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Thèse de Doctorat

Spécialité Neurosciences

présentée à *l'Ecole Doctorale en Sciences Technologie et Santé (ED 585)*

de l'Université de Picardie Jules Verne

par

Giulia BUCCHIONI

pour obtenir le grade de Docteur de l'Université de Picardie Jules Verne

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in empathy for pain and pain anticipation*

Soutenue le 16 décembre 2015, après avis des rapporteurs, devant le jury d'examen :

M. Lorenzo PIA, Associate Professor	Rapporteur
M. Salvatore CAMPANELLA, Chercheur qualifié FNRS	Rapporteur
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Résumé

L'empathie nous permet de comprendre et de réagir aux sensations des autres individus. Dans le modèle de l'empathie pour la douleur, une personne qui regarde une situation douloureuse peut réagir par de comportements de type prosociaux orientés vers les autres ou par des réponses d'évitement du type de celles enregistrées en réponse à une menace. De récentes études ont montré que les représentations partagées impliquées dans le traitement des stimuli douloureux pourraient être préférentiellement liées à des processus de fuite devant une menace. Le but principal de cette thèse était d'étudier les comportements d'approche/ évitement et *freezing* qui se produisent soit en observant la douleur des autres, soit pendant l'anticipation de la douleur.

Dans un premier temps, deux tâches manipulant la prise de perspective ont permis d'analyser l'influence du genre et de la nature du lien entre l'observateur et l'individu dans le modèle de l'empathie pour la douleur. Les résultats obtenus montrèrent des cotations supérieures concernant le niveau de douleur, des temps de réaction (expérience 1) inférieurs et des index de réponses d'évitement (expérience 2) plus grands pour la perspective consistant à imaginer que le sujet représenté sur la photographie était la personne la plus aimée.

Dans l'expérience 3, nous avons analysé le comportement de rigidification (*freezing*) au niveau du système corticospinal du participant pendant l'observation de stimuli douloureux selon des conditions dans lesquelles le sujet devait adopter une perspective en première et en troisième personne. Un effet de rigidification spécifique de la douleur fut rapporté uniquement lorsque le sujet adopta une perspective en première personne.

Dans une quatrième expérience, l'effet de rigidification, normalement présent en réponse aux stimuli douloureux fut aussi rapporté dans le cadre de l'anticipation de la douleur. Interprétées sous le prisme du modèle de l'empathie pour la douleur, ces données suggèrent que le développement d'une même empathie pourrait contribuer à l'anticipation de la douleur pour soi-même.

En conclusion, nos études suggèrent que ce sont principalement les mécanismes cognitifs de prise de perspective qui modulent la réponse empathique et que la perspective de la personne la plus aimée est la condition de perspective induisant la réponse empathique la plus forte. Au contraire les modulations corticospinales de plus bas niveau son principalement observées lorsque le sujet adopte une perspective en première personne.

Abstract

Empathy is a multidimensional concept allowing us to understand and react to other people feelings. Regarding empathy for pain, a witness looking at a painful situation may react to other-oriented and prosocial-altruistic behaviors or self-oriented withdrawal responses. Recent studies suggest that shared representations of affective processing of painful stimuli may be more representative of general self-oriented mechanisms of threat avoidance. The main aim of this thesis was to study approach/avoidance and freezing behavioral manifestations that co-occurring along with both others' pain observation and during the anticipation of pain.

Firstly, in two perspective-taking tasks we investigated the influence of the type of relationship between the witness and the observed person in pain. Results showed that higher pain ratings, lower reactions times (experiment 1) and greater withdrawal avoidance postural responses (experiment 2) were attributed when participants considered the depicted character as their most loved person.

In experiment 3, we analyzed the freezing behavior in the subject's corticospinal system while subject was observing painful stimuli in first-and third-person perspectives. Results showed that the pain-specific freezing effect only pertained to the first-person perspective condition. An empathy for pain interpretation suggests that empathy might represent the anticipation of a real painful stimulation in oneself. In the interpretation of experiment 4 results, we found that the freezing effect normally present during a painful electrical stimulation was also present in the anticipation of pain.

In conclusion, our studies suggest that cognitive perspective-taking mechanisms mainly modulate the empathic response and the most loved person perspective seems to be prevalent. In addition, more basic pain-specific corticospinal modulations are mainly present in the first-person perspective and it seems to not be referred to the empathy components.

List of contributions

Publications

Bucchioni, G., Lelard, T., Godefroy, O., Ahmaidi, S., Krystkowiak, P., & Mouras., H. The postural correlates of the empathic pain response: the influence of perspective-taking. *In preparation*.

Fossataro, C*, **Bucchioni, G.***, D'Agata, F, Mouras., H., Krystkowiak, P., & Garbarini Francesca. Pain anticipation induces freezing effects as in the actual pain: evidence from corticospinal modulation during classical conditioning paradigm. *In preparation*. (*These authors equally contributed to the study)

Bucchioni, G.*, Fossataro, C.*, Cavallo, A., Mouras, H., Neppi-Modona, M., & Garbarini, F. Empathy or Ownership? Evidence from corticospinal modulation during pain observation. Submitted to *Journal of Cognitive Neuroscience*. (*These authors equally contributed to the study)

Bucchioni, G., Lelard, T., Ahmaidi, S., Godefroy, O, Krystkowiak, P., & Mouras, H. (2015). Do we feel the same empathy for loved and hated peers? *PlosOne*, 10(5). DOI:10.1371/journal.pone.0125871

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Oral communications / Posters

Fossataro, C.*, **Bucchioni, G.***, Bruno, V., D'Agata, F., & Garbarini, G. Pain expectancy induces freezing effects as in the actual pain: evidence from corticospinal modulation during classical conditioning paradigm. Oral Communication at the 23° Annual Meeting of SIPF. Lucca, Italy (2015, November).

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Bucchioni, G., Fossataro, C., Cavallo, A., Krystkowiak, P., Godefroy, O., Mouras, H., Garbarini, F. Does empathy is the only framework to explain the corticospinal modulation during pain observation? Poster presented at the *12eme Colloque Société des Neurosciences*, Montpellier, France (2015, May).

Krystkowiak, P., **Bucchioni, G.**, Lelard, T., Montalan, B., Longin, E., Ahmaidi, S., Godefroy, O., & Mouras, H. Postural correlates of the empathic pain response: the importance of perspective. Poster. *EFNS-ENS*, Istanbul, (2014).

