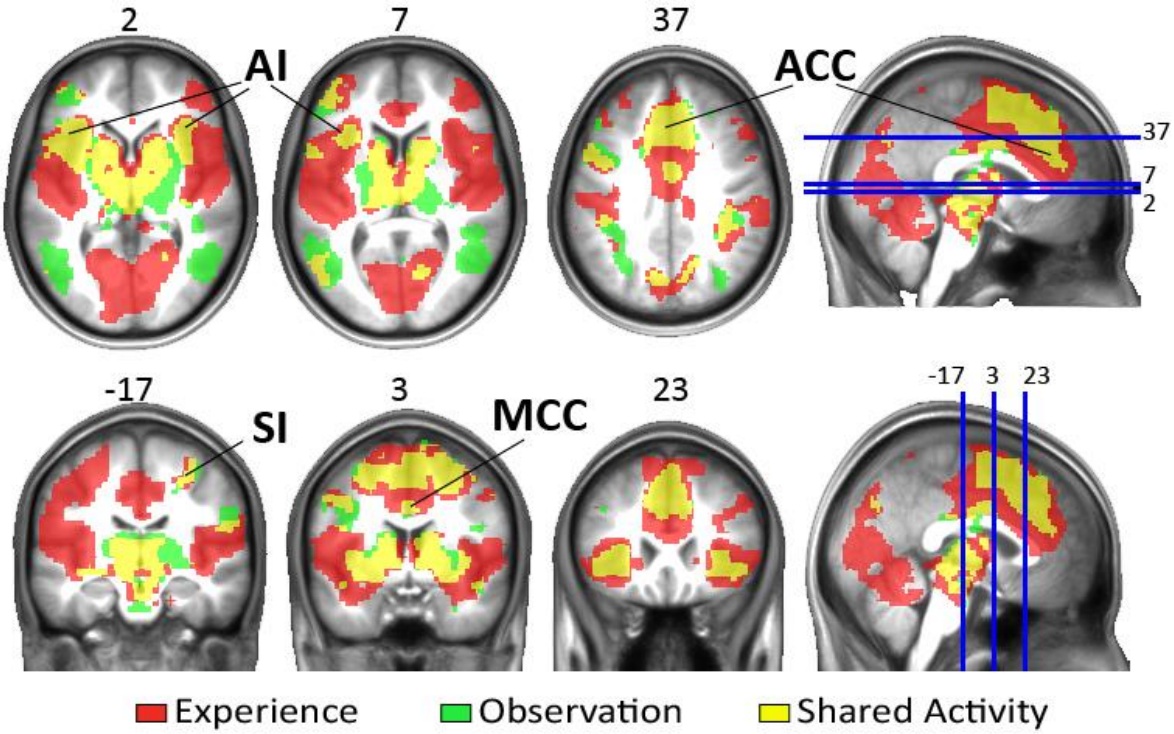

Figure S2. All brain results of the Experience task contrast ExpHighPain (red) and Observation task contrast ObsHighPain (green). Results are corrected for multiple comparison (qFDR=0.05, k=10). In yellow is the shared activity between the two tasks.

Face minus Hand						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
1	1039	52	-36	4	9.97	R Middle Temporal Gyrus
		50	-14	-16	6.88	R Middle Temporal Gyrus
		52	4	-22	5.96	R Middle Temporal Gyrus
		48	-30	-4	5.81	R Superior Temporal Gyrus
		58	-4	-22	5.47	R Middle Temporal Gyrus
		56	-4	-16	5.12	R Middle Temporal Gyrus
		48	10	-28	4.64	R Medial Temporal Pole
2	552	-54	-2	-22	5.89	L Middle Temporal Gyrus
		-58	-10	-18	5.45	L Middle Temporal Gyrus
		-54	-22	-8	5.08	L Middle Temporal Gyrus
		-52	4	-16	5.07	L Temporal Pole
		-58	-28	-4	4.8	L Middle Temporal Gyrus
		-60	-36	0	4.14	L Middle Temporal Gyrus
		-48	-18	-16	4.09	L Middle Temporal Gyrus
3	198	54	28	8	6.3	R IFG (p. Triangularis)
		54	28	-4	4.44	R IFG (p. Orbitalis)
4	89	22	-6	-18	8.7	R Hippocampus

Face minus Hand						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
5	48	-44	-72	40	6.99	L Angular Gyrus
		-48	-72	34	5.76	L Angular Gyrus
		-52	-68	32	5.15	L Angular Gyrus
6	47	42	-70	46	5.28	R Angular Gyrus
		52	-60	44	4.69	R Angular Gyrus
		48	-70	38	4.51	R Angular Gyrus
		50	-68	36	4.35	R Angular Gyrus
		56	-62	30	4.3	R Angular Gyrus
		54	-64	34	4.27	R Angular Gyrus
7	47	36	8	-26	5.17	R Temporal Pole
		32	0	-20	4.64	R Amygdala
		32	2	-24	4.62	R Amygdala
8	40	24	-44	10	5.21	N/A
9	36	2	42	-20	5.73	R Rectal Gyrus
10	27	10	-50	34	4.69	R MCC
11	20	46	18	20	4.22	N/A
12	16	-36	10	-28	5.48	L Temporal Pole
13	12	6	-96	12	5.5	N/A
		6	-94	18	4.7	R Cuneus
14	11	-30	-2	-18	4.4	L Amygdala

Table S9. Activation table of the contrast *Face minus Hand*. Coordinate and t-values of the cluster peaks are reported. The results are corrected for multiple comparison (qFDR b 0.05, k=10).

Hand minus Face						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
1	60989	-58	-22	34	16.17	L SupraMarginal Gyrus
		-28	-80	24	14.22	L Middle Occipital Gyrus
		-48	-68	-2	14.17	L Middle Occipital Gyrus
		-34	-88	14	13.96	L Middle Occipital Gyrus
		40	-80	12	13.70	R Middle Occipital Gyrus
		22	-68	-12	13.21	R Lingual Gyrus
		-36	-84	16	13.19	L Middle Occipital Gyrus
		-28	-86	16	13.15	L Middle Occipital Gyrus
		34	-80	22	12.89	R Middle Occipital Gyrus
		-50	-66	6	12.60	L Middle Temporal Gyrus
		-22	-66	-10	12.38	L Lingual Gyrus
2	956	-42	40	30	4.13	L Middle Frontal Gyrus
		-36	46	30	4.02	L Middle Frontal Gyrus

Hand minus Face						
#	**Cluster side**	**MNI x/y/z**			**T-values**	**Anatomical description**
		-38	44	28	3.94	L Middle Frontal Gyrus
		-48	40	22	3.78	L Middle Frontal Gyrus
		-30	32	24	3.65	L Middle Frontal Gyrus
		-48	44	8	3.41	L IFG (p. Triangularis)
		-42	42	8	3.18	L IFG (p. Triangularis)
		-28	46	38	3.15	L Superior Frontal Gyrus
		-32	54	24	3.15	L Middle Frontal Gyrus
		-42	50	8	3.04	L Middle Frontal Gyrus
		-42	34	10	2.96	L IFG (p. Triangularis)
3	363	48	44	-12	4.28	R IFG (p. Orbitalis)
		38	42	-2	3.41	N/A
		40	54	16	3.32	R Middle Frontal Gyrus
		48	48	2	3.29	R Middle Frontal Gyrus
		40	54	-2	3.26	R Middle Orbital Gyrus
		46	52	6	2.96	R Middle Frontal Gyrus
		48	46	16	2.93	R Middle Frontal Gyrus
		44	44	14	2.89	R Middle Frontal Gyrus
4	153	-2	18	4	4.06	N/A
		6	20	4	3.53	N/A
5	91	14	-2	-4	3.55	N/A
		20	-6	0	2.72	R Pallidum
6	61	-20	16	-10	3.13	L Putamen
7	32	40	40	36	3.25	R Middle Frontal Gyrus
8	21	18	28	-4	2.70	N/A
9	19	-8	38	20	2.60	L ACC
10	17	12	-36	-30	2.97	N/A
11	16	-16	28	-8	3.14	N/A
12	12	8	38	16	2.47	R ACC
13	12	-10	-18	-32	2.66	N/A
		-8	-16	-28	2.53	N/A
14	10	10	14	32	2.43	R MCC

Table S10. Activation table of the contrast *Hand minus Face*. Coordinate and t-values of the cluster peaks are reported. The results are corrected for multiple comparison (qFDR = 0.05, k=10).

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APPENDIX

The role of the dorsal Somatosensory Cortex in understanding observed pain

In our previous work (Gallo *et al.* 2018) we tested the role of the somatosensory cortex in prosocial decision making. We let the participants witness a person receiving a noxious stimulation, using the same Face and Hand videos described here in the main text. The stimuli used showed the injured body part of the person in pain and this is thought to recruit the somatosensory regions (Keysers, Kaas and Gazzola 2010; Lamm, Decety and Singer 2011; Christov-Moore and Iacoboni 2016).

We demonstrated using EEG that a dorsal portion of the left SI (dSI-L), which is activated by experience of pain on the right hand, can predict the amount donated to help a person in pain both when the pain is expressed by facial expression and when it's expressed by the reaction of the affect body part (Face videos and Hand video respectively; EEG study**)**, and by using TMS over SI (TMS study). This activity is necessary to adapt the helping behaviour to the pain witnessed, but only when the pain is expressed as movement of the affected body part. In the third experiment, we asked the participants to watch the same Face and Hand videos plus the Color video, also in this case as control task, while the activity of SI was interfered by HD-tDCS (HD-tDCS rating study). As in the task described in the main text, participants were asked to rate how intense was the pain suffered by the person in the Face and Hand videos and how much the intensity of the saturation of the Color videos changed. The brain stimulation interfered with the participants' ability to correctly rate the intensity of the pain but only when the hand reaction showed the information about the intensity of the inflicted pain (Hand video). We integrated the results of the three experiments and concluded that SI activity is necessary for prosocial decision making by helping transform observed reactions of affected body-parts into accurate perceptions of pain that are necessary for decision making.

The fMRI experiment described in the main text is conceptually the same paradigm used in the HD-tDCS rating study and it uses the same videos as stimuli. The all brain fMRI analyses described in the main text did not seem to support the involvement of SI in observation of others' pain. One possible explanation for this, is that the correction from multiple voxel comparison hided the results in this area. Therefore in this appendix we deepened the finding reported in the main text by focusing specifically on the dSI-L region of interest (ROI) analysed in our previous study.

We tested if the BOLD activity extracted from dSI-L is modulated by rating of observed intensity of pain in the Face and Hand videos (Pain videos, summed) and the brain the activity is corrected for cognitive processes related to the rating task but not to pain (Color videos)

We also tested the activity related to rating pain not corrected for the control task, also and separately for the two kind of videos.

TMS and HD-tDCS studies suggested a functional difference in dSI-L activity for the Face and the Hand videos. For this reason we directly compared the activity of this region related to rating when observing the Face versus when observing the Hand videos.

Methods

dSI-L region of interest

We interrogated the same brain area tested in Gallo *et al.* 2018/Chapter 2 EEG study. The procedure to identify the region is describe in details in Chapter 2. Also, it is the same procedure used to identify the ROIs described in this work in the "Supplementary Information: Pain shared activations: a priori anatomical regions of interest approach".

Briefly, we were interested in the portion of SI that is modulated by own experience of pain and also by observation of another person in pain. We defined anatomical SI-L with the Anatomy toolbox for SPM as the combination of four cytoarchitectonic maximum probability maps for each hemisphere: BA3a, BA3b, BA2 and BA1 (Eickhoff et al., 2005). To identify sub-region of the anatomical ROIs that are modulated by the rating in the Experience task, we used SPM imagecalc logic function '&' between the anatomical ROIs and the significant voxels of the contrast *ExpPainModulator*. This procedure leaded to 2 ROIs: a ventral and a dorsal SI-L cluster. The dorsal region has been proven functionally relevant in responding to the same stimuli (Gallo et al, 2018), so we concentrated on the dorsal cluster for further analyses.

GLMs and contrasts of interest

Depending on the hypothesis under test, we applied to the fMRI data the GLM described in the main text, in which the ratings given are used as linear modulator and must include the correction for control task (from now on referred as GLM-1, See paragraph Methods: Data analysis: fMRI analysis: Observation task GLM for detailed explanation) or the GLM described in the Supplementary Information section, in which the rating are used to define High and Low rating trials and whose design can calculate results for each condition separately contrasting High and Low rating trials (from now on referred GLM-2, see Supplementary Information: High vs Low Rating Design).

In the Observation task, the main contrast of interest was *ObsPainModulator*, which included the Face video modulator + Hand video modulator -2*Color modulator *(*calculated on GLM-1). For each participant, parameter estimates of the Observation Task contrast of interest, *ObsPainModulator*, were extracted from each ROIs using the Marsbar toolbox for SPM (http://marsbar.sourceforge.net).

We also extracted the contrasts representing the BOLD signal parametrically modulated by the subjective rating of the *Face Mod minus Color Mod*, *Hand Mod minus Color Mod* videos were calculate. These two contrasts would identify if dSI-L brain activity was specifically modulated by the two conditions (Face or Hand video).

Then we tested if the BOLD signal extracted from dSI-L can differenciate between functional relavance of the areas in modulating of others' pain, as the brain stimulation technique recorded, or if, as the EEG signal, would not. The contrast extracted to answer this question is *Face Mod – Hand Mod (*calculated on GLM-1).

Finally, we tested the effect on dSI-L activity of perceiving the Pain videos as having High or Low rating. The contrast extracted to answer this question was *Face High-Rating - Face Low-Rating, Hand High-Rating - Hand low-Rating and Color High-Rating - Color low-Rating* (calculated on GLM-2).

The extracted data were then analyzed using single sample t-test. Analysis were performed in both a frequentistic and a bayesian framework using JASP (https://jasp-stats.org).

Contrast	Mean	St.D.	T	pval	BF_{10}
ObsPain modulator	-0.13	0.84	-0.76	0.46	0.27*
Face Mod minus Color Mod	-0.06	0.45	-0.68	0.50	0.26*
Hand Mod minus Color Mod	-0.06	0.51	-0.65	0.52	0.25*
Face Mod minus Hand Mod	0.00	0.46	0.05	0.96	0.21*
Face High-Rating minus Face Low-Rating	0.23	1.52	0.77	0.71	0.28*
Hand High-Rating minus Hand Low-Rating	0.18	1.54	0.60	0.45	0.25*
Color High-Rating minus Color Low-Rating	0.11	1.48	0.37	0.56	0.23*

Results

Descriptive analyses and results are reported in Table A1.

Table A1. Descriptive statistic of the contrasts extracted from the ROIs, results from single sample Student t-test (T and pval, 2 tails) and Bayesian single sample t-test (BF_{10}). Student t-test degree of freedom are equal to 25 for all test. For the Bayesian single sample t-test we used JASP default prior Cauchy scale=0.707. * indicates moderate support for the null hypothesis.

Discussion

In this series of analyses we tested the role of dSI-L in rating other people pain. After encouraging results obtained by studies using EEG and brain stimulation techniques, the fMRI data analysed here did not support the previous finding: dSI-L does not appear to be modulated by rating, not even in the contrast High-Rating minus Low-Rating, which design was demonstrated successfully able to modulate SI in previous fMRI studies (Nummenmaa *et al.* 2008; Lamm, Decety and Singer 2011a; Morrison *et al.* 2013; Christov-Moore and Iacoboni 2016).

The most probable explanation for the lack of replication of our own and other labs results is that the time resolution of fMRI is not adapt to measure the activity of SI in this specific task, with these particular stimuli. The stimuli employed in our current study have a total duration of 2 s. During the first second no painful stimulation is depicted and only in the following second the person in the videos shows signals of distress. In our previous EEG study, the time windows in which the brain activity that correlated with the helping behaviour and evoked by the Face and Hand video were maximum 146 and 32ms respectively. This duration is on a completely different scale, and approximately ten time shorter than the 1700ms duration of our fMRI all brain volume acquisition (TR, see in main text Method, MRI data acquisition for details).

Previous studies were able to register fMRI SI activity related to observation of pain. Often the stimuli used where static picture illustrating a hand in painful situation (Nummenmaa *et al.* 2008; Lamm, Decety and Singer 2011; Morrison *et al.* 2013) of duration varying from 2 to 9 sec depending on the studies, therefore in line with fMRI time resolution. Other studies used short videos but in block design (opposite to the event-related design employed in our study), therefore the brain activity in SI would have time to sum-up during the block presentation and be detected by fMRI signal (Christov-Moore and Iacoboni 2016).

The null results of our study might be a direct consequence of the inability of our stimuli to evoke in SI a difference in BOLD signal strong enough to be detected by fMRI. Unfortunately this hypothesis cannot be directly tested.

Another possibility is that the changes in BOLD signal related to rating another person's pain in our stimuli are strong enough to be detected by fMRI but masked by changes related to the motor activity of pressing the button to give the rating, which is not correlated to the observed pain.

By design, participants were instructed to give their rating as soon as they felt able to judge the intensity of the observed rating. This means that the two events (judging the pain and pressing the button) were always as close in time as possible. The BOLD signal evoked by a motor event is extremely powerful compared to the signal evoked in the motor-sensory circuit by the observation of an event. The motor event might have masked our signal of interest.

To test this hypothesis we designed a second version of the rating task in which participants were requested to wait some seconds before giving their answers. Moreover, the method in which the participants gives the rating changed. In this second version, a VAS scale appeared of the screen and participants had to move the cursor to the step indicating the rating chosen. The scale was in 8 points and its extremes were randomly flipped between trials so that participants could not anticipate the motor movement required to give their rating. The experiment is currently ongoing. If the results will be able to confirm precedent literature results, it might be affirmed that the design of our original study was sub-optimal to record changes in d-SIL activity evoked by observed pain because of the brain activity related to motor response was masking it. We would then proceed and test if the activity is more finely and linearly modulated by the rating, if it is sensitive on how the other's pain is depicted. With the current design is not possible to answer these questions. Further studies are needed to replicate and deepen our understanding of the role of SI in perception of other's pain.

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Chapter 4:
Bayesian statistics show a lack of change in excitability following bi-hemispheric HD-tDCS over the primary somatosensory cortices

Authors

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A detailed description of each author contribution can be found in the chapter *Reference list and authors' contribution*

Abstract

High Definition transcranial Direct Current Stimulation (HD-tDCS) is a method meant to explore the causal structure-function relationship of brain areas, developed to improve the spatial resolution of tDCS, but the validity of tDCS results is currently under intense debate. The goal of this study is to validate a new HD-tDCS protocol for bilateral modulation of the Somatosensory Cortex (SI). The new montage is meant to increase the focus of the stimulation while limiting the area of the scalp covered by electrodes. We aim to characterize the effect of the stimulation in terms of directionality, consistency and reproducibility. We aim to leverage a 1x1 montage to most focally stimulate the primary somatosensory cortex (SI) and measure modulation via Somatosensory Evoked Potentials (SEP) triggered by median nerve stimulation. The results of Experiment1 suggest that our montage increases the amplitude of the SEP component N30. In Experiment2, we aim to replicate our finding and to assess the duration of the modulatory effect on N30 over time. Data from Experiment2 fails to replicate N30 modulation. A sequential Bayesian analysis performed on N30 data from both experiments indicates that the effect fluctuates across participants, without a clear homogenous directionality. This study sets boundaries on the effect size that can be expected for this montage and illustrates the need to include replication samples or larger sample sizes to avoid overestimating effect sizes. We conclude that our montage has insufficient effect size for use in moderately sample-sized experimental studies and clinical applications.

Introduction

The studies of Priori and his colleagues (Priori *et al.* 1998) followed by Nitsche and Paulus (Nitsche and Paulus 2001) have suggested that low direct electrical currents applied over the scalp can influence brain excitability and produce substantial aftereffects on cortical excitability, lasting from minutes to hours. Combined with its low cost, simple application and portability, this has led to an increasing use of transcranial direct current stimulation (tDCS) in a wide variety of settings (DaSilva *et al.* 2015; Lefaucheur 2016; Lewis *et al.* 2016; Sellaro, Nitsche and Colzato 2016; Tatti *et al.* 2016; Bikson *et al.* 2017; Lefaucheur *et al.* 2017). Unfortunately, in its standard form, using large electrodes (most commonly between 25-35 cm^2), the effects of tDCS are too diffuse to attribute effects to specific brain regions (Datta *et al.* 2009; Dmochowski *et al.* 2011; Nitsche, Bikson and Bestmann 2015). High Definition transcranial Current Stimulation (HD-tDCS) was created to increase spatial selectivity and probe the function of specific brain regions (Datta *et al.* 2009; Borckardt *et al.* 2012; Caparelli-Daquer *et al.* 2012; Edwards *et al.* 2013). HD-tDCS uses small ring electrodes 3-5mm in diameter, with the most popular montage being a *4 × 1 ring* with a central electrode (anode or cathode) over the targeted area, surrounded (at 3–7.5 cm radius) by four reference electrodes (Datta *et al.* 2009; Edwards *et al.* 2013; Kuo *et al.* 2013; Villamar *et al.* 2013a; Filmer, Dux and Mattingley 2014; Roy, Baxter and He 2014; Alam *et al.* 2016). This increases focality (Tergau *et al.* 2007) up to 80% compared to standard tDCS (Datta *et al.* 2009; Alam *et al.* 2016),(Datta *et al.* 2008; DaSilva *et al.* 2015; Alam *et al.* 2016). Simulations using the Finite Element Method (FEM) suggested that an increment of focality can be reached by lessening the number of return electrodes: using only one cathode and one anode should support the most focal stimulation possible, albeit at the cost of depth current penetration (Alam *et al.* 2016).

Here we aim to leverage a 1x1 montage to most focally stimulate the primary somatosensory cortex (SI) and measure such modulation via Somatosensory Evoked Potentials (SEP) triggered by median nerve stimulation. SI is active both when a person perform an action or feels pain and when we witness someone else doing the same, meaning it has mirror proprieties and it is thought to be part of the mirror

system, a network of brain regions responsible for understanding others' by mean of simulation. While other regions of this system lay deep into the brain and are not accessible to non-invasive brain stimulation, SI superficial location makes it a perfect target for brain stimulation and therefore to assess if its activity is necessary to understand others'.

We designed the protocol to stimulate the hand knob of SI in both hemispheres simultaneously. This is because there is evidence for a bilateral receptive field in the Brodmann 1 and 2 sub-regions of SI (Iwamura et al. 2002), although the right hemisphere has sometimes been found to dominate in the perception of emotion (not including pain) from facial expressions (Adolphs et al. 2000; Carr et al. 2003; Lamm, Decety and Singer 2011; Cui et al. 2015; Ashar et al. 2017), and hand-movement (Christov-Moore and Iacoboni 2016). In chapter 2 we showed the signal originating from mirroring our left SI to the right hemisphere significantly explaining the donation. For the hand region of SI results for the two hemispheres are very similar in line with a more bilateral activity (Chapter 2 Figure 3D and Supplementary Information).

Different SEP components are thought to reflect activity from different sub-regions of SI: N20 and P24 the first cortical components, from Brodmann area 3b (Allison *et al.* 1989, 1991; García Larrea, Bastuji and Mauguière 1992; Valeriani *et al.* 1998; Valeriani, Le Pera and Tonali 2001), N30 from precentral (Waberski *et al.* 1999) or postcentral cortical regions (Valeriani *et al.* 1998), and P45 from area 1 or 2 (Allison et al. 1992). To modulate the hand knob of SI bilaterally, we place two anodes over the hand representation of SI (between C3 and CP3, and between C4 and CP4), and two cathodes between CZ and CP1 and CZ and CP2 (Figure 1A), so that the current flow would be parallel to the central sulcus (Rawiji *et al.* 2018). We aim to characterize the effect of the stimulation in term of directionality (increase vs. decrease of SEP), consistency (across participant) and reproducibility (across studies).

In Experiment 1, we measured the change in cortical excitability in SI by comparing SEPs before and after the tDCS stimulation. A meta-analysis suggests that for tDCS over sensorimotor cortices the anode causes an enhancement of cortical excitability(Jacobson, Koslowsky and Lavidor 2012) and thus we expect an *enhancement* of the SEP components (Creutzfeldt, Fromm and Kapp 1962; Rahman *et al.* 2013; Fertonani and Miniussi 2017) from our montage. Because the effectiveness of tDCS is debated (Horvath, Forte and Carter 2015; Woods *et al.* 2015; Learmonth *et al.* 2017; Rumsey *et al.* 2017), we performed a second experiment (Experiment 2) aimed at replicating the results of Experiment 1, and at investigating the duration of tDCS effects.

Methods

Participants

Thirty-two participants took part in Experiment 1 (17 females, average age=25±5 years old) and 17 in Experiment 2 (10 females, average age=22±3). None reported neurological, psychiatric, or other medical problems or any contraindication to brain stimulation (Rossi *et al.* 2009; Rossini *et al.* 2015). All were right-handed, naïve to the purpose of the experiment and free to withdraw at any point. No discomfort or adverse HD-tDCS effects were reported by participants or noticed by the experimenters. All studies have been approved by the Ethics Committee of the University of Amsterdam, the Netherlands (protocol number 2015-BC-4070 and 2014-EXT-3829).

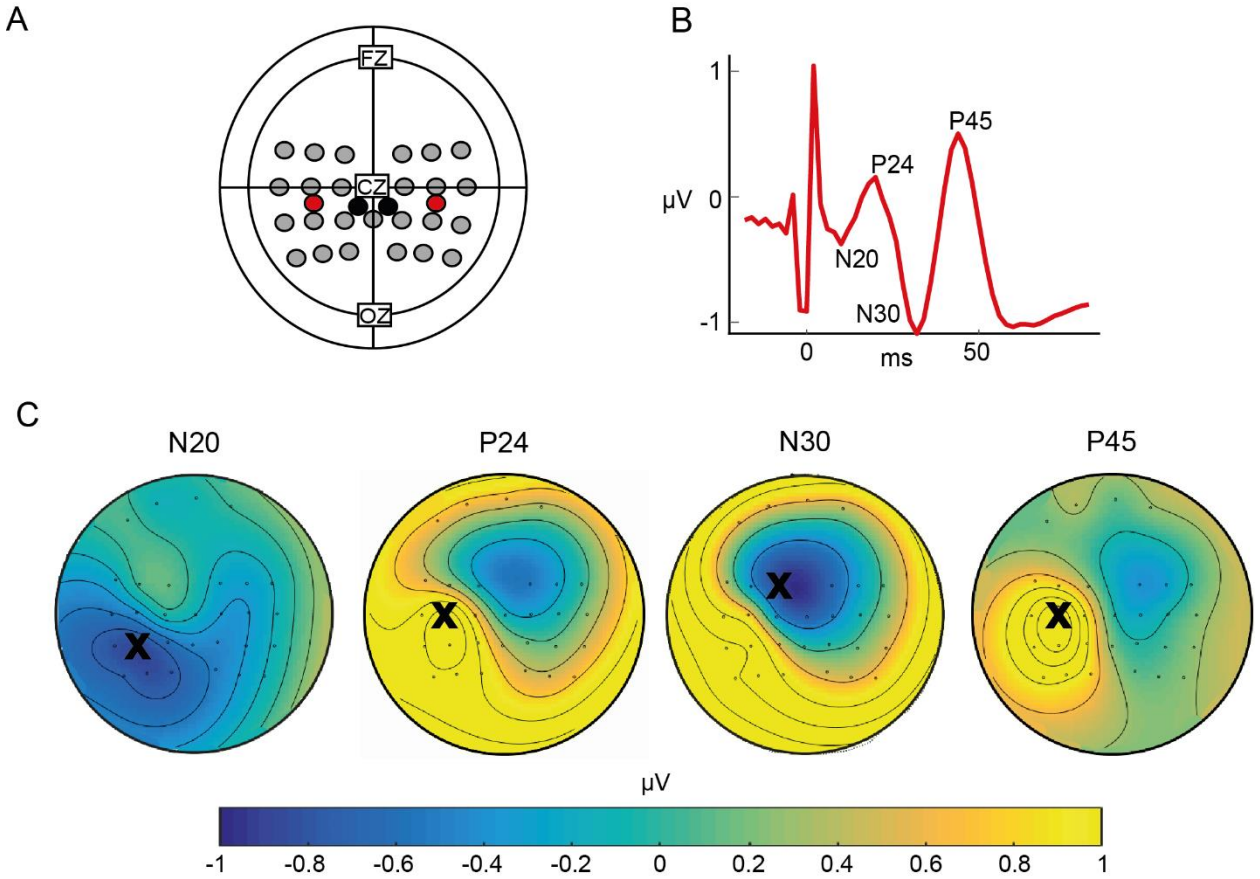

Figure 1. A) HD-tDCS montage and EEG electrodes. Grey circles are the EEG electrodes. Red circles represent HD-tDCS anodes, and black circles HD-tDCS cathodes. B) SEP obtained by averaging across experiments the signal recorded in C3 at T_0, before HD-tDCS stimulation. C) Scalp topography of the SEP components obtained by averaging the T0-grand average of experiment 1 and 2 in the appropriate time-windows. Bold Xs indicate the location of the electrodes chosen as peak of the component.