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Bucchioni, G., Lelard, T., Krystkowiak, P., Montalan, B., Longin, E., Ahmaidi, S., Godefroy, O., & Mouras, H. Influence de la menace posturale sur les réponses posturales à des stimuli visuels aversifs. Oral Presentation. *Journée amiennoise de la recherche*, Amiens, (2013, Octobre).

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Acronyms list

AAT: Approach-Avoidance Task

ACC: Anterior Cingulate Cortex

ADM: Abductor Digiti Minimi

AI: Anterior Insula

aMCC: anterior Medial Cingulate Cortex

AP: Anteroposterior

APB: Abductor Pollicis Brevis

BAS: Behavioural Approach System

BA: Broadman area

BES: Basic Empathy Scale

BDI: Beck Depression Inventory

BIS: Behavioral Inhibition System

CS: Corticospinal

COP: Center Of Pressure

COP-AP: Center Of Pressure in the averaged Anterior / Posterior position

path[COP]-AP: Center Of Pressure sway Path in the Anterior / Posterior axis

DA: Dopamine

DLPFC: Dorsolateral Prefrontal Cortex

dmPFC: dorsal part of the medial Pre-Frontal Cortex

ECG: Electrocardiogram

EDA: Electrodermal Activity

EEG: Electroencephalography

EMG: Electromyography

ERPs: Event-Related Potentials

FCR: Flexor Carpi Radialis

FDI: First Dorsal Interosseous

FFFS: Fight-Flight-Freeze System

fMRI: functional Magnetic Resonance Imaging

HR: Heart Rate

IAPS: International Affective Picture system

IASP: International Association for the Study of Pain
IFG: Inferior Frontal Gyrus
IRI: Interpersonal Reactivity Index
IS: Innate Stimuli
MEG: Magnetoencephalography
MEPs: Motor-Evoked Potentials
ML: Mediolateral
MNS: Mirror Neurons System
MV: Mean Velocity
mPFC: medial Pre-Frontal Cortex
M1: Primary Motor Cortex
NA: Noradrenaline
OMHF: Other-Most-Hated-Familiar
OMLF: Other-Most-Loved-Familiar
OS: Other-Stranger
OSP: Optimal Scalp Position
pACC: posterior Anterior Cingulate cortex
PAG: Periaqueductal gray area
PET: Positron Emitting Tomography
PF/PFC: Prefrontal Cortex
PI: Posterior Insula
pSTS: posterior Superior Temporal Sulcus
Pun: Punishments
Rew: Rewards
rmPFC: rostral part of the medial Pre-Frontal Cortex
rMT: Resting Motor Threshold
RMS: Root Mean Square
rTPJ: right Temporal-Parietal Junction
RTs: Reaction Times
SA/ATT: Selective Attention/Action Tendency Task
SEM: Standard Error of the Mean
SG: Substantia Gelatinosa
SI: Primary Somatosensory Cortex

SII: Secondary Somatosensory Cortex
SCR: Skin Conductance Response
SD: Standard Deviation
spTMS: single-pulse Transcranial Magnetic Stimulation
SRC: Stimulus-Response Compatibility task
STAI: State-Trait Anxiety Inventory
STS: Superior Temporal Sulcus
SO: Soleus
TA: Tibialis Anterior
TE: Thenar Eminence
TMS: Transcranial Magnetic Stimulation
TP: Temporal Poles
TPJ: Temporal-Parietal Junction
TOM: Theory of Mind
VBM: Voxel Based Morphometry
VTA: Ventral Tegmental Area
5HT: Serotonin

Résumé étendu

L'empathie est un processus complexe qui joue un rôle social essentiel. Elle nous permet de comprendre et de réagir aux états émotionnels et sensoriels des autres personnes (Thompson 2001), non seulement celles que nous connaissons et dont nous sommes les plus proches, mais également celles jamais rencontrées auparavant.

Dans le cadre du développement moral, Hoffman (2000) a proposé que l'empathie ne se caractérise pas uniquement par des composantes affectives et cognitives, mais par une composante motivationnelle qui encourage les comportements prosociaux en réponse à la détresse des autres.

En général, l'empathie est évoquée pour sa valeur adaptative et évolutive et les chercheurs ont concentré leur attention principalement sur l'aspect prosocial du comportement humain (par exemple : aider les autres) et sur les avantages indirects pour la personne qui ressent de l'empathie : les individus sont encouragés à aider les autres quand cette aide est bénéfique pour eux-mêmes (Batson *et al.*, 2005; de Waal, 2008; Hamilton 1964).

Des études récentes proposent que l'empathie pourrait non seulement être associée aux comportements orientés à l'approche des autres, mais aussi à une motivation auto-protectrice de retrait pour échapper à une situation dangereuse (Goubert *et al.*, 2005, Singer & Klimecki, 2014). De plus, de nouveaux résultats sur les représentations partagées de la douleur soutiennent que les circuits neuronaux activés pendant l'observation de la douleur des autres pourraient représenter la base neurale d'un mécanisme d'évitement de menace au lieu d'une véritable représentation de la douleur affective (Decety, 2015). En outre, l'activation des représentations partagées impliquées dans le traitement des stimuli douloureux pourraient être préférentiellement liées à des processus de fuite devant une menace (Avenanti & Aglioti, 2006). Selon l'Association Internationale pour l'Etude de la Douleur (*IASP*), la douleur est une expérience désagréable, subjective et émotionnelle associée à des lésions tissulaires réelles ou potentielles (Tracey & Mantyh, 2007). L'expérience de la douleur est un mélange complexe de composants sensorielles et affectives et motivationnelles (Fernandez & Turk, 1992 ; Price *et al.*, 1987). La douleur peut induire des réactions motrices afin de réduire le niveau de douleur (par exemple : le retrait d'un membre d'une surface chaude pour arrêter la stimulation délétère et pour préserver l'intégrité du membre en question ; Sullivan *et al.*, 2006) ou des réactions

d'évitement (par exemple : éviter de réaliser des mouvements principalement chez les patients souffrant de douleurs chroniques ; Crombez *et al.*, 1999).