General protocol

Both experiments consisted of a sham and an active HD-tDCS sessions over SI distributed over two days. Each session started with the calibration of the SEP system, and the recording of a first baseline SEP measurement (t0). Participants then received sham or active HD-tDCS, depending of the pre-assigned order of the conditions, which was followed by a second SEP recording in Experiment 1 (t1), and a total of seven additional recordings in Experiment 2. To reduce the variability in participants' state (Antal *et al.* 2007; Benwell *et al.* 2015; Silvanto and Cattaneo 2017), during the HD-tDCS stimulation we showed emotionally-neutral videos and induced tactile sensations on the dorso of both hands (obtained through electrical stimulation (square wave pulses, frequency of 1 Hz, duration of 2000 μs). For the entire duration of the experiment, participants sat comfortably in an armchair with their hands resting on a pillow, in a semi-darkened room in front of the computer screen. Before leaving the participants completed a tDCS side-effect questionnaire.

HD-tDCS stimulation protocol

HD-tDCS was applied with a 9-channel stimulator (tDCS MXN-9 HD Stimulator, Soterix Medical Inc, US). To bilaterally stimulate SI we used two high-definition 5.5 mm electrodes (Villamar *et al.* 2013) over the right and two over the left hand knob of the somato-motor cortex (Figure 1A). One anode was placed between P3 and CP3 (left side), and one between P4 and CP4 (right). The two cathodes were placed along the central sulcus between CZ and CP1 (left), and CP2 (right). The electrode holders (HD-M, Soterix Medical Inc, US) were embedded in standard EEG caps (ActiCAP,

Brain Products GmbH, Germany**).** In the active stimulation, the current was delivered with a ramp-up time of 30s, held at 0.75 mA in both anodes (1.5 mA in total, Bikson *et al.* 2017) for 18 min, and ramped down over 30s. In the sham condition, the current was ramped up and back down at the beginning and at the end of the 18 min and kept around 0 mA during this period (Villamar *et al.* 2013a). The participant's skin was cleaned by means of a Q-tip soaked in alcohol, and gel was used as interface between the skin and the electrodes. The impedance measured before the stimulation was below 10 kΩ (Edwards *et al.* 2013; Villamar *et al.* 2013a) .

SEP stimulation protocol

SEP stimulations were obtained via non-painful electrical stimulation (Stimulator Model DS7A, Digitimer Ltd, UK) of the median nerve at the right wrist (square wave pulses, personalized stimulus intensity, frequency of 3 Hz and duration of 500 µs). The intensity, determined beforehand in each session, was set to elicit a consistent and visible twitch of the thumb, and was kept constant before and after HD-tDCS.

An opaque panel covered participant's hands during medial nerve stimulation, preventing a modulation of SEP response through observation (Voisin *et al.* 2011). In Experiment 1, the SEP recording consisted of one block of 500 medial nerve stimulations right before and one after the HD-tDCS stimulation. In Experiment 2, the number of medial nerve stimulation was increased to 1000 per block, in order to better differentiate the signal from noise (Passmore, Murphy and Lee 2014), and 8 blocks were recorded: t0 before the tDCS stimulation, t1 directly after stimulation, t2 after 15 min from the end of stimulation, t3 after 30min, t4 after 45 min, t5 after 60 min, t6 after 90 min and t7 after 120min. During the medial nerve stimulation, participants were instructed to fixate their eyes on a white cross on a black screen.

EEG Data Acquisition and Preprocessing

Electrophysiological recordings were obtained from 27 Ag/AgCl scalp electrodes (10-20 International System; Figure 1A). The ground electrode was positioned in FPz. The impedance was kept below 5 kΩ. The signal was acquired at a 500 Hz sample rate and stored on a disk for off-line pre-processing (actiCHamp and PyCoder v1.0.8, BrainVision LLC, USA). Digitalized signal has been analysed using the FieldTrip Toolbox (Oostenveld *et al.* 2011) and customized MATLAB (Mathworks Inc., Natick, MA, USA) scripts. For each SEP recording, data before the first medial nerve modulation and after the last were removed, leaving a long segment of data containing only medial nerve stimulations. Signal was demeaned, detrended and filterer between 2 and 200 Hz. Using Independent Component Analysis (ICA), eye blinks, eye movements and stimulator artefacts were identified and rejected (24/26 components kept on average). The data was then segmented in trials containing one single event, 100ms prior to each medial nerve stimulation and 300ms after. Trials containing muscle artefacts were identified (cut off: value 6 z point above average) and excluded from further analysis (496/500 trials kept on average) using the Fieldtrip automatic rejection routine.

ERPs of each repetition were obtained by averaging the data and correcting for baseline (average time between 50 and 30ms before medial nerve stimulation).

Data Analyses

At the group level, the signal recorded in sham and active sessions at t0 were averaged to identify SEP components (T0-Grand Average). The early SEP components known in literature as N20, P24, N30 and P45 (Allison et al. 1991, 1992; Valeriani et al 2001) were clearly identifiable in the T0-Grand Average of both experiments. Visual inspection of the scalp topographies (Figure 1C) and the information

available in literature (Allison *et al.* 1989; Valeriani, Le Pera and Tonali 2001; Bufalari *et al.* 2007) guided the selection of peak electrodes and of component time-windows. We defined N20 as the most negative peak between 18 and 22ms after medial nerve stimulation recorded in the electrode P5, component P24 as the most positive peak between 22 and 26ms recorded in C3, N30 as the most negative peak between 28 and 36ms recorded in FC1 and P45 as most positive peak between 42 and 48ms recorded in CP3. For illustrative purposes we averaged the T0-Grand Average (in C3) obtained from the two experiments in Figure 1B.

At the individual level, for each participant and block (time-repetition), we calculated the SEP components of interest in the electrode selected in T0-GrandAverage as the maximum or minimum peak (according to the component polarity) present in the time-windows selected at the group level (Bufalari *et al.* 2007).

HD-tDCS effect analyses

To assess the effect of the experimental manipulation, for sham and active stimulation sessions, we subtracted, for each participant and ERP component separately, the baseline measurement (t0) from each measurement after active or sham HD-tDCS. Given in Experiment 1 we only have one SEP recording after HD-tDCS the index of stimulation effect for a component becomes: $(t1_{active}\text{-}t0_{active})$ - $(t1_{sham}\text{-}t0_{sham})$. These values were used at the group level to test whether stimulation had a consistent effect across participants. Because the anodes were placed above the source of the SEP components, we expected HD-tDCS to enhance SEP components - i.e. to increase negativity for N20 and N30 and positivity for P20 and P45. As the distribution of indices did not significantly deviate from normality (Table 1, Figure 1), in Experiment 1 we tested our hypotheses with four frequentist one-tail t-tests, one for each component (H_1 for P24 and P44: index>0; H_1 for N20 and N30: index<0). As in many cases, this lead to non-significant t-values, to quantify evidence for null hypothesis, we complement the frequentist approach with Bayesian t-tests in Jasp 9.2 (https://jasp-stats.org/) and default Cauchy prior (r=0.707) unless otherwise specified. We first looked for evidence for the null for the one-tailed effects we expected. If the Bayesian t-test brought evidence for H_0 (i.e. index >=0 for N20 and N30, or index <=0 for P24 and P44) a second Bayesian two-tailed t-test was performed to quantify evidence specifically for H_0: index=0. Using the JASP formalism, the indices next to Bayes Factors (BF) indicate what hypothesis is in the nominator and denominator. For two tailed tests, BF_{10} is $p(data|H_1)/p(data|H_0)$ and $BF_{01}= p(data|H_0)/p(data|H_1)$. $BF_{10}>3$ thus is moderate evidence for an effect, $BF_{10}<1/3$ for the absence of an effect, and the reverse is true for BF_{01}. BF values around 1 indicate the data is similarly likely under H_0 and H_1, and cannot adjudicate in favour of either. For one-tailed tests, the index representing H_1 is indicated by a "–" if H_1: index<0, and a "+" if H_1: index>0. So BF_{-0} represents $p(data|H_1{:}index<0)/p(data|H_0{:}index>=0)$.

Experiment 2 was collected to replicate Experiment 1 and to investigate the duration of HD-tDCS effects. The Shapiro-Wilk test for normality rejected the notion that the N30 component was drawn from a normal distribution (Table 1), which became normal after removal of participants #8. There was no significant deviation from normality for the other components. To investigate replicability of the results of Experiment 1, we first computed both a frequentist and Bayesian analysis on the index $(t1_{active}\text{-}t0_{active})$ - $(t1_{sham}\text{-}t0_{sham})$ removing participant #8 for the N30 component and using all 17 participants for the other components, and then ran a non-parametric Wilcoxon signed-rank test on the full sample for each component separately. Based on Experiment 1, we expected to find an increase of negativity only for N30. To investigate whether the effect of HD-tDCS on N30 lasts over time we performed a repeated measures Bayesian ANOVA with 7 indices, one for each time point acquired after stimulation: $(ti_{active}\text{-}t0_{active})$ - $(ti_{sham}\text{-}t0_{sham})$ with i=1 to 7. We also performed Bayesian t-tests on each of these time

points to assess whether they deviated from zero in the expected direction. Because the increase of negativity for N30 was not replicated in Experiment 2 for any time point, follow-up Bayesian analyses were implemented to understand the reasons for the non-replication. A sequential analysis was performed on all the four ERP components to see how the evidence in support for or against our effect accumulate over participants across the two experiments. To explore whether effects might be detectable for any of the SEP components, we also performed a 4 components x 7 time points repeated measures Bayesian ANOVA. All Bayesian ANOVAs were conducted in JASP with default priors.

Results

Experiment 1

Traditional frequentist t-tests showed a significant increase in the negativity for N30 after HD-tDCS. No significant effects were observed for the other components (all p_{unc}>0.3). A one-tailed Bayesian t-test confirms the frequentist results showing moderate evidence for an increase of negativity for N30, and indicates a moderate to strong evidence for a lack of an increase of negativity for N20, and of positivity for P24 and P44. Follow-up Bayesian tests confirm a moderate evidence for the absence of any changes in the N20, P24 and P44 components. Table 1 and Figure 2 summarize these results.

		Mea	**SD**	**Norm.**		**t-**	**1**	***d***	**BF_{-0} or BF_{+0}**		**BF_{01}**		**Wilcoxon**	
		(µV)		W	p		p_{unc}		Cauch	Exp1	Cauch	Exp1	z	p
Experiment 1	$N20_3$	0.09	1.5	0.9	0.2	0.32	0.6	0.06	0.15		**5.04**		0.2	0.5
	$P24_{32}$	-0.08	1.3	0.9	0.4	-	0.6	-	0.15		**5.03**		-	0.5
	$N30_3$	-0.46	1.0	0.9	0.1	-	**0.0**	-	**4.27**		0.46		-	**0.0**
	$P44_{32}$	0.03	1.6	0.9	0.5	0.19	0.4	0.02	0.21		**5.27**		0.2	0.3
Experiment 2	$N20_1$	0.57	1.1	0.9	0.4	2.13	0.9	0.52	0.09	0.46	0.67	0.50	2.0	0.9
	$P24_{17}$	0.06	1.0	0.2	0.8	0.25	0.4	0.06	0.30	0.96	**3.90**	1.25	0.4	0.3
	$N30_1$	0.05	0.6	0.8	**0.0**								0.9	0.8
	$N30_1$	0.17	0.4	0.9	0.2	1.57	0.9	0.39	0.11	0.10	1.42	**8.45**	1.5	0.9
	$P44_{17}$	-0.36	0.8	0.9	0.3	-	0.9	-	0.11	0.43	1.22	0.86	-1.8	0.9

Table 1. For each of the ERP component of interest the table reports: the mean value in µV of the HD-tDCS effect index [(t1active-t0 active) - (t1sham -t0 sham)]; its standard deviation (SD); the Shapiro-Wilk W- and p-values of the Normality test (Norm.); the t- and uncorrected p- value from the frequentist statistic (H1: index<0 for N20 and N30, and index>0 for P24 and P44) and the Cohen's *d* effect size. Thereafter we provide the results of the one- and two-tailed Bayesian analysis. This includes the Bayes factor (BF) in favour of the expected directional effect (H_1 index<0 for N20 and N30, and index>0 for P24 and P44, indicated with BF_{-0} and BF_{+0} respectively). We then present the Bayes factor from a two-tailed Bayesian t-test in favour of the absence of any effect (BF_{01}). In all cases the Bayesian analyses has been run using the default Cauchy prior (Cauchy). For Experiment 2, BF were additionally calculated using the posterior distribution from Experiment 1 as an informed prior (Exp1). The last two columns report the z- and p-value from the non-parametric Wilcoxon signed-rank directional test (index<0 for N20 and N30, and index>0 for P24 and P44). Sample sizes indicated as subscript of each component. In bold are the significant results, and the evidence in favour of the predicted or unpredicted effects.

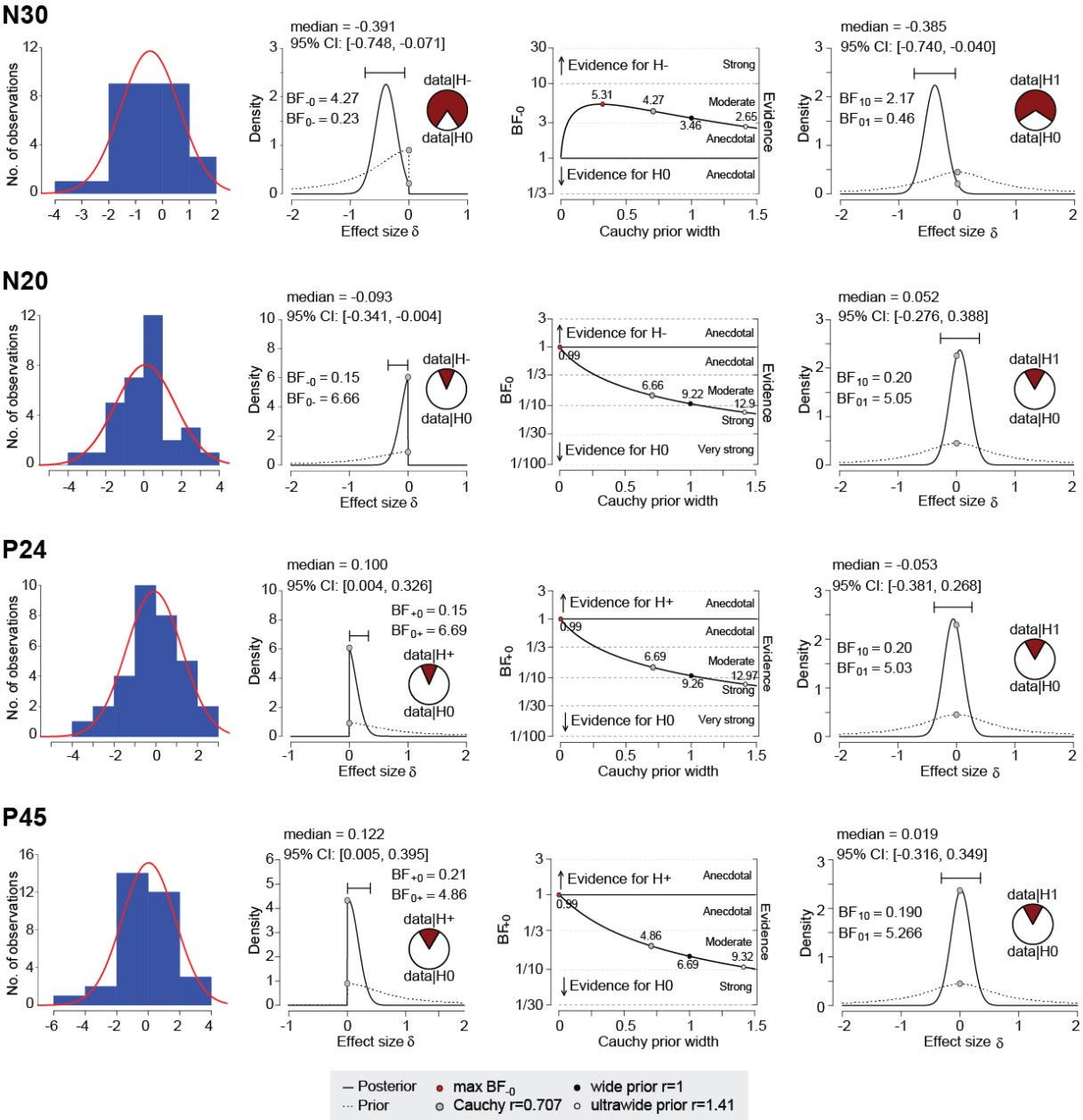

Figure 2. **Bayesian results for Experiment 1**. First column: distribution of the ERP components across participants. Second column: shape of the prior distribution (dotted line) based on our hypothesis, and the posterior resulting distribution (thick line). For Experiment 1 the default Cauchy prior was used, and the fact that the prior is set to zero on one side of each graph expresses the directionality of the hypothesis. The cake illustrates the relationship of the probability of the data given H_1 (either an increase of negativity H_- or an increase of positivity H_+) in dark red, and the probability of the data given H_0 (i.e. either evidence for no effect or for the effect in the opposite direction). Posterior median and 95% confidence intervals (CI) of the effect size are also indicated. Third column: robustness of the Bayes Factor against modifications in the width of the prior. Fourth column: results of the two-tails Bayesian t-test. Conventions as in second column.

Experiment 2

A frequentist one-tailed t-test on $N30_{16}$, for which based on the results of Experiment 1 we expected to find an increase of its negativity, did not reveal significant results (Table 1). A Bayesian t-test with the default prior indicates evidence against an increase in N30 negativity (Table 1 and Figure 2). From the results of the Bayesian two-tailed test of Experiment 1 we observed that the posterior distribution was

approximately normal with median at -0.39 (Figure 1, fourth column), and a SD of about 0.17 (CI/4). To take full advantage of the Bayesian framework, which allows to use of informed prior, we repeated the t-test by using a normal distribution with mean -0.39 and standard deviation 0.17 as prior. Although this prior shifts the posterior towards the expected negative effect size, results still confirm a moderate evidence for the changes in N30 negativity to be equal to zero. The non-parametric Wilcoxon signed-rank test on the full $N30_{17}$ data set from Experiment 2 confirms the lack of reproducibility of a significant decrease in negativity for N30 (Table 1).

For the other components, frequentist and non-parametric analyses show a consistent lack of significant difference between tDCS and sham stimulation, and Bayesian results consistently show a lack of evidence in support of a change after tDCS stimulation (Table 1).

Considering our initial aim to test whether the increase of negativity in Experiment 1 would last over time, despite not replicating the effect of HD-tDCS on N30 at t1 in Experiment 2, we took advantage of the multiple recordings to test whether the reduction in negativity would be present at any other time points. A Bayesian repeated measure ANOVA on $N30_{16}$ indicates evidence for a lack of difference between time points (the variable time as a factor has a $BF_{inclusion}$=0.23, providing moderate evidence against inclusion of time). Results of Bayesian t-tests ran for each time point separately finally indicate that there is no evidence for an increase in N30 negativity following tDCS stimulation at any time points recorded during Experiment 2 (Figure 4 and Table 2). The non-parametric Wilcoxon signed-rank test performed on the full sample separately for each time point supports the Bayesian results, indicating that none of the time point significantly differs from zero (all p>0.16).

To test if there is any evidence that tDCS transiently altered any of the SEP components, we performed a Bayesian repeated measure ANOVA including all the time points for each ERP component (Component x Time Point as within subject factors). This revealed that the best performing model was the one only including the factor component (BF_M=1269). The BF for including the factor time or the component x time interaction are $BF_{inclusion}$=0.002 and $BF_{inclusion}$=0.000015 showing that the data

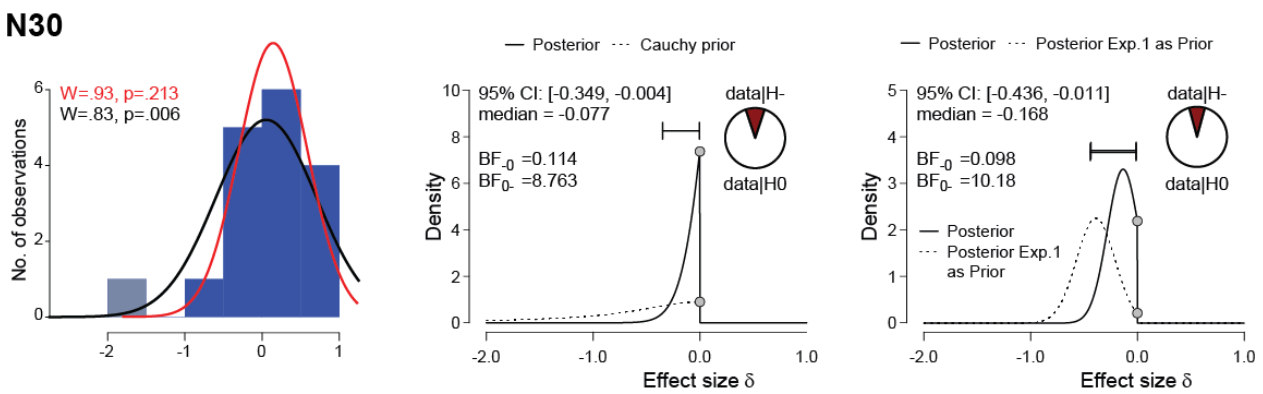

Figure 3. Bayesian results for Experiment 2. Distribution of the N30 components values in Experiment 2 with the normality curve fitted for the whole sample (N=17) in black and after removal of participant 8 (grey bar) in red (left graph). The middle and right most graphs show the posterior and prior distribution for the N30 component computed removing participant 8 using a default Cauchy distribution as prior (middle graph) or the posterior distribution resulting from the data of Experiment 1 (right graph). Conventions as in Figure 2.

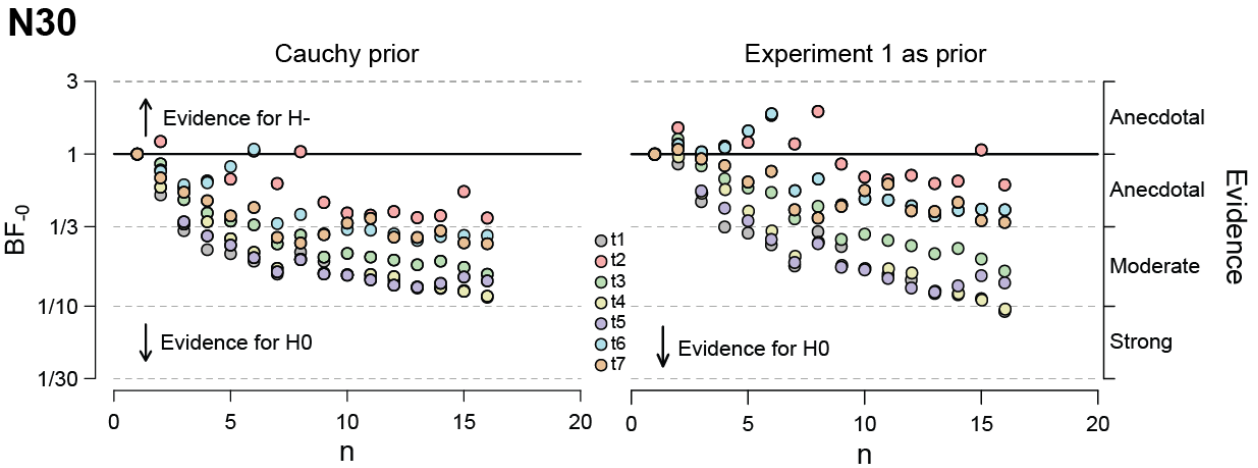

Figure 4. Bayesian sequential analysis for different time points. The graphs illustrated cumulative updates in BF estimation as individual participants are added to the analysis for the N30 ERP component in Experiment 2. The computation is done separately for each time point after stimulation (t1 to t7). Left: estimation calculated using the default Cauchy prior. Right estimation computed using the posterior distribution from Experiment 1 at t1 as informed prior.

provides strong evidence against the hypothesis that indices calculated at subsequent time points different from each other. The results of follow up Bayesian t-test and Wilcoxon signed rank test (Table 2) all confirm the lack of a tDCS effect at any time point.

To check whether the lack of reproducibility of the effect for the N30 component was due to a general difference in the distribution of our indices between the two experiments, we ran a Bayesian ANOVA with the four components as repeated measures and experiment as between subject factor. The ANOVA indicates that the best model is the null model (BF_M=6.12), with BF in favour of the inclusion of the factors all being less than 0.21. A non-parametric comparison of the N30 indices across experiments also indicates a lack of significant difference across experiments (p=0.1, W=-1.63).

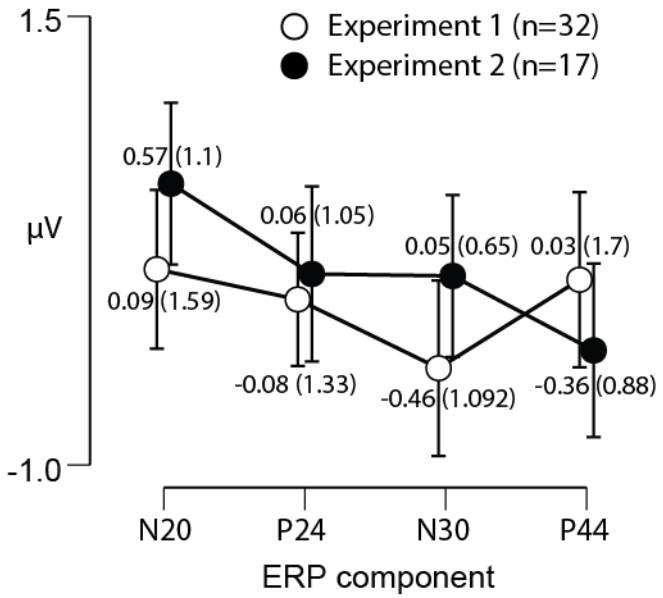

Figure 5. Experiment 1 and 2 comparison. The graph reports the mean indices [(ERPcomponent$_{t1}$ active-ERPcomponent$_{t0}$ active) - (ERPcomponent$_{t1}$ sham - ERPcomponent$_{t0}$ sham)] for each ERP component and the two Experiment separately. The numbers close to the dots indicate the mean and standard deviation values, while the error bars are the 95% confidence interval.