Il a été montré que la stimulation électrique du cortex moteur primaire (*MI*) peut avoir une fonction antalgique : elle est efficace pour réduire la douleur (Cruccu *et al.*, 2007) suggérant ainsi que le système moteur pourrait influencer les sensations somatosensorielles (Peyron *et al.*, 2000). Pour ces raisons, la douleur paraît étroitement liée au système moteur (Farina *et al.*, 2003). En outre, les études sur l'empathie pour la douleur ont mis en évidence des corrélats corticospinaux des réponses de *freezing* ou de fuite pendant l'observation de la douleur d'autres personnes (Avenanti *et al.*, 2005). Il a été aussi suggéré que cette modulation du système corticospinal pendant l'observation de la douleur des autres peut refléter l'anticipation de la douleur sur soi-même (Avenanti & Aglioti, 2006), induisant des comportements moteurs de rigidification (*freezing*) et d'évitement (Avenanti *et al.*, 2005).

Aujourd'hui, la réaction du système moteur chez l'Homme lors de l'observation de la douleur chez les autres n'est pas clairement caractérisée. Cette thèse s'inscrit dans le cadre des neurosciences sociales et son objectif principal est d'étudier l'effet de l'empathie pour la douleur et l'anticipation de la douleur sur les comportements humains et le système moteur humain. Dans la première partie, nous avons analysé le rôle que la nature du lien social avec des personnes connues connues peut avoir sur l'empathie pour la douleur et ses effets sur le comportement moteur (comportement d'évitement et de *freezing*), en nous focalisant sur l'étude du comportement postural et de la modulation des corrélats corticospinaux associés. L'empathie pour la douleur a souvent été interprétée comme l'anticipation de la douleur sur nous-même (Avenanti & Aglioti, 2006; Avenanti *et al.*, 2005; Jackson, *et al.*, 2005; Singer & Frith, 2005) mais les corrélats corticospinaux de ce comportement n'ont pas encore pu être mis en évidence. Cette problématique de recherche a été abordée dans la seconde partie de cette thèse.

Au début de cette thèse seront présentées les théories et les différentes études qui décrivent les caractéristiques de l'empathie et des comportements d'approche et de défense (retrait et *freezing*) avec une attention particulière pour les théories sur l'empathie et en particulier celle utilisant le modèle de travail de l'empathie pour la douleur. Ensuite, nous présentons les différents comportements moteurs (comportements d'approche, d'évitement et de *freezing*). Puis, nous exposerons plus précisément le but de la thèse et les études expérimentales qui ont été menées. Enfin, nous discuterons les résultats obtenus.

I. L'empathie et l'empathie pour la douleur

Dans la littérature, il est possible de trouver des termes différents liés au concept d'empathie, comme la sympathie (aussi dénommée « préoccupation empathique » ; Batson *et al.*, 2007), la compassion, la détresse personnelle / émotionnelle / empathique. Souvent, ces termes sont utilisés de manière interchangeable, mais ils sont considérés comme des synonymes de l'empathie (Eisenberg & Eggum, 2009). Plus particulièrement, la sympathie est une réponse émotionnelle morale orientée vers les autres (Batson, 2009) induite par « l'appréhension de l'état ou et de la condition émotionnelle d'autres personnes » (Eisenberg & Eggum, 2009, p.71). La sympathie peut avoir pour origine un processus cognitif comme la prise de perspective (Eisenberg *et al.* 1991 ; Eisenberg & Eggum, 2009) et une réponse empathique (Eisenberg *et al.*, 2006). Il est important de remarquer que l'empathie n'est pas nécessairement associée à une motivation prosociale (Singer & Lamm, 2009). Récemment le terme de « *compassion* » a été préféré à celui de « *sympathie* » parce qu'il permet une description plus ample des états émotionnels, comme « sympathie », « pitié » et « préoccupation empathique » (pour plus d'informations voir revue, Goetz *et al.*, 2010).

Singer et Klimecki (2014) décrivent la compassion comme une sensation *pour* et non une sensation *avec* les autres et la présentent comme l'état affectif le plus présent dans les comportements aidants naturels et dans les professions comme thérapeute, infirmière et médecin. Au contraire, l'empathie peut conduire à une réaction affective orientée vers soi qu'on appelle, selon les différents auteurs, détresse personnelle ou empathique (Decety & Lamm, 2009 ; Eisenberg & Eggum, 2009 ; Singer & Klimecki, 2014). Elle correspond à un état émotionnel aversif ressenti par l'observateur quand il est en face de l'état négatif de la personne observée : l'observateur en général ressent anxiété et inconfort (Batson, 2009). Eisenberg *et al.* (2006) décrivent les individus qui réagissent avec détresse personnelle à l'inconfort des autres comme des personnes incapables de réguler leur propre réponse émotionnelle. Ils ont une motivation égoïste à réduire leurs sensations négatives (Batson, 1991) et la motivation prosociale en est réduite (Tice *et al.*, 2001). Par exemple, Lamm *et al.*, (2007) ont montré que l'observation de la douleur des autres conduit à une détresse personnelle plus grande quand le sujet doit se projeter explicitement dans la situation douloureuse d'un autre, soulignant l'influence du « soi » dans la détresse personnelle. Goubert *et al.* (2005) proposèrent un modèle des processus psychologiques impliqués dans l'empathie pour la douleur où la sympathie (ou compassion ; Singer & Klimecki, 2014) et la détresse personnelle / empathique étaient

considérées comme des composantes affectives du modèle. De même, la sympathie et la détresse personnelle / empathique ont été caractérisées par des réponses de motivation comportementales distinctes.

Ce modèle d'empathie pour la douleur incorpore différents types de réponses empathiques conduisant à différents comportements: ignorer la douleur des autres ; mettre l'attention sur la protection de soi, ou prendre des mesures pour réduire la douleur de l'autre personne et appliquer un comportement prosocial. Spécifiquement, ce modèle décrit comment les sous-processus qui sont nécessaires à l'empathie, ou qui sont induits en même temps qu'elle, permettent à l'empathie même de se mettre en place. Ces processus permettent de recueillir des informations sur les sensations, les pensées, les intentions et les émotions d'autres personnes. Deux types de mécanismes neuronaux sont associés à l'empathie : *bottom - up* (comprendre les états affectifs et mentaux d'autres personnes, en les mimant) et *top-down* (faire des inférences sur les sentiments et les pensées des autres personnes ; Decety & Lamm, 2006). Donc, l'empathie est influencée soit par le mécanisme *bottom – up*, soit par le mécanisme *top – down* (Decety & Lamm, 2006). Ces mécanismes pourraient tous deux causer des émotions similaires chez l'observateur. Par exemple, quand nous regardons une femme qui coupe des légumes et qui, soudainement et accidentellement, se coupe le doigt avec le couteau, nous devrions être préoccupés par sa douleur (principalement en raison des influences *bottom – up*) ou par la pensée de la douleur expérimentée par l'observateur (principalement en raison des influences *top – down*). Goubert *et al.* (2005) incorporent dans leur modèle l'influence des facteurs contextuels comme la nature de la relation entre l'observateur et la victime ou le participant.

La théorie de l'empathie impliquant le mécanisme de couplage perception-action de Preston et de Waal (2002) décrit l'idée selon laquelle un mécanisme d'imitation des autres, automatique et inconscient, existe et permet l'activation d'une émotion chez l'observateur qui est similaire à celle ressentie par la personne ressentant la douleur. L'empathie est la réponse affective à la perception directe ou à l'imagination des états affectifs des autres (Singer & Lamm, 2009). Des études d'imagerie fonctionnelle ont montré que les paradigmes d'empathie pour la douleur induisent une activation constante des régions du cerveau impliquées dans l'expérience de la douleur physique dont l'insula antérieure (*IA*), le cortex cingulaire antérieur médial (*aMCC*) et le cortex cingulaire postérieur antérieur (*pACC*). Ces régions sont associées aux composantes affective et motivationnelle du traitement des stimuli douloureux (par exemple, impulsions et désirs de faire cesser ou d'éviter la stimulation nuisible ; Decety & Jackson, 2004 ; Decety & Lamm, 2006; Hein & Singer, 2008). Le modèle de représentations

partagées de l'élaboration affective des stimuli douloureux du cerveau soutiennent que pendant l'observation de la douleur de l'autre, il y a une prévalence dans l'activation des circuits *bottom-up* et une superposition partielle des représentations de soi et des autres. Une superposition complète entre les représentations de la douleur de soi-même et de la douleur de l'autre peut conduire à une réponse aversive qui consiste en une détresse émotionnelle / empathique (Decety & Grèzes, 2006). D'autre part, Decety (2011) propose qu'il puisse exister d'autres explications à l'activation de ses régions pendant des tâches d'observation de la douleur. L'empathie est un phénomène complexe à étudier et les activations neurales associées peuvent permettre de mieux décrire le traitement pour les stimuli aversifs.

Fox *et al.*, (2013) ont récemment rapporté activation importante dans l'ACC, l'insula, le cortex somatosensoriel et le striatum dorsal quand des sujets de confession juive observaient des personnes antisémites qui subissaient une douleur par rapport à des personnes tolérantes et sympathiques dans ces mêmes conditions. Or, ces zones sont fonctionnellement connectées à l'amygdale, aux régions frontales et aux régions de récompense. Comme ce réseau est impliqué dans le processus de régulation émotionnelle, ces auteurs proposent que voir des individus souffrants induit peut-être préférentiellement les circuits liés à l'exposition à une situation dangereuse plutôt que ceux liés à une réponse empathique (Fox *et al.*, 2013). Ces résultats soutiennent l'hypothèse que les représentations partagées des traitements affectifs – motivationnels des stimuli douloureux, peuvent être dû à des mécanismes généraux d'autoprotection pour éviter une menace plutôt qu'aux caractéristiques sensorielles de la douleur (Iannetti & Mouraux, 2010 ; Decety, 2015).

La théorie de l'esprit (*TOM*) caractérise une dimension plus cognitive de l'empathie. En effet, il est nécessaire de différencier nos émotions de celles des autres et de comprendre que l'esprit des autres peut être différent du nôtre (Decety & Jackson, 2004; Decety & Grèzes, 2006; Brass *et al.*, 2009) pour arriver à déduire le contenu de l'esprit des autres. Les corrélats neuraux de la *TOM* inclut le cortex préfrontal médial (*mpPFC*) qui est impliqué lorsque nous pensons à nous-même et à d'autres états mentaux (Amodio & Frith, 2006 ; Gallagher & Frith, 2003 ; Macrae *et al.* 2004). La partie postérieure du sillon temporal supérieur (*pSTS*) et la jonction temporo-pariétale (*TPJ*) et les lobes temporaux sont également impliqués dans la *TOM* et les paradigmes de prise de perspective (Frith & Frith, 2003 ; Brass *et al.*, 2009). Ces mécanismes peuvent moduler la qualité de l'empathie sur la base de nos expériences. Decety *et al.* (2008) ont analysé les aspects liés à *TOM* dans le cadre de l'empathie pour la douleur chez les enfants en bonne santé en enquêtant sur la nature de l'intention d'infliger du mal aux autres. Le réseau

neuronal de la matrice de la douleur empathique (*aMCC*, *ACC*, *PAG*, *Insula*) est activé chez les adultes et, de manière intéressante, les auteurs ont signalé que, quand les enfants observaient un individu infliger intentionnellement une douleur à une personne, ils avaient enregistré une plus grande activation dans les régions impliquées dans les interactions sociales, les comportements moraux, les régions *TOM* qui comprennent *TPJ*, le cortex médial orbitofrontal, le cortex paracingulaire et l'amygdale.

Beaucoup de facteurs peuvent moduler le niveau d'empathie ressenti par l'observateur comme l'*intensité de la stimulation* et l'*implication des émotions* (De Vignemont & Singer, 2006). De plus, le niveau de détresse de l'observateur pourrait être modulé par le type de relations sociales qu'il entretient avec les autres. Nous pouvons ressentir plus ou moins d'empathie pour la personne observée, en fonction de la manière dont nous percevons la personne observée semblable à nous (Batson *et al.*, 1997) et si nous la considérons comme une partie de notre groupe (Yabar *et al.*, 2006). Par exemple, Cheng *et al.* (2010) ont montré que l'activité dans les zones associées à la douleur empathique *ACC* et *AI* était augmentée lorsque la perspective adoptée était celle d'une personne appréciée par rapport à celle d'une personne étrangère pendant l'observation de stimuli visuels douloureux. De plus, les auteurs ont signalé des évaluations plus élevées de l'intensité et du caractère désagréable de la douleur pour les perspectives « soi » et « autre personne aimée » par rapport à la perspective « autre personne inconnue ».