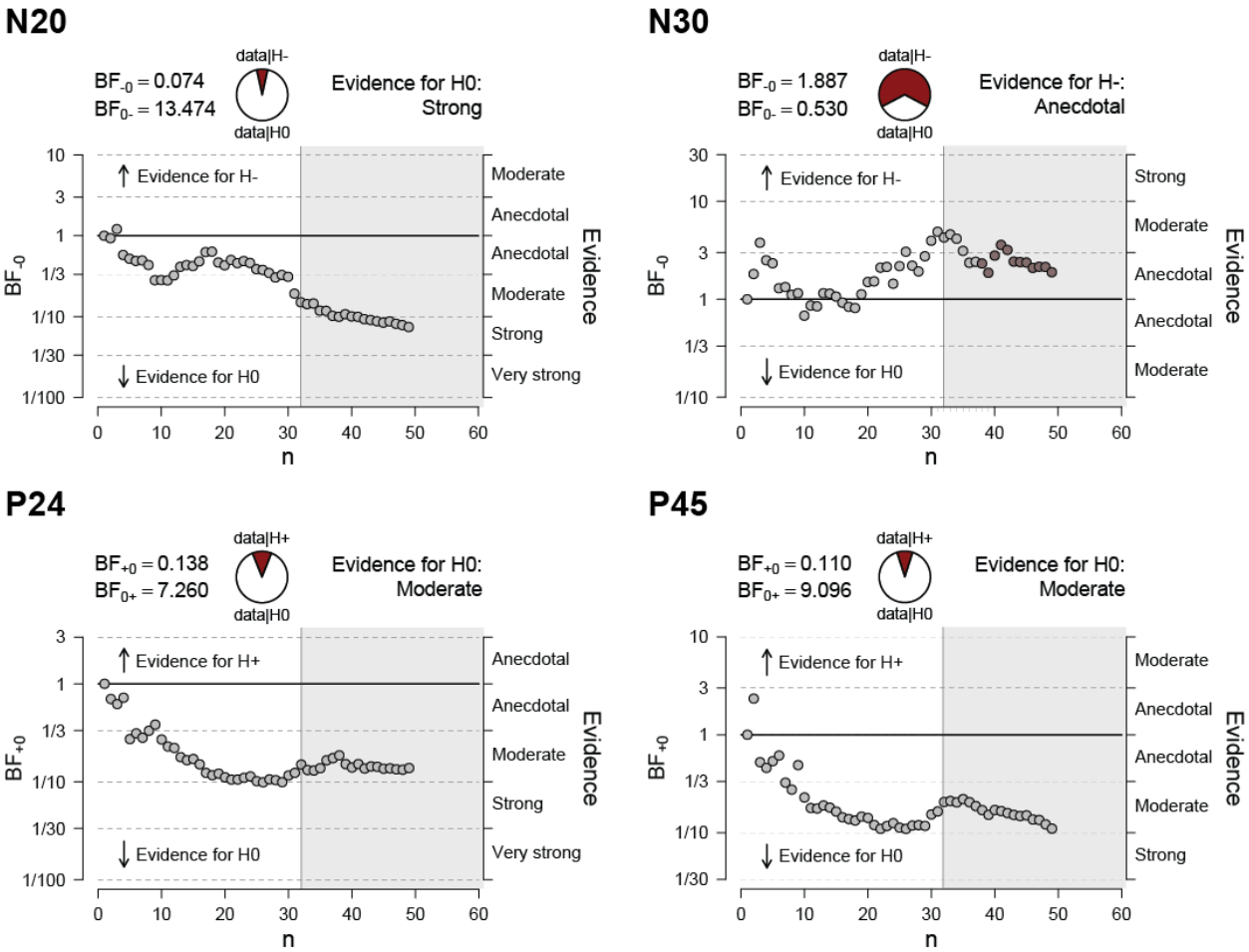

Figure 6. Bayesian sequential analyses. Cumulative probabilities of the data given our hypotheses for each added participant. The grey area indicates the pool of participant coming from Experiment 2. The beginning of darker dots in the N30 plot indicates that from that point onward (n=38) the N30 distribution violates normality (Shapiro-Wilk W=.94 with p=.042).

To have a better understanding of our data, and of why the effect found in Experiment 1 did not replicate, we pooled the data of the two experiments together and ran a sequential analysis, which calculates the evidence for H_1 sequentially (and cumulatively) for each added participant: results at n=x consider the data including the first x participants, results at n=x+1 those including the first x+1 participants, and so forth. Figure 6 shows that while for N20, P24 and P44 the evidence in favour of a null effect keeps accumulating over participants in a relatively constant manner reaching a moderate (for P24 and P44) to strong (for N20) strength, this is not the case for N30. The sequential analysis on N30 indicates that the effect fluctuates across participants, without a clear homogenous directionality. More specifically while some participants contribute toward the predicted effect (increase in the N30 negativity), others contribute to the evidence in the opposite direction, leaving the evidence in favour of the increase of negativity anecdotal at best. In particular, this analysis shows that for N30, we happened to stop collecting data in Experiment 1 at the peak of the evidence. To be noted that the distribution of the N30 components starts to violate normality after participants 37 (32 subjects from Experiment 1 + the first 5 participants of Experiment 2; from participant 38 the Shapiro-Wilk test for normality W=.94 with p=.042), making the interpretation of this analyses for N30 more tentative. Table 3 reports the results of a 2-tailed Bayesian t-test, which are useful for planning future experiments.

	Median	95% CI	BF_{10}	BF_{01}
N20	0.17	-0.10, 0.44	0.32	3.13
$N30_{49}$	-0.27	-0.55, 0.01	1	1
$N30_{37}$	-0.32	-0.65, 0.01	1.24	0.81
P24	-0.02	-0.3, 0.24	0.16	6.36
P45	-0.07	-0.34, 0.21	0.18	5.72

Table 3. Effect size estimates from full sample. The table indicates for each component the median effect size (Cohen d) of the index at t1 together with the 95% confidence interval (CI) as resulting from a Bayesian t-test, together with the Bayes factors of a Bayesian 2-sided t-test (indices different from 0). Considering that the distribution of the N30 indices becomes not normal from participant 38, the values are reported both for the full not-normal sample ($N30_{49}$) and the reduced normal sample ($N30_{37}$). Note that in all cases effect sizes are small at best, and CI always include the zero line, suggesting that there is no confidence that the effect size differs from zero.

Discussion

After initial enthusiasm, the effectiveness of currently used tDCS protocols is increasingly debated (Neuling, Rach and Herrmann 2013; Helfrich *et al.* 2014; Chhatbar and Feng 2015; Horvath, Forte and Carter 2015; Padberg *et al.* 2015; Woods *et al.* 2015; Ruhnau *et al.* 2016; Medina and Cason 2017; Parkin *et al.* 2018; Vöröslakos *et al.* 2018). Recently, a protocol of good practices for rigor and reproducibility in tDCS research has been published (Rumsey *et al.* 2017). We designed our studies to conform to those guidelines and tried to replicate our own results. Even though Experiment 1 results seemed to suggest that our montage modulated SI excitability, the effects of the stimulation were small (Cohen d estimated around 0.4) and characterized by individual variability. A lack of reproduction of the effect in Experiment 2 would perhaps not be entirely alarming, as the smaller study would have been expected to detect an effect size of 0.4 in only half the cases (power analysis, n=17, d=0.39, alpha=0.05, power=0.46). Bayesian sequential analyses though revealed that the results at the end of Experiment 1 were a singular peak of significance in a set of data. This points towards such a small effect size (d=0.27) that one would need to conduct studies with 87 participants to achieve 80% at alpha=0.05. As a result, this kind of tDCS protocol would be highly impractical in most cognitive neuroscience or clinical contexts.

The bias towards publishing positive results in the scientific literature can lead to a substantial literature with limited evidential value (Medina and Cason 2017). This problem is particularly felt in the non-invasive brain stimulation field. The widespread use of frequentist statistic means one can provide evidence only for the presence of an effect, but not its absence. While $p<0.05$ arguably provide publishable evidence for a role of a brain region in a task, non-significant P-value do not translate into a likelihood that the null hypothesis is true (Wagenmakers *et al.* 2017, 2018), i.e. that a region is not involved in a task. A series of articles have stressed these limitations and proposed alternatives (Nienborg and Cumming 2009; Kruschke 2011; Johnson 2013; Nuzzo 2014; Halsey *et al.* 2015; Simonsohn 2015). The Bayes factor hypothesis testing in particular allows researchers to quantify the relative evidence for and against the null hypothesis, and to monitor it continually as data accumulate (Wagenmakers 2007; Rouder 2014). In this study, after identifying a HD-tDCS modulation of N30 in Experiment 1 through frequentist approach, one could have been tempted to publish the result, leading others to invest in HD-tDCS to study the increasingly recognized function of SI in higher cognitive functions (Keysers, Kaas and Gazzola 2010; Bolognini *et al.* 2014; Gallo *et al.* 2018). Since we did not replicate the results in Experiment 2, we took advantage of the Bayesian framework. The sequential analysis on N30 indicates that the effect fluctuates across participants, without a clear homogenous directionality, with the contribution of some participants moving the evidence toward the predicted

effect (increase in the N30 negativity), and others' contributing to the evidence in the opposite direction, leaving the evidence in favour of the increase of negativity at best anecdotal, and effect sizes clearly too small to be of much use in cognitive neuroscience. In particular, this analysis shows that for N30, we happened to stop collecting data in Experiment 1 at the height of the evidence. Our montage thus produces insufficient effect size for use in moderately sample-sized experimental studies and clinical applications.

We designed the HD-tDCS montage tested in this study with the goal of maximizing the focality of the stimulation over SI. The risk of increasing focality is to loose current penetration (Datta *et al.* 2008; DaSilva *et al.* 2015; Alam *et al.* 2016). Intuitively, this is because reducing the distance between anode and cathode reduces the brain volume that would be affected but makes the scalp a shorter circuit. Moreover the positioning of the electrodes so that the current flows parallel to the central sulcus might have exasperate variability between participant's response to the modulation, because it has been suggested to produce non-uniform current directions across the cortical surface (Rawiji *et al.* 2018). Thus, the arguably neglectable effect size we found does not challenge tDCS in general, but our specific montage in particular. We are nevertheless keen to share our experience with our montage because we feel it has been an instructive and sobering example of how risky it is to take small effects in tDCS at face value without adding some more participants to see if the effect consolidates.

Our results highlight the need for robust replication of positive tDCS results to better understand the efficacy of, and mechanisms involved in, non-invasive brain stimulation. Further studies should be carried out to explore the potential of focality of HD-tDCS protocols, and the use of Bayesian statistics can provide us with the means to provide evidence in favour of either an effect or HD-tDCS or a lack thereof.

Acknowledgement

We thank Tatjana Maskaljunas and Balint K. Lammes for helping with the data collection.

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Chapter 5:
General discussion

The crucial role of SI vicarious activity in prosocial behaviour

Observing another person in pain elicits vicarious activity in the brain, which is similar to that seen when experiencing pain ourselves. This vicarious pain activity is correlated with empathic traits and it is believed to be the neural correlate of empathy for pain (Keysers, Kaas and Gazzola 2010; Lamm, Decety and Singer 2011). Seeing others in pain teaches us to avoid the pain-eliciting environment or stimulus (de Vignemont and Singer 2006; Benuzzi *et al.* 2008a). It has a threat value that can elicit a motor response, such as freezing or fleeing (Avenanti *et al.* 2005), and it can promote prosocial behaviour, such as providing care to the person in pain (Goubert *et al.* 2005; Benuzzi *et al.* 2008a; Decety 2010).

Empathy for pain has a multidimensional nature that is thought to involve affective, cognitive, and somatosensory-motor processes (Davis, 1983; Avenanti & Aglioti, 2006; Gallese, 2001; Preston & de Waal, 2001). Affective and cognitive processes have their neural correlate in limbic areas of the brain, namely the Insular cortex and the Cingulate cortex, the involvement of which are well established. Somatosensory-motor processes rely on the motor and somatosensory cortices, but their role is a matter of debate. One hypothesis is that it is only when participants directly witness an intense, localised, harmful somatic event as the cause of the other person's pain that they vicariously activate their somatosensory cortices (in addition to the affective representation in other regions of the brain) (Keysers, Kaas and Gazzola 2010).

To test this idea, we designed two kinds of stimuli that each depicted a person receiving a painful stimulation on her right-hand, but which differed in their SI recruitment. The 'hand videos' showed a hand receiving a slap with a belt, highlighting the sensory component of pain. The 'face videos' depicted a person with a pained facial expression, after receiving electrical stimulation to the hand. The stimuli were designed to emphasise the unpleasantness of pain (i.e., the affective component of pain).

As noted in Chapter 2, we identified, using fMRI, the regions in SI that correlated with variation in subjective perception of pain caused by noxious stimulations on the right hand. This pointed to two different clusters: a dorsal cluster, coherent with the hand representation, and a ventral, coherent with the face representation. Using EEG, we assessed whether vicarious activity in these clusters correlated with helping behaviour, measured by the sum of money the observer chose to give away to lessen the intensity of pain stimulation of the other person's hand. In the dorsal cluster, this correlation was observed for both stimuli—in effect, whether the information about the victim's pain was delivered by their hand reaction or their facial expression. However, the activity of the ventral cluster predicted the behaviour only when the victim's facial expression was visible. As the theoretical framework of emotional contagion and vicarious activity would predict, the activation of brain areas involved in experiencing pain during the observation of other people's pain helped the observer to 'feel' what the other person was experiencing by inducing a similar psychological state.

In the following experiment, we interfered with the activity of the SI hand knob by means of repetitive TMS. This disrupted the relationship between the pain witnessed and the sum of money donated to help, but only when the painfulness was indicated by the victim's hand movement. We interpreted this result as proof of the essential contribution of SI in transforming the sight of the observed kinematics into an accurate sense of how painful the particular stimulation was for the other. Although activity in the hand representation in SI predicted the amount of money donated in the trials in which the facial expression alone carried the information about the pain felt by the victim (face videos), interfering with the hand

representation activity did not affect the helping behaviour in trials in which participants watched the face videos. The information coded during the face videos in the dorsal SI, the portion of SI that represented the hand sensation, may be an epiphenomenon, possibly generated by our participants' knowledge that the stimulation was happening to the hand, thus it was not fundamental to understanding its intensity. (Alternative interpretations are discussed in Chapter 2.) The results are coherent with the notion that pain information takes different paths based on the stimulus from which it is derived (Keysers, Kaas and Gazzola 2010; Lamm, Decety and Singer 2011). We suggest that, when the suffering of the victim is expressed by facial expression, manipulating the brain activity of the somatosensory face representation may affect the helping behaviour. The contribution of this area to understanding emotional facial expressions has been demonstrated in the literature (Adolphs *et al.* 2000; Oberman, Winkielman and Ramachandran 2007; Wood *et al.* 2016a; Paracampo *et al.* 2017). Future research should neuro-modulate brain activity in ventral SI in addition to the hand representation we targeted here, while measuring willingness to help, to further investigate the dissociation suggested by our results. As explained in Chapter 5, we tested a novel HD-tDCS montage, the goal of which was to increase the spatial resolution of the brain stimulation. To assess the effectiveness of the montage, we tested its capability in modulating the cortical excitability of the hand region. Unfortunately, the results were not sufficiently reliable to proceed with testing the hand/face dissociation in our costly helping paradigm.

The crucial role of SI vicarious activity in other's pain perception

In the first two experiments described in Chapter 2, we used stimuli designed to differently engage the sensory motor network of pain and vicarious pain, and we showed that the putative face representation in SI is activated only when the stimulus showed a facial expression of pain, while the hand representation was active both when the participants witnessed the facial expression and the hand receiving the noxious stimulation. We focused on the vicarious activity of SI with the goal of leveraging its superficial position to apply brain stimulation and to determinate its functional relevance in prosocial behaviour. When the hand receiving the noxious stimulation was the source of information about the unpleasantness of the stimuli, the hand representation was necessary to modulate helping behaviour toward the victim and interfering with the brain region had an effect on the participants' behaviour.