II. Les comportements d'approche / évitement et de *freezing*

Porges (2003) a défini comme « *neuroception* » les circuits neuronaux impliqués dans l'évaluation des risques menaçant la survie dans l'environnement. Après une telle évaluation, le système neural organise la réponse de défense la plus appropriée au contexte environnemental associé: combattre, rester immobile ou fuir (Akitsuki & Decety, 2009). Les comportements défensifs devraient être inhibés pour être remplacés par des comportements sociaux (Carter *et al.*, 2011) permettant les interactions et même les comportements prosociaux. Conformément aux théories sur l'évolution des comportements sociaux, leur origine phylogénétique dépend de l'évolution des systèmes sympathique et parasympathique qui permettent une évaluation rapide et inconsciente des stimuli externes et en conséquence une réponse rapide du système moteur tout en maintenant les états homéostatiques viscéraux (Porges, 2001). Un important facteur qui peut moduler les comportements d'aide est d'avoir ou

ne pas avoir la possibilité d'échapper facilement à une situation dangereuse. De plus, des comportements de type *rigidification* (« *freezing* ») automatiques peuvent être adoptés pendant l'observation de stimuli visuels douloureux. Ce type de réponse est enregistré normalement lors de l'expérience de la douleur en première personne: une inhibition de la réponse du système moteur est enregistrée quand les stimuli douloureux sont délivrés à la main des sujets (Farina *et al.*, 2003 ; Urban *et al.*, 2004). Avenanti *et al.* (2005, 2006, 2009, 2010) ont également mis en évidence cette réponse dans l'observation de stimuli douloureux. Les auteurs interprètent cette réponse comme une réponse empathique à la douleur des autres.

Les théories d'approche / évitement humain décrivent comment les comportements sont motivés par des stimuli inconditionnés appétitifs et aversifs (Lang *et al.*, 1998). Il est important de noter que même si, dans la littérature, il y a une divergence dans leur définition, les concepts d'« approche / évitement », « approche / retrait » et « appétitif / aversif » sont souvent confondus, indiquant le niveau de la complexité des processus motivationnels en fonction de leur valence positive ou négative (Elliot & Church, 1997 ; Elliot *et al.*, 2013). La *reinforcement sensitivity theory* (Corr, 2008 ; Gray, 1973 ; Gray & McNaughton, 2000) met en évidence la composante du recompense et punishment des stimuli sur l'émotion et sur les réponses motivées d'approche et d'évitement. Trois systèmes neuropsychologiques semblent impliqués : le Système Comportemental d'Approche (*BAS*) qui est associé avec des motivations appétitives positives et des comportements d'approche. Carver et White (1994) ont subdivisé ce système en trois sous-systèmes : (i) *BAS Reward Responsiveness*, sensible à la récompense ; (ii) *BAS Fun Seeking* qui cherche de nouvelles récompenses ; (iii) *BAS Drive* qui est orienté vers un but et qui, pour les auteurs, est le plus important et utile des trois sous-systèmes. Les deux autres systèmes sont associés aux réponses d'évitement (Gray & McNaughton, 2000).

L'observation des sentiments des autres personnes, spécialement de ceux négatifs comme la douleur, induit la production d'états émotionnels empathiques chez l'observateur. Scherer (2005) a décrit l'émotion comme une condition qui induit des réactions à différents niveaux : variations neurophysiologiques, réponse du système moteur, cognition, motivation et sensations subjectives. De la même façon, Lang (1995) a défini les émotions comme des dispositions d'action : elles préparent rapidement l'organisme à potentialiser les réponses appropriées aux stimuli émotionnels. Cette description générale sur les émotions et leurs effets sur les comportements de motivation pourrait s'adapter aussi au cas spécifique des émotions empathiques, c'est-à-dire la détresse empathique personnelle et la compassion. Dans ce contexte, les émotions empathiques pourraient se traduire par des réponses comportementales

d'approche / évitement aux stimuli externes (Goubert *et al.*, 2005). En effet, plus généralement, il semble qu'il y ait des liens étroits entre le comportement d'approche / évitement et les émotions empathiques positives / négatives. Bechara et Damasio (2005), dans leur hypothèse des « marqueurs somatiques », ont concentré l'attention sur le rôle que jouent les émotions dans la focalisation de l'attention sur des résultats d'action possible, en aidant à choisir la réaction appropriée. En effet, les émotions sont l'expression de l'activation de trois systèmes : système nerveux central, systèmes nerveux autonome somatique et périphérique ; le comportement final (Lang & Bradley, 2008).

III. Objectifs de la thèse

Le but principal de ce projet de recherche a été l'étude des manifestations des comportements d'approche / évitement et de *freezing* qui se vérifient à la fois avec l'observation de la douleur des autres et pendant l'anticipation de la douleur. Il est possible d'utiliser différentes techniques pour étudier les comportements d'approche / évitement et de *freezing*. Dans la présente étude, nous avons utilisé les temps de réaction (*RTs*) et la cotation de la douleur d'autrui ; la posturographie ; les mesures physiologiques et la stimulation magnétique transcranienne (*TMS*) associées à l'enregistrement des potentiels évoqués moteurs (*MEPs*).

IV. Méthodes

IV.I. Les sujets expérimentés

Les participants ayant pris part aux quatre études ont été sélectionnés selon des critères différents. Tous étaient volontaires ; étudiants provenant de l'Université de la Picardie Julius Verne et de l'Université de Turin. Le nombre total des sujets qui ont participé aux quatre expériences a été de 167 (82 hommes ; et 85 femmes) ; tous étaient droitiers conformément au *Standard Handedness Inventory* (Briggs & Nebes, 1975).

Les critères d'inclusion étaient les suivants : (i) âgés de 18 à 40 ans ; (ii) avoir une acuité visuelle normale, ou corrigé à normale ; (iii) pour l'expérience de stimulation magnétique transcranienne (*TMS*), n'avoir aucune contre-indication à la *TMS* (Wassermann 1998, Rossi *et al.*, 2009). Pour éviter d'éventuels dommages liés à la *TMS*, un questionnaire leur a été présenté avant le début de la session expérimentale. Les participants ont été exclus de l'expérience s'ils / elles : étaient épileptiques (ou quelqu'un de leur parents avait cette maladie) ; avaient eu une

syncope ; avaient eu un trauma à la tête ; avaient des problèmes d'audition ou étaient équipés d'implants cochléaires ; avaient une pièce métallique dans le cerveau / le crane ; avaient un neurostimulateur installé ; avaient un *pacemaker* ; avaient un dispositif pour l'infusion de médicaments ; devaient prendre des médicaments ; étaient enceintes.

En outre, on a leur demandé de remplir différents questionnaires standardisés selon les différentes études de cette recherche. Ces questionnaires comprenaient : *The Beck Depression Inventory (BDI)* ; Beck et Beamesderfer, 1974 ; expérience 1) ; *The State- Trait Anxiety Inventory (STAI)* ; Spielberger *et al.*, 1983 ; expériences 1, 2, 4), *The Basic Empathy Scale (BES)* ; Joliffe et Farrington, 2006 ; expériences 1, 2) , *The interpersonal Reactivity Index (IRI)* ; Davis, 1983 ; expérience 3) et une mesure ad hoc sur la *body-ownership* (expérience 3) et une mesure ad hoc aussi sur l'anticipation (à la douleur) des participants (expérience 4).

Tous les participants ont signé un consentement informé avant leur inclusion dans l'expérience dont ils ne connaissaient pas le but. Chaque expérience a été conduite conformément aux standards éthiques de la Déclaration de Helsinki et approuvée par le Comité d'Investigation Locale (pour l'Université de Picardie Jules Verne : Comité de Protection des Personnes, Nord- Ouest II, Amiens, France ; pour l'Université de Turin: Comité Ethique de l'Université de Turin).

IV.II. Enregistrement des données

Dans ce travail de doctorat, différents types de stimuli, procédures de collectes et analyse de données ont été utilisées. La présentation de ces stimuli a été contrôlée et randomisée grâce au logiciel E-prime (version 2.0, Psychology Software Tools, Inc., Pittsburg, PA) dans un PC et les données ont été analysées après les avoir recueillies.

Les corrélats de l'activité du système nerveux pendant les comportements appétitifs / aversifs par une série de marqueurs physiologiques (Blair *et al.*, 2004). Dans cette thèse, les réponses physiologiques autonomes électriques enregistrées sont l'activité musculaire (enregistrée par électromyographie, *EMG*), l'activité cardiaque (mesurée par électrocardiographie, *ECG* et le rythme cardiaque, *HR*) et l'activité électrodermale (enregistrée par la réponse galvanique de la peau, *SCR*). En revanche, l'enregistrement de ces marqueurs a le désavantage principal de donner des informations de nature indirecte sur l'activité du cerveau (Sequeira *et al.*, 2009).

Une autre méthode pour étudier les comportements d'approche / évitement et de *freezing* utilisée au cours de cette thèse est l'étude du contrôle postural. Cette méthode semble permettre une analyse plus large des comportements rapides de *freezing* et de type approche-évitement ; on peut l'utiliser pour vérifier une réponse globale du système moteur dans une position unique, et quantifier une variété de paramètres décrivant l'équilibre naturel et postural du corps. Le contrôle postural a été principalement analysé à l'aide d'une plate-forme de force permettant d'enregistrer les variations de position du Centre de Pression (*COP*) dans les directions antéropostérieure (*AP*) et médiolatérale (*ML*) quand l'individu se trouve en face de stimuli visuels. Ces stimuli présentent la main et le pied droits réalisant des gestes communs avec soit des conséquences douloureuses (par exemple : coincer son doigt dans une porte) soit non douloureuses (par exemple : fermer une porte sans y glisser un doigt ; Jackson, *et al.*, 2005, 2006).

Enfin, la *TMS* associée à l'enregistrement des amplitudes pic à pic des *MEPs* permet de révéler d'une manière indirecte l'excitabilité du système corticospinal quand le participant se trouve directement face à des stimuli douloureux (stimulation *TMS* associée à de stimulations électriques douloureuses) ou quand il observe des vidéos où les stimuli douloureux sont administrés à la main observée.

V. Partie expérimentale

D'abord, au cours de deux tâches qui utilisaient des points de vue différents, nous avons analysé l'influence de la nature de la relation entre le témoin et l'individu soumis au stimulus douloureux.

V.I. Première expérience : réponses comportementales dans la tâche de l'empathie pour la douleur

Dans cette première étude, nous avons porté notre attention sur les aspects du comportement relatifs à l'évaluation de la douleur d'autrui. En particulier nous avons étudié les facteurs qui modulent l'empathie pour la douleur, pour exemple le genre du témoin qui regarde les images douloureuses et nous avons pris en considération les perspectives adoptées de la part de l'observateur pendant l'expérience. Les perspectives étaient les suivantes : « Soi », « Personne inconnue (*OS*) », « Personne connue, la plus aimée (*OMLF*) » et « Personne connue, la plus détestée (*OMHF*) ». De précédentes études ont focalisé l'attention sur l'influence du lien

entre l'observateur et la perspective par lui-même adoptée quand il observe des comportements douloureux d'autrui (Cheng *et al.*, 2010 ; Jackson *et al.*, 2005; 2006). Ces études ont adopté les premières deux ou trois perspectives précédemment présentées (*Soi*, *OS* et *OMLF*) sans utiliser la quatrième (*OMHF*). Plus spécifiquement, nous avons étudié l'influence du genre du témoin et de la nature de la prise de perspectives sur les aspects du comportement (estimation de la douleur et temps de réaction) et de la cotation de la douleur de la personne observée.

Les résultats ont montré que les cotations de la douleur attribuées pendant l'observation d'images douloureuses dans la perspective *OMLF* étaient les plus hautes par rapport aux trois autres perspectives (*Self*, *OS*, *OMHF* ; $p < 0.0001$). A l'égard des temps de réaction, les participants évaluaient la douleur observée de manière plus rapide dans les perspectives *OMLF* et *Self* que dans les autres deux (*OS*, *OMHF*) ($p < 0.001$). De plus, à propos de l'influence du genre du témoin, les données ont suggéré que les sujets féminins étaient plus sensibles que les masculins dans la cotation de sa douleur et de celle de la personne qu'elles aiment le plus. Les résultats de cette étude ont suggéré que le genre du participant et la perspective adoptée pendant l'observation de situations douloureuses influencent l'empathie pour la douleur et ses réponses associées.