In the third experiment described in Chapter 2, we tackled the question of *how* SI contributes to prosocial behaviour. Participants watched the same stimuli presented in the costly behaviour paradigm and a third type of stimuli in which no pain was depicted. We asked them to rate how painful the stimuli were for the person in the videos or the extent to which the saturation of the control stimuli changed. Interfering with SI activity by means of an established HD-tDCS protocol improved the accuracy of the ratings, but only when the painfulness was deduced from the hand movement. These results suggest that SI functionally contributes to accurately perceiving another person's pain by simulating it internally. This information is then sent to other regions, in which the prosocial decision-making process takes place.

This result supports others described in the literature that highlight the fundamental role of SI in other's pain perception (Avenanti *et al.* 2005; Jackson, Rainville and Decety 2006; Bufalari *et al.* 2007; Aziz-Zadeh and Damasio 2008; Valeriani *et al.* 2008; Benuzzi *et al.* 2008b; Cheng *et al.* 2008; Betti *et al.* 2009; Perry, Troje and Bentin 2010; Whitmarsh *et al.* 2011; Riečanský *et al.* 2014; Hoenen, Lübke and Pause 2015). It does so by using ecological video clips of the same person receiving a noxious stimulation, specifically created to differentially engage SI. However, contrary to much of the literature,

which usually compares low and high perceived intensities, our stimuli cover a spectrum of intensities, enabling us to test a more fine-grained relationship between SI activity and perceived intensity. Finally, we used a control task in which pain was not present, and requested the same process (rating intensity) from the participants. In this way, we found that the modulation was not generally related to the task, but specific to the pain content.

Thanks to this series of experiments, we were able to demonstrate that vicarious brain activity (i.e., the recruitment of regions involved in our own somatosensation while witnessing somatosensory states of others) plays a role in how we perceive the distress of others and how changes in perception influence prosocial behaviour. Our results support the idea that we must represent someone else's pain as if it were our own to correctly interpret what the other person is feeling. This information is then transmitted elsewhere in the brain, where the decision about the help to offer takes place. We demonstrated that the vicarious activity of the somatosensory cortex, likely coding for the sensory discriminative information about the noxious stimulation (e.g., the location, intensity, mechanical quality), has a crucial role and it is part of the network necessary for understanding other's feelings, but only if there is no other available information about the unpleasantness.

Functional meaning of vicarious activity

With the series of studies described in Chapter 2, we investigated the role of SI in prosocial behaviour and other's pain perception. In Chapter 3 explains that we took advantage of fMRI spatial resolution to expand the study of vicarious pain activity to the entire network of regions defined by subjective experience of pain.

There is a body of literature exploring the notion that watching another person in pain activates regions that are also active when we are in pain ourselves, but recently this idea has been challenged by new findings. Critics argue that experimental designs used to investigate shared brain activity often do not allow us to discriminate between activity related to pain and activity related to unspecific cognitive processes (Krishnan *et al.* 2016). Moreover, most studies do not test whether the activation is generalisable for different kinds of noxious stimulations (Woo *et al.* 2014; Chang *et al.* 2015; Krishnan *et al.* 2016).

With the study described in Chapter 3, we sought to deepen our knowledge of shared activity for pain and to characterise shared activity using more strict criteria (Chapter 3).

We identified a cluster of voxels crossing the AI/fronto-insular cortex, whose activation was common to experience and observation of pain, linearly modulated by the intensity, and which was not active for stimuli not depicting pain, independent of how the pain was inflicted. Although the exact location of the cluster was more frontal than is usually reported in the literature, it was sufficient to slightly relax the threshold of the correction for the multiple comparison, to observe how it expanded in the Insula and not in the frontal lobe.

Other regions traditionally included in the mirror system, namely the bi-lateral anterior insula (AI) and anterior-middle cingulate cortex (ACC, MCC) (Gallese, Keysers and Rizzolatti 2004; Singer *et al.* 2004; Jackson, Meltzoff and Decety 2005; Lamm *et al.* 2007; Corradi-Dell'Acqua, Hofstetter and Vuilleumier 2011a; Fan *et al.* 2011; Lamm, Decety and Singer 2011; Corradi-Dell'Acqua *et al.* 2016; Zaki *et al.* 2016a; Carrillo *et al.* 2019), were not found to be specifically for pain. ACC and MCC were indeed part of the shared activity network in our results, only when the control task was not taken into the equation (see Chapter 2 'Supplementary High vs Low Rating Design'). Particularly surprising was the lack of results for SI, which was suggested to be functionally relevant for the task and specific for

pain in the series of experiments described in Chapter 2. The inconsistency of these results is discussed later in this chapter.

Indeed, the number of voxels satisfying our criteria in the Observation task was unusually low. These voxels are the results of the contrast obtained by the sum of the BOLD activity linear modulated by the ratings of pain in the face and hand videos, after subtracting the BOLD activity linearly modulated by ratings of change of saturation in the control task. In Chapter 2, we discussed some theoretical and methodological explanations for this. From a theoretical point of view, we suggested that the stimuli of the three categories being randomised and, in the context of an experiment clearly about pain, showing—albeit not fully in focus—a picture of the same person receiving the noxious stimulation in both videos, might have had a strong influence on the brain activity induced by the control task. It is known that the context in which a stimulus is presented influences how it is elaborated; and in the theoretical framework of embodied cognition models, people do not represent concepts in isolation, but within the relevant situational context (Barsalou 1999; Wilson-mendenhall *et al.* 2009; Oosterwijk *et al.* 2017). From a more methodological perspective, it is important to highlight that, when we apply to the data a design that does not contain a linear regressor but which tests stimuli rated as high pain (contrasted with stimuli indicated as low pain), the face videos result in the network usually described in the literature, while the hand and colour videos failed to show any voxel surviving the correction for multiple comparisons. The Observation task was not designed for this analysis and, as a consequence, the division of the trials into high and low rated is *a posteriori* and somewhat artificial. Nevertheless, the lack of results for the hand and colour videos suggests more fundamental limitations in the design of the experiment. To disentangle these possible theoretical and methodological issues, we are running the Observation task with an independent pool of participants. In this new experiment, the participants do not receive painful stimulation themselves, and we cluster stimuli belonging to each category (face, hand, and colour videos) in blocks of trials, separated by breaks. We hope that this will mitigate the effect of the embodied context 'pain'. Results from this data will be able to answer some of the remaining questions.

Interestingly, while the results of applying the General Linear Model approach to fMRI data converge when assigning a role for the insula in both the experience and observation of pain, recent studies using Multi Voxel Patter Recognition have produced contradictory findings. Some have successfully identified patterns sensitive and specific to both experienced and observed pain (Corradi-Dell'Acqua, Hofstetter and Vuilleumier 2011b), while others have not, claiming dissociability of the mechanisms (Krishnan *et al.* 2016). The classification algorithms used in this kind of analysis tend to focus on discriminative features and ignore those features shared across the categories (Haynes 2015). Decades of work on mirror neurons for actions show that just 10% of neurons involved in performing an action are recruited when observing that action (Gallese *et al.* 1996; Keysers *et al.* 2003; Mukamel *et al.* 2010). A classification algorithm is less likely to focus on this common neural substrate to discriminate between intensities and more likely to rely on signals from the other 90%. A region could contain neurons that code for both variation in intensity of experience and observation of pain, but the MVPA may not detect them if it concentrates on the other 90%.

It is important to emphasise that our results are based on voxels analyses, and a voxel can contain millions of neurons. It is impossible to know, using an fMRI methodology, whether the signal is recording the same population of neurons responding to the two tasks, or whether this signal could originate from neurons that are simply spatially intertwined with those involved in the pain experience (Keysers and Gazzola 2009; Zaki *et al.* 2016b). Until we have systematic single cell data for the experience and observation of pain (Hutchison *et al.* 1999), it will remain unclear whether neurons represent felt and observed pain reliably (Zaki, Wager, Singer, Keysers, & Gazzola, 2016b). In the same

fashion, it is unclear whether the linear relationship between BOLD activity level and observed pain intensity attributed to voxels in the insula exists at the neuron level or the population level. Spatial resolution is an intrinsic limitation of fMRI studies, which is not an adequate method of exploring the neuro-correlate of function at the cell levels.

Intracranial recordings can provide crucial converging information in cases such as these. Electro physiologic data gave some important contribution on the role of insula in own pain perception (Hauck, Lorenz and Engel 2008). Recent studies found that event locked activity is unspecific to pain, touch or sound (Liberati *et al.* 2016), but gamma frequency power recorded from the area is preferential for nociception (Liberati *et al.* 2017). In the same fashion, intracranial recording have the time and space resolution to disentangle if the contribution of the insula to empathy for pain is modality specific or not, and its sensitivity to empathic pain.

In a currently ongoing study, we ask epileptic patients, which are planned to have micro-electrodes implanted in the insula as part of their epilepsy treatment, to watch and rate our hand and face video. We will analyse time-frequency bands power recorded and the spike activity recorded from the same micro-channels. These analyses will give valuable insights about the sensitivity of the neurons and the relationship between spike activity and behaviour.

An important limitation of human electro-physiology experiments is the location of the electro-physiological recordings. The spread of the electrodes and their exact location are strictly dictated by clinical factors. In a study recently published by our laboratory, Carrillo and colleagues used a previously established model of emotional contagion in which a rat observes a conspecific experience painful electroshocks (Jeon et al. 2010; Atsak et al. 2011; Kim et al. 2012; Gonzalez-Liencres et al. 2014; Keum and Shin 2016; Carrillo et al. 2019), while multi- and single-unit activity is recorded via chronically implanted probes in the rats' ACC. The results indicate the existence of neurons that respond for both experienced and observed shock, but not for fear (Carrillo *et al.* 2019). More research is necessary to explore how this relates to animal prosocial behaviour, to other regions of the rat's brain, and to the human brain and behaviour.

Inconsistency between SI activation in EEG and fMRI data

The involvement of SI in other's pain perception has been at the centre of debate due to inconsistencies in the findings (Keysers, Kaas and Gazzola 2010; Lamm, Decety and Singer 2011; Betti and Aglioti 2016). Interestingly, inconsistency emerges in our work between the studies presented in Chapters 2 and 3.

As described in Chapter 2, we tested the involvement of SI in prosocial behaviour when viewing the stimulus of the person in pain, measuring the activity using EEG. This technique revealed a small time-window in which the activity of the portion of SI representing the hand predicted the size of the donation given by the participants, when the pain was represented by both the hand movement and the facial expression. When the face was the source of information, a ventral portion of SI was also modulated by the donation. Interfering with SI activity using TMS had an effect on the relationship between the amount of pain witnessed and the donation, confirming the crucial functional role of this region in our paradigm. We also proved the functional involvement of this region in pain perception: disturbing its activity while the participants were performing the rating of the other's pain had an effect on their

performance. This occurred only when the pain was deduced from the hand movement and it was specific for pain, thus it did not affect the rating of stimuli unrelated to pain.

In the Chapter 3 Appendix, we explored the involvement of SI in other's pain perception. Rather than recording the EEG signal, we recorded the BOLD signal when participants were performing the rating task on the two kinds of stimuli (one depicting a person in pain and the other being the control, in which no pain was shown). When we interrogated SI using an ROI approach, this failed to prove the involvement of the region in judging the intensity of observed pain. The whole-brain approach was not successful either: no voxel belonging to SI was identified to be linearly modulated by observed pain intensity.

There may be multiple reasons for this incongruence between the EEG, neuro stimulation studies, and fMRI recordings. The nature of the stimuli used in our tasks could be accentuating one of the major differences between the EEG and fMRI technique: time resolution. To portray the noxious stimulations given to the actor, we used two seconds of footage. The first of these two seconds served as a baseline, with no pain shown; while in the second half of the clip, the actress made movements of the hand or face (depending on the kind of stimuli), related to the painfulness of the stimulation. The EEG highlighted brief but significant time-windows of activity related to donations while watching the clips, specifically 32 and 18 ms when the pain was deduced from the hand movement and 86, 146 and 72 ms when the facial expression changed. The length of these clusters is two orders of magnitude below the time resolution of our fMRI, which is 1.7 s. It is possible that the cerebral activity informing of the videos' perceived intensity was too short to be captured by the slow BOLD signal changes.

Another reason for our failure to find significant predictors of the ratings in SI is that we had to contrast the emotional stimuli with our control task. This was because of our decision to develop a more spontaneous task in which participants could rate the video as soon as they had determined the level of observed pain. As a consequence, each button was associated with a pre-determined pain value. Subjects used the same finger to press a specific button each time, therefore each pain-value was associated with a specific finger movement. With such a design, it is impossible to fully regress out motor activity from a single button press event, and the impact of the motor preparation and movement are taken into account by direct contrasts of different conditions (i.e., face, hand, and colour videos). The experimental conditions had the same motor responses for rating intensities, but the content of the rating (pain vs. colour saturation) was different. It could be, therefore, that we over-estimated the expected perceptual differences compared to the motor responses. In the follow-up experiment, therefore, we controlled for this limitation.

This reasoning assumes that the time window identified for predicting the donation also predicted the perceived intensity of pain. In the study, the donation given was highly correlated with the rating assigned to the videos by an independent pool of participants, thus it is reasonable to assume that the time-window also correlated with the perceived intensity assigned to the video. However, this was not directly tested and could create noise when comparing the results.

Very different aspects of brain activity are measured by EEG and fMRI. The former is a direct measure of voltage fluctuations resulting from ionic current within the neurons of the brain, originating from post-synaptic potential; while fMRI is an indirect measure of the cerebral activity. It images the change in blood flow (hemodynamic response) related to energy use by brain cells. Although studies have shown that spatial maps of fMRI BOLD activity in response to different types of sensory stimulation roughly correspond to EEG and MEG responses in the respective primary sensory cortices (Singh *et al.* 2002; Brookes *et al.* 2005; Im *et al.* 2007; Rosa *et al.* 2010), integrating results across methods is

challenging because the signals measured by these instruments differ in spatial and temporal sensitivity, as well as in the manner in which they combine the underlying neuronal population activity (Logothetis and Wandell 2004; Buzsáki, Anastassiou and Koch 2012).

The relationship between fMRI and EEG measurements is far from being understood. fMRI has a better spatial resolution than EEG, but the time lag between synaptic activity and changes in blood flow fundamentally limits its temporal resolution, and thus its ability to discriminate during the sequence of neural activity in complex cognitive tasks (Magistretti et al., 1999). In contrast, electrophysiological recordings of EEG are capable of identifying the cortical regions activated during different stages of task performance. If these sequential activations also partially overlapped in time, in the time resolution frame of fMRI, these regions would presumably be lumped together by fMRI into a larger region of activation (Crone, Sinai and Korzeniewska 2006).

Other scholars go further and support the hypothesis that such fundamental functional difference must suggest that the measurements are based on different aspects of the neural population response (Hermes, Nguyen and Winawer 2017). Recent studies and models have suggested that the BOLD signal represents the asynchronous broadband component of the electric signal generated by neural activity and not the event-related one (Niessing *et al.* 2005; Winawer *et al.* 2013; Hermes, Nguyen and Winawer 2017).

In the first experiment in Chapter 2, we specifically interrogated the signal fluctuations time-locked to the videos, the event-related potentials. Focusing attention on the frequency domain may solve the incongruence between our EEG and fMRI results. Unfortunately, EEG losses sensitivity at higher frequencies and is thus unsuitable for recording the higher gamma band activity that could best correlate with fMRI. Other techniques, such as Magnetoencephalography (MEG) or intracranial recoding, may be more suitable for this task.

Finally, we used beam-formed source reconstruction guided by fMRI results to identify the EEG activity originating in the somatosensory cortex. Although this is a state-of-the-art method for EEG source activity reconstruction, we used a template of the head to calculate the beam-former and not a personalised head model for each participant. This, together with the intrinsic level of error present in each measurement, could have meant that the signal recorded was not, in fact, originating from SI, but rather from the neighbouring areas.

A final possibility we must consider is that our findings in SI in the EEG signal could have been a false positive. Replicating a similar task with EEG would shed light on the reliability of our finding.

Modulation of the vicarious activity

We explored the neural correlate of prosocial behaviour and other's pain perception. We aimed to overcome the correlational nature of neuroimaging studies by pairing them with brain stimulation techniques. As detailed in Chapter 2, we used repetitive TMS to interfere with SI activity while performing a costly helping paradigm and offline HD-tDCS over SI to modulate other's pain perception.

The 'interventional nature' of brain stimulation has added a new dimension to human brain mapping, opening up new possibilities for probing causality at the system level of sensory, cognitive, and motor brain networks. This unique feature in the field of cognitive neuroscience depends primarily on the ability of brain stimulation techniques to interact transiently with the stimulated brain area, modifying its activity and interrogating its function. Its functional impact is due to its ability to transiently alter

neuronal function (Miniussi et al., 2013), modifying information processing dependent on the activity of the involved neurons (Siebner *et al.* 2009; Silvanto and Cattaneo 2017).

Brain stimulation techniques can effectively activate neuronal outputs that project from the stimulated site to other distant areas of the brain. This means that they can modify ongoing neuronal activity within complex neuronal circuits, not only those at the site of stimulation.

Remote effects of TMS on the vicarious activation activity related to the observation of another person have been observed in multiple studies (Driver *et al.* 2009; Siebner *et al.* 2009; Reithler, Peters and Sack 2011).

A recent study combining TMS over SI and fMRI recordings identified brain regions exchanging action-specific information with SI by mapping voxels away from the coil, with TMS-induced BOLD contrast changes that mirror those under the coil. These remote effects have been interpreted as evidence for a causal backward influence from SI to the rest of the action-specific network (Valchev *et al.* 2016).

Both TMS and (HD-)tDCS techniques induce remote effects from the site of stimulation to the rest of the network involved in the task under study. For this reason, target sites should be considered nodes within a widespread network of interacting brain regions, where perturbing or boosting processing of one element can also influence several others (Reithler, Peters and Sack 2011). This is an important factor to consider when interpreting the results of the brain stimulation studies described in Chapter 2. In our design, it is not possible to determine whether the behavioural effect observed after stimulation is directly caused by SI activity manipulation or if whether it is the indirect result of remote activation changes.

Electrical stimulation techniques are not only affected by the phenomenon of the remote stimulation effect, but they also suffer limited spatial resolution of the direct stimulation. In fact, in magnetic stimulation, the area directly affected is limited to the region under the TMS coil, while the area affected by the electric stimulation includes the region below and between electrodes (Datta *et al.* 2009; Kuo *et al.* 2013; Villamar *et al.* 2013; Filmer, Dux and Mattingley 2014). This is regarded as an important drawback of the more traditional form of tDCS, which applies current through two large electrodes that are often placed far apart (Villamar *et al.* 2013; Antal *et al.* 2017). HD-tDCS was developed to enhance the spatial resolution of the stimulation. Compared to standard tDCS, it uses smaller electrodes and the distance between them is reduced: typically a positive one surrounded by four negative, in a radius of 5/7 cm (Kuo *et al.* 2013; Villamar *et al.* 2013; Roy, Baxter and He 2014).

These methods produce electric fields with length scales of the order of several centimetres, which span anatomically and functionally distinct human brain circuits. Despite this improvement, focal stimulation of target cortical regions not involving stimulation of neighbouring anatomical areas is difficult to achieve with this technique (Datta *et al.* 2009; Dmochowski *et al.* 2011).

We are particularly interested in understanding the contribution of SI to understanding other's pain when the affected body part is visible and the pain must be deduced by its movement, in contrast to when the unpleasantness of the painful experience is highlighted. To pursue this, we designed the hand and face videos. Using fMRI, we localised regions in the left SI that encode the intensity of pain, as experienced here by the group of participants. This evidenced two clusters in the left SI: a dorsal cluster corresponding to the hand representation of SI and a ventral cluster with a dorso-ventral extent similar to the face representation of SI. EEG data suggested that dSI was active when witnessing stimulation on another person's hand, predicting the helping behaviour, independently of how the pain was conveyed. However, vSI did the same only when the other person's pain was conveyed by facial

expression. Owing to the TMS over the hand representation of SI, we were able to confirm that this area is necessary for perceiving the intensity of other's pain and adapting prosocial behaviour toward the person in pain. It would be extremely valuable to identify the functional role of vSI while exposed to the stimuli, but the TMS on the ventral region of SI causes movement in the facial muscles, and these twitches would be likely to distract the participant, thus affecting emotion recognition (Oberman, Winkielman and Ramachandran 2007; Wood *et al.* 2016b) and creating ambiguous and difficult to interpret results when applied to the face stimuli.

In Chapter 4, we described a novel montage designed to deliver as focally as possible a stimulation of the hand knob in SI in both hemispheres and that induced reliable changes in cortical excitability. The montage pushed the boundaries of HD-tDCS, using only one small electrode positively charged above the region of interest and one return electrode close to the vertex. We designed the protocol to simultaneously stimulate dSI in both hemispheres.

There is evidence for a bilateral receptive field in the Brodmann 1 and 2 sub-regions of SI (Iwamura et al. 2002), although the right hemisphere has been found to dominate in the perception of emotion (not including pain) from facial expressions (Adolphs et al. 2000; Carr et al. 2003; Lamm, Decety and Singer 2011; Cui et al. 2015; Ashar et al. 2017) and hand movement (Christov-Moore and Iacoboni 2016). In Chapter 2, we show that the signal originating in mirroring our left ROIs to the right hemisphere significantly explained the donation. Results from the hand region of SI in the two hemispheres are very similar, in line with more bilateral activity (Chapter 2 Figure 3D and Supplementary Information). For the more ventral, putative face region of SI (vSI), the responses appear stronger on the right hemisphere, in line with previous findings (Adolphs et al. 2000).

If successful, our bilateral montage would have had the potential to positively enhance dSI activity while inhibiting vSI on both hemispheres (and vice versa).

We tested the modulation of cortical excitability (measured by Somatosensory Evoked Potential) induced by the novel protocol and re-tested its stability. In the first study, the montage was found to modulate SI, but this result failed the replicability test. We suggest that the effect of the stimulation was weak and too susceptible to individual subject variability to support strong claims about the modulation of cortical excitability. Further studies should be carried out to explore the boundaries of HD-tDCS protocols.

Another non-invasive brain stimulation technique has recently gained attention for it potential to very focally stimulate the brain and reach deeper structures of it. This technique is called 'transcranial Focus-Ultra-Sound' (tFUS) and it uses a single-element-focused transducer through the human skull to modulate brain activity. Recent studies have suggested that, when SI is the target, it has a spatial resolution in the order of a few millimetres (Legon *et al.* 2014; Mueller *et al.* 2014; Lee *et al.* 2015). Ultrasound can noninvasively modulate neural targets deep in the brain. In a recent study, Legon and colleagues claim to have successfully targeted and neuromodulated the ventro-posterior lateral nucleus of thalamus, showing the high spatial resolutions and deep focal lengths that this technique offers (Legon *et al.* 2018).

In relation to the present work, the emergence of focused ultrasounds as a focal neuro-modulation method (Mueller et al. 2014; Lee et al. 2015; Lee et al. 2016) could solve some of the limitations intrinsic to the use of TMS and HD-tDCS. First, this would enable a more focused stimulation of the hand knob of SI and the dissociation from neighbouring regions, such as facial representation in the same region. This would disentangle the contribution of the two regions suggested by our results, without the muscle artefacts that are inevitable with TMS. HD-tDCS, as used in our third experiment,

also has the advantage of not causing muscle twitches, but it lacks the focality to argue with confidence that one can disentangle the contribution of the face and hand region located only 2 cm away.

Second, it would allow direct interference with the affective pathway of pain elaboration in regions such as the insula and the cingulate cortex, which have been repeatedly demonstrated to be involved in observing another in pain or in helping behaviour, but which fall outside the target range of TMS and (HD-)tDCS.

Conclusion

Prosocial behaviour is an extremely important aspect of human social life. Both human beings and animals have been seen to help conspecifics, even at a cost to themselves. The theory suggests that this behaviour is motivated by 'feeling with/for the other'. This occurs when vicariously activating one's own pain brain network to represent the other's distress, allowing for a direct understanding of the pain and perhaps a sharing of the negative motivational value of that vicarious pain. Here, we provide evidence that the brain areas involved in feeling pain are necessary to optimally deploy helping where it is most needed—in effect, to tie one's help to the level of pain experienced by the other. These areas transform the sight of bodily harm into an accurate sense of how much pain the victim is experiencing. We use the knowledge represented in these regions to adapt our decisions to meet the needs of others. Contributing to the current debate on the role of empathy in helping behaviours, this study demonstrates that empathy-related brain activity indeed promotes helping by allowing us to detect the intensity of distress in others and thus appropriately adapt our assistance. Furthermore, we explored the functional relevance of the vicarious activations, specifically testing for sensitivity, generalisability to pain, and observation of others in pain, using a novel design and fMRI measurements. Finally, we aimed to improve the field of brain stimulation by designing a novel HD-tDCS protocol.

A key aspect of this work is that we employed different techniques to study the same subject, using the same stimuli and similar paradigms. This created a unique opportunity to reflect on the specific advantages and disadvantages of the methods employed. It also supported reflection on how methodological differences can drive inconsistent results in the literature. Evidence from experiments employing various techniques and analytical procedures provides complementary information about the phenomenon under investigation. This is extremely important in circumstances in which any given technique can only provide a very selective and distorted perspective. In such cases, it is only by relating results to those acquired through other techniques that researchers can address the questions being posed (Bechtel 2002) Two or more approaches combined, if properly selected, will complement one another well. Together, they can provide evidence for points of view that none could defend individually.

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Chapter 2

Gallo Selene, Paracampo Riccardo, Müller-Pinzler Laura, Severo Mario C., Blömer Laila*, Fernandes-Henriques Carolina*, Henschel Anna*, Lammes Balint K.*, Maskaljunas Tatjana*, Suttrup Judith*, Avenanti Alessio, Keysers Christian, Gazzola Valeria. The causal role of the somatosensory cortex in prosocial behaviour. *Elife* 2018;**7**:e32740.

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Severo Mario C.: stimuli preparation and data collection of EEG experiment.

Blömer Laila, Fernandes-Henriques Carolina*, Henschel Anna*, Lammes Balint K.*, Maskaljunas Tatjana*, Suttrup Judith**: data collection of experiment "Pain Localizer" or HD-tDCS experiment and manuscript revision.

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Keysers Christian: Project planning, manuscript writing and revision.

Gazzola Valeria: Project planning, experiment design, stimuli preparation of experiment "Pain localizer", EEG Experiment, TMS Experiment, HD-tDCS experiment and manuscript writing and revision.

Chapter 3

Gallo Selene, Maskaljunas Tatjana, Lammes Balint K., Fernandes-Henriques Carolina, Suttrup Judith, Keysers Christian, Gazzola Valeria. Shared brain activity in rating own and others' pain. In preparation and adapted from *Brain Stimulation*, *10*(2), 466. http://doi.org/10.1016/j.brs.2017.01.366

Gallo Selene: Project planning, experiment design, stimuli preparation, data collection, data analyses and manuscript writing.

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Chapter 4

Gallo Selene, Baaijen Thijs J., Suttrup Judith, Fernandes-Henriques Carolina, Keysers Christian and Gazzola Valeria. Bayesian statistics show a lack of change in excitability following bi-hemispheric hd-tdcs over the primary somatosensory cortices. Submitted and adapted from *Brain Stimulation*, *8*(2), 351. http://doi.org/10.1016/j.brs.2015.01.134

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Keysers Christian: Project planning, experiment design and manuscript revision.

Gazzola Valeria: Project planning, experiment design, data analyses and manuscript writing.

Funding

Funder	Grant reference number	Receiver
Nederlandse Organisatie voor Wetenschappelijk Onderzoek	VIDI: 452-14-015	Valeria Gazzola
Brain and Behavior Research Foundation	NARSAD young investigator 22453	Valeria Gazzola
H2020 European Research Council	ERC-StG-312511	Christian Keysers
Nederlandse Organisatie voor Wetenschappelijk Onderzoek	VICI: 453-15-009	Christian Keysers
Nederlandse Organisatie voor Wetenschappelijk Onderzoek	NIHC: 056-13-017	Christian Keysers

Summary in English

In this work, we explored the neurocorrelate of prosocial behaviour and empathy for pain, with specific attention to the functional relevance of the vicarious activity.

Prosocial behaviour is an extremely important aspect of human social life. Human beings and animals have been seen to help conspecifics, even when this has a cost for themselves. Research suggests that this behaviour is motivated by 'feeling with/for the other'. In this process, one's own pain brain network is vicariously activated to represent the other's distress, allowing for a direct understanding of the pain and, perhaps, a sharing of the negative motivational value of that vicarious pain.