V.II. Deuxième expérience : réponses posturales dans la tâche de l'empathie pour la douleur

Les résultats de la tâche du comportement obtenus dans l'étude précédente, nous ont menés à rechercher les réactions d'approche / évitement et de *freezing* du témoin à situations douloureuses d'autre personnes. La seconde étude analyse les paramètres posturaux pendant l'observation de scènes douloureuses dans les quatre perspectives précédemment décrites (*Self*, *OS*, *OMLF* et *OMHF*). Plus particulièrement, nous avons pour but de comprendre dans quelle direction l'observateur d'événements douloureux touchant d'autres personnes se dirigeait selon les quatre perspectives ; par exemple, s'il s'approchait, s'écartait ou s'il se rigidifiait (*freezing*) devant des situations douloureuses infligées à d'autres personnes. Pour l'enregistrement de paramètres posturaux, nous avons analysé la position moyenne antéropostérieure du centre de pression (*COP-AP*), de plus, nous avons calculé le delta entre les situations douloureuses et non douloureuses pour le paramètre *COP-AP* (*delta COP – AP*). Pour les comportements de type *freezing* (longueur du chemin du centre de pression en la direction antéro – postérieure ; *chemin COP – AP*) pendant une tâche de contrôle postural quand l'observateur regardait des images de

stimuli similaires à celles utilisées pour l'expérience comportementale précédente et alors que les participants adoptaient une des quatre perspectives (*Self*, *OS*, *OMLF* et *OMHF*).

Les résultats ont suggéré que les sujets avaient tendance à adopter une position d'évitement, quand ils étaient en face d'un individu selon la perspective *OMLF* subissant une situation douloureuse par rapport à l'individu selon une perspective *OMHF* dans la même situation douloureuse ($p=0.01$). La valeur du delta douleur-non douleur en ce qui concerne la perspective *OMLF* était significativement plus faible par rapport à toutes les autres perspectives (*Self*, *OS*, et *OMHF*), et ce, à la troisième seconde de la présentation des stimuli ($p<0.01$) : la position antéropostérieure moyenne du centre de pression (COP-AP) était évidemment plus réduite par rapport aux autres perspectives. Selon Goubert *et al.* (2005) et Singer et Klimecki (2014) cette réaction de retrait peut être associée à la protection de soi-même et à l'évitement de réponse de menace (Yamada & Decety 2009) : regarder une personne aimée qui souffre, cause une détresse personnelle plus forte chez l'observateur. Afin d'éviter ce sentiment négatif et de se placer dans des situations potentiellement dangereuses, le sujet s'éloigne d'une situation perçue comme dangereuse et négative. Par rapport au comportement de *freezing*, nous avons enregistré une réduction significative du *chemin COP-AP* relativement à la perspective *OS* par rapport aux autres perspectives (*Self*, *OMLF* et *OMHF* ; $p<0.01$).

V.III. Troisième expérience : réponses corticospinales dans l'empathie pour la douleur

Dans la seconde partie de ce projet de thèse, dans le cadre d'une collaboration avec l'Université de Turin (département de psychologie, SAMBA Lab), nous avons pu étudier l'effet *freezing* à l'échelon corticospinal. Dans une première étude, nous avons examiné cet effet sur le témoin d'une situation douloureuse ; dans la seconde expérience nous avons focalisé notre attention sur son étude pendant l'anticipation d'un événement douloureux.

Dans un troisième temps, nous avons étudié l'effet *freezing* dans les perspectives soi-même / autre, qui sont relatives à l'empathie pour la douleur. Précédemment, Urban *et al.* (2004) avaient rapporté une inhibition corticospinale (effet *freezing*) associée à une stimulation douloureuse. Spécifiquement, pendant une stimulation électrique périphérique appliquée à un doigt de la main, l'amplitude pic à pic des potentiels moteur évoqués (*MEPs*) (induite par la stimulation magnétique transcranienne (*TMS*) sur le cortex du premier moteur) enregistrée du même muscle de la main, avait été réduite significativement. Plus récemment, Avenanti *et al.* (2005) ont trouvé que la simple observation de stimuli douloureux transmis à la main d'un

personnage représenté dans une perspective en première personne induit une modulation du système corticospinale de l'observateur (Avenanti et al, 2005) à l'instar de ce qui est enregistré lors d'une stimulation douloureuse directe. Cet effet a été interprété comme étant à la base physiologique de l'empathie. Même si ce paradigme a été répliqué de nombreuses fois (Avenanti *et al.*, 2006 ; 2009 ; 2010), dans chacun étude la main pénétrée par une aiguille a été présentée selon une perspective en première personne (celle selon laquelle nous percevons normalement les parties de notre corps). Toutefois, il devient crucial de se demander ce qu'il arrive quand la main est présentée selon une perspective en troisième personne (celle dans laquelle nous apercevons les parties du corps d'autres personnes).

Dans cette étude nous avons examiné si les réponses spécifiques douloureuses du cortex moteur survenant pendant l'observation de la douleur, comme il l'a été suggéré précédemment, représenter les bases physiologiques de l'empathie, ou, si, au contraire, elles peuvent être mieux expliquées par un mécanisme *d'incarnation (embodiment)* corporelle. Ce processus d'incarnation (plus communément connu sous le terme anglais d'*embodiment*) est représenté par les mécanismes neurocognitifs qui modulent l'expérience d'identité de soi-même et qui sont à la base du soi pendant l'observation d'événements sensoriels touchant les parties du corps d'autres personnes. Par la manipulation de la perspective selon laquelle la main du modèle recevant douleur est observée, nous avons trouvé une modulation au niveau corticospinale et spécifique de la condition douleur seulement uniquement lorsque les stimuli étaient présentés selon une perspective en première personne ($p < 0.05$), induisant de façon automatique à une sorte d'*embodiment*. Aucune modulation ne fut trouvée dans la perspective troisième personne, la seule dans laquelle nous apercevons normalement les autres. Nous avons interprété cet effet dépendant de la perspective selon une conception «affective» du *body-ownership* (De Vignemont, 2014) qui suggère que le corps que je considère comme m'appartenir est celui auquel je fais attention.