In Chapter 2, we focus on the role of the somatosensory cortex in the costly helping paradigm. First, using EEG, we address whether variation in the region activity when seeing another person in pain can predict variation in helping, measured as a variation of monetary donation. Participants watched two variations of the stimulus of a person receiving a noxious stimulation on her right hand. The stimuli were intended to highlight the somatosensory component of the painful stimulation in different ways, and we assessed how this influenced the engagement of the somatosensory vicarious activity. We then applied TMS, a non-invasive brain stimulation technique that uses a focused magnetic field to temporarily interfere with the Primary Somatosensory cortex (SI) brain activity. This manipulation disrupted the relationship between the intensity of the pain witnessed and the amount of money given to help. Finally, to understand how SI activity may be related to prosocial behaviour, we interfered with our volunteers' own hand representation while asking them to indicate how much pain they thought the person in the video was suffering: if their rating was not in line with what the participants were shown, SI was deemed to be contributing to transforming the visual information to a more accurate understanding of the intensity of the pain.

This series of experiments provided evidence that the brain areas involved in feeling pain are necessary to optimally deploy helping where it is most needed—in effect, to tie ones helping to the level of pain experienced by another. These areas transform the sight of bodily harm into an accurate sense of the victim's pain. We use the knowledge produced in these regions to adapt our decisions to the needs of others. In the current debate about the role of empathy in helping behaviours, this study demonstrates that empathy-related brain activity indeed promotes helping by allowing us to detect the intensity of other's distress and adapt our assistance accordingly.

Chapter 3 details how, using fMRI, we sought to deepen our understanding of the functional meaning of shared brain activity. We explored the functional relevance of the vicarious activations, specifically testing for sensitivity, generalisability to pain, and observation of others in pain, employing a novel design and fMRI measurements. We identified a cluster of voxels crossing the Anterior Insula (AI)/fronto-insular cortex, the activation of which fulfilled all the requirements. The role of the AI in empathy for pain is well recognised in the literature, but its specificity for the task is a matter of debate between scholars. We built on the knowledge of AI functional meaning and confirmed that this region showed a pattern of activity common to experience and observation of pain, linearly modulated by the intensity, not active for stimuli not depicting pain, and independent of how the pain was inflicted. Surprisingly, other regions traditionally indicated in empathy for pain did not meet the criteria we imposed. Additional tests are required to understand the lack of results in other brain regions and the validity of the results described in Chapter 3. More experiments are also need to understand the role of

AI and in particular its specificity for pain, as opposed to being activated by more general negative valence.

Chapter 4 describes how we aimed to improve the field of brain stimulation by designing a novel HD-tDCS protocol to bilaterally and focally modulate the cortical excitability of the hand knob of SI. The effect of the stimulation was weak and too susceptible to individual subject variability to allow us to make strong claims about the modulation of cortical excitability. Further studies should be carried out to explore the boundaries of HD-tDCS protocols. This might be exported to the study of empathy of pain and help to disentangle the contribution of SI from the neighbouring areas.

Taken together, these results provide new insights into the relationship between vicarious activation and prosocial behaviour and the functional meaning of this kind of brain activity. Moreover, they provide an opportunity to reflect on how different techniques affect the development of knowledge.

Samenvatting in het Nederlands

In dit proefschrift hebben we onderzocht wat de neurale correlaten zijn van prosociaal gedrag en empathie voor pijn, met specifieke aandacht voor de functionele relevantie van de gedeelde representaties van deze neurale activiteit.

Prosociaal gedrag is een uiterst belangrijk aspect van het sociale leven van de mens. Mensen en ook dieren helpen medegenoten in nood, zelfs als dit voor henzelf een negatieve uitkomst heeft. Theorieën suggereren dat dit gedrag wordt gemotiveerd door het “voelen met/voor de ander”. Dit gebeurt door ons eigen pijn netwerk in de hersenen te activeren en zo het leed van de ander in onze eigen hersenen te simuleren, waardoor een direct begrip van de pijn mogelijk is en wellicht de negatieve motiverende waarde van deze pijn kan worden gedeeld.

In hoofdstuk 2 hebben we gekeken naar de rol van de somatosensorische cortex in een "helpende" taak waaraan een prijs zit. Met behulp van EEG hebben we allereerst bestudeerd of variatie in de regionale hersenactiviteit tijdens het observeren van iemand anders in pijn de variatie in het help gedrag kan voorspellen, door de variatie in gelddonatie te meten. Proefpersonen bekeken hiervoor twee verschillende soorten stimuli van een persoon die pijnlijke stimulaties op haar rechterhand ontving.

De stimuli hadden als doel de verschillende somatosensorische aspecten van de pijnlijke stimulatie te belichten en we evalueerde hoe deze verschillende aspecten invloed hebben op de betrokkenheid van de somatosensorische cortex bij plaatsvervangende activiteit. Daarna gebruikten we TMS, een niet-invasieve hersenstimulatie techniek dat een gefocust magnetisch veld gebruikt, om tijdelijk de SI-hersenactiviteit te verstoren. Deze manipulatie verstoorde de relatie tussen de intensiteit van de geobserveerde pijn en de hoeveelheid geld dat werd gedoneerd om te helpen.

Ten slotte, om te begrijpen hoe SI activiteit gerelateerd kan zijn aan prosociaal gedrag, interfereerde we met de eigen hand representaties van onze vrijwilligers in de hersenen terwijl ze moesten aangeven hoeveel pijn zij dachten dat de persoon in de video had: als hun beoordeling niet gelijk was aan wat zij daadwerkelijk zagen dan droeg de SI bij aan het transformeren van de visuele informatie naar een correct begrip van pijn intensiteit.

Deze reeks aan experimenten leverde bewijs op dat de hersengebieden die betrokken zijn bij het voelen van pijn nodig zijn om optimaal hulp te kunnen inzetten waar dat het hardst nodig is, d.w.z. help gedrag is nauw verbonden aan het niveau van pijn dat wordt ervaren door een ander. Deze gebieden transformeren het zien van lichamelijk letsel in een nauwkeurig gevoel voor de hoeveelheid pijn die het slachtoffer ervaart. We gebruiken de kennis die wordt vergaard uit deze regio's om ons besluit aan te passen aan de behoeften van anderen. We gebruiken de kennis in deze regio's om ons gedrag aan te passen aan de behoeften van anderen. In het huidige debat over de rol van empathie bij het helpen van anderen, toont deze studie aan dat empathiegerelateerde hersenactiviteit inderdaad het helpen van anderen stimuleert door ons in staat te stellen de intensiteit van nood bij een ander persoon te detecteren en zodoende onze hulp daarop aan te passen.

In hoofdstuk 3, verdiepten we onze kennis over de functionele betekenis van gedeelde hersenactiviteit door gebruikt te maken van fMRI. We onderzochten de functionele relevantie van de gedeelde representaties, met specifieke aandacht voor de sensitiviteit, de generaliseerbaarheid, en de observatie

van pijn, door gebruik te maken van een nieuw design en fMRI metingen. We identificeerden zo een cluster van voxels dat de AI/fronto-insulaire cortex kruist, waarvan de activatie aan alle genoemde eisen voldeed. De rol van de AI in empathie voor pijn is goed bestudeerd in de literatuur maar de specificiteit van de gebruikte taak is onderhevig aan debat door wetenschappers. We bouwden voort aan de functionele kennis van de AI en bevestigden dat deze regio dezelfde activiteit toont bij het zelf voelen van pijn en het zien van iemand anders in pijn, wat lineair kan worden gemoduleerd door pijn intensiteit, niet actief is voor stimuli die geen pijn laten zien en onafhankelijk is van hoe de pijn werd gegeven. Verrassend genoeg overleefden andere gebieden die traditioneel gezien worden als deel van het empathie voor pijn systeem de opgelegde criteria niet. Aanvullende testen zijn nodig om dit gebrek aan resultaten van andere hersengebieden en de validiteit van resultaten omschreven in hoofdstuk 3 te begrijpen. Meer experimenten zijn ook nodig om beter de rol van de AI the begrijpen voornamelijk de specificiteit voor pijn ten opzichte van activatie door een meer generale negatieve valentie

In hoofdstuk 4 hadden we als doel het veld van hersenstimulatie te verbeteren door het ontwerpen van een nieuw HD-tDCS protocol om bilateraal en focaal de corticale exciteerbaarheid van de hand representatie van SI te moduleren. De effecten van de stimulaties waren te zwak en te ontvangbaar voor individuele variabiliteit van de proefpersonen om sterke uitspraken te kunnen maken over de modulatie van corticale exciteerbaarheid. Aanvullende studies moeten worden uitgevoerd om de grenzen van HD-tDCS protocollen te verkennen. Dit zou dan kunnen worden uitgebreid naar het onderzoeken van empathie voor pijn en zo helpen de bijdrage van SI uit de aangrenzende gebieden te ontwarren.

Samengevat geven de resultaten nieuw inzicht in de relatie tussen plaatsvervangende activiteit en prosociaal gedrag en de functionele betekenis van dit soort hersenactiviteit. Bovendien biedt het een kans om na te denken over hoe het gebruik van verschillende technieken de voortgang van kennis beïnvloedt.

Acknowledgement

Writing the acknowledgment section of my thesis has been one of the “happy thoughts” I hold on to in difficult moments. This work would have not be possible without the many people who helped me along the way. Sit comfortably, it’s going to be a long one.

First of all I would like to thank Valeria and Christian, two heads for one Social Brain Lab. Thank you for guiding me along this journey. Christian, thank you for all the precious input, the brainstorming, the encouragements to do more and to do better but also thank you for allowing me to say “no” sometimes. Valeria, thank you for all the above and more! Especially thanks for believing in me when I did not, for the long walks on the grass, for being open to crazy ideas, for your bottomless creativity, for riding with me this emotional roller-coaster and having a lot of laughers along the ride.

A big gigantic thank you to the master and bachelor students who worked with me on this project. Thank you for enduring all the trainings, the meetings, my stress, for learning all the incredible things we learnt together: without you guys, this would have not been possible. In order of appearance: Mario, thank you for recording the stimuli and all the lies we told our participants; Balint, thank you for creating the color videos and being supportive through the first fMRI experiment, for your caring attitude and Disney song singing; Carolina, thank you for taking over torturing people in the fMRI and the great sense of humor; Thijs, thank you for your eagerness to learn, all your questions and your crazy work collecting SEP data; Nouschka, thank you for begin patience with us and having fun inventing new experiments; Tatjana, thank you for learning fMRI preprocessing before me and to keep me with the feet on the ground when needed; Laila, thank you for the energy you brought in the lab; Hanna, thank you for learning so much so fast, for the reading suggestions and for the unicorn names.

Thank you to all the members of the Social Brain Lab, so many unique and special people in a big open office! You made this time fun and unforgetful. A special thanks to (in random order) Abdel, the last of the gentlemen; Leonardo, the best cappuccino in town; Filippo, a great storyteller/poet/piano player; Ritu for never say no to a coffee; Rajeev for always saying yes to helping out, Maria for teaching me so much just by demonstration (wink); Kalli for bringing the synesthesia back into my life; Rajat for explaining the fourier transformation in week #1 and never stop explaining me new things (and more to go!); Teresa thank you for always being there and for the support and the many laughers; Chiara thank you for the cat pictures and the adventures (especially in California); Judith thank you for being the go to person in and outside the lab, for being so chill and so fun at the same time and a great traveler companion; Camilo, for your sparkling humor, Julen thanks for the interesting discussion, Laura, thank you for taking over the TMS challenge and the good vibes while analyzing the Insula recording; YingYing thank you for the insightfull chats about science but especially for the ones not about science, thank you for your lovely dark humor and for the best karaoke duo ever. Riccardo, Rune, Karina, Alessandra thank you because your friendship is worth all the hassle to have gone through a PhD for meeting you. Thank you for the many laugher, the crazy adventures, for cheering and suffering with me about this thesis and about all the things in life.

Thank you to all the people outside the lab that with their support, caring and providing distraction made this stressed PhD student a happy one. In Amsterdam: Nacho, my party buddy!; Linda, supporting the art always!; Jacopo, for teaching me the art of political correctness; Pablo for introducing me to the fantastic burning world. Outside Amsterdam: Simpatico Team – gli amici di sempre, per sempre. Sara, for all the driving on the phone and all the moments we shared, and together with Stefano, thank you

guys for little Tommaso. Thank you guys for coming to visit me as often as you can, you make my life brighter. Eleonora, we shared every part of our life since the last 15 years, and this PhD was no exception. Thank you for making me feel never alone; Valentina and Morgan, thanks for the consultation for the font of this thesis. Thank you Psycho friends: we approached psychology together and ended up doing all sort of different things – still, is always so incredible when we get together! Thank you Martina del mio cuor, because in this years you have been a mirror, a confident, a reviewer, the first test for new ideas, and a co-author, but above all such an amazing friend. Annalisa, thank you because even though we failed to phone each other very often, it does not matter and each time we meet is like no time had passed.

I started this PhD as a Miss and I finish it as a Mrs. Together with a husband I got an amazing family in law. Thank you Lisel, Jürgen and Chris for your warmth and support, for always being so nice to me, for asking about my research and for understanding when I did not want to talk about it.

Thanks to my family in Sicily, the Lusiani family in Binasco, the Spini family in Cavamanara. Thank you zia Raffa for being the first one to encourage my critical thinking. Thank you Nonna Lina for taking the effort of understanding my job, Nonno Luigi for all the jokes. A thought goes to Nonna Vannina e Nonno Saro, who proudly made the best with what they had and whose life example and love will live forever.

Never ending gratidute goes to my parents and my brother. Thank you for letting me go and thank you for always being happy to having me back. You are my rocks and my roots. Thank you for the incredible example of strength and creativity and resourcefulness, thank you for showing me that, with the right attitude and willingness, you can do everything.

Thank you Maximilian for being every day the best part of my day, for the incredible support throughout this journey, for listening to me, always have words of encouragement. You should get a PhD Ad Honorem. Thank you for being so fun to share life with.

Thank you Krocat, for trying very hard to teaching me the concept of boundaries and unconditional love. You are so similar to my PhD: so appealing, often so close I can almost grasp it but so hard to get.

Finally, thanks yoga for saving my mental health, thanks to red wine, mezcal, techno music, Amsterdam under the sunshine, Amsterdam under the rain.