V.IV. Quatrième expérience : réponses corticospinales dans une tâche d'anticipation de la douleur

Enfin, nous avons conduit une étude sur l'anticipation de la douleur. Des études précédentes avaient mis en évidence que le réseau neural activé pendant l'observation de la douleur des autres et les circuits activés pendant l'expérience de l'anticipation de sa propre douleur semblaient se recouvrir partiellement sur le plan spatial (Morrison *et al.* 2004). Tenant compte des interprétations nouvelles sur les représentations partagées de la douleur, les circuits

neuraux activés pendant l'observation de la douleur des autres pourraient représenter les bases neurales du mécanisme d'évitement de la menace (Decety, 2015) et l'activation de ces circuits pourrait se concrétiser par le développement d'une réponse de type défensive pour éviter les situations dangereuses (Avenanti & Aglioti, 2006). Selon ce point de vue, une interprétation possible de l'empathie pour la douleur est qu'elle pourrait représenter l'anticipation de la douleur pour soi. Par exemple, Porro *et al.* (2003) ont étudié en IRMf l'anticipation de la douleur sans pour autant permettre de clarifier les rôles respectifs du cortex moteur primaire et de la modulation corticospinale. De plus, des études ont montré que les stimuli douloureux induisaient soit une forte augmentation des réponses de conductance de la peau (*SCR*), soit une modulation fine de l'excitabilité corticospinale, illustrant une inhibition significative du *MEP* enregistrée au niveau de la partie du corps à laquelle les stimuli ont été délivrés (Urban *et al.*, 2004). Dans cette dernière étude, à l'aide d'un paradigme de conditionnement classique, nous avons étudié la modulation de l'excitabilité corticospinale, en particulier l'enregistrement de l'effet *freezing* pendant l'anticipation de l'envoi d'un stimulus électrique douloureux. La simple anticipation d'un stimulus douloureux induit une inhibition significative au niveau corticospinal ($p=0.01$), à l'instar de ce qui se produit lors de l'exposition à des stimuli douloureux. L'enregistrement de l'anticipation de la réponse au niveau corticospinal pourrait représenter un processus de préparation à une réponse de type défensive de l'organisme en réponse à l'exposition à un événement dangereux et probable pendant l'observation d'un événement douloureux.

VI. Conclusions

Les résultats de cette thèse suggèrent que les processus cognitifs de prise de perspective (analysés dans les deux premières études du présent projet) pourraient moduler la réponse de l'empathie pour la douleur. Plus spécifiquement, quand les participants ont adopté la perspective de la personne la plus aimée pendant l'observation de stimuli douloureux, nous avons enregistré une plus grande réponse au niveau comportemental, moteur (représenté par une réaction de retrait mesurée en posturographie) et physiologique. Au contraire, la modulation corticospinale spécifiques de la douleur (effet *freezing*) était principalement présente dans l'observation de la perspective à la première personne, la seule dans laquelle nous observons des parties de notre corps. Dans cette situation, les réponses corticospinales à la douleur des autres ne semblent pas être rapportées aux composants de l'empathie. De plus, ces données ont

été confirmées ultérieurement par une étude portant sur l'anticipation de la douleur : une inhibition corticospinale a été aussi enregistrée pendant l'anticipation de la douleur sur soi-même.

En conclusion, ce projet contribue à mieux comprendre les processus moteurs (comportements de retrait et de rigidification ou *freezing*) réalisés pendant l'observation de situations douloureuses, soulignant le fait que les relations sociales et les réponses autoprotectrices pourraient moduler ces comportements.

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Preamble

Empathy is a complex construct that plays an essential social role, allowing us to understand and react to another person's emotional and sensory states (Thompson, 2001). It is an essential mechanism to feel other's emotional experience and allow social interactions. Empathy helps us not only to understand and share affective states with the people closest to us, but also with someone we have never met before. Hoffmann (2000) theorized that, in the frame of moral development, empathy has not only affective and cognitive components but also motivational ones, and the latter support prosocial behaviors when other's distress is perceived. Usually, empathy is studied for its adaptive and evolutionary value and researchers mainly focused their attention on the prosocial side of human behavior, such as helping others) and also on the indirect benefits for the empathizer: people are encouraged to help others when it is also good for themselves (Batson *et al.*, 2005; de Waal, 2008; Hamilton 1964). Recently studies proposed empathy might be associated not only with other-oriented approach behaviors but also with a self-protective withdrawal motivation to escape from dangerous situations (Goubert *et al.*, 2005; Singer & Klimecki, 2014). Moreover, new interpretations about the shared representations of pain, support the neural circuits activated during the observation of others' pain might represent the neural basis of threat avoidance mechanism instead of the representations of affective pain (Decety, 2015). Besides, the activation of these circuits might co-occur with defending response for preventing dangerous events (Avenanti & Aglioti, 2006). The International Association for the Study of Pain (IASP) defines pain as an unpleasant subjective, sensory, and emotional experience associated with actual or potential tissue damage (Tracey & Mantyh, 2007). The experience of pain is a complex mix of sensory and affective – motivational components (Fernandez & Turk, 1992; Price *et al.*, 1987). Pain can induce protective actions that may lead to pain reduction (such as the withdrawal of a limb from a hot surface in order to stop the noxious stimulation and to preserve the limb's integrity (Sullivan *et al.*, 2006) and avoidance reactions (such as, avoidance of movements mainly present in chronic pain patients) (Crombez *et al.*, 1999). Moreover, it has been shown that the electric stimulation of the primary motor cortex (M1) may have a therapeutic function: it is efficacious in pain reducing (Cruccu *et al.*, 2007), suggesting motor system might influence somatosensory sensations (Peyron *et al.*, 2000). For these reasons, pain seems to be closely linked to the motor system (Farina *et al.*, 2003). Recently, empathy for pain studies highlighted freezing or escaping

responses in the corticospinal system during the observation of other's pain (Avenanti *et al.*, 2005). Moreover it has been suggested that this modulation of the corticospinal system during the observation of other's pain may reflect the anticipation of pain in the self (Avenanti & Aglioti, 2006), inducing freezing-like and avoidance motor behaviors (Avenanti *et al.*, 2005).

Nowadays, it is still not completely clear how the human motor system react to the observation of other's pain. This thesis is in the frame of social neuroscience and its main purpose is to investigate the effect of empathy for pain and pain anticipation on human behavior and human motor system. In the first part of the present thesis the role of the self / other distinction on empathy for pain and its effect on the observer's motor correlates has been analyzed. Specifically approach / avoidance and freezing behaviors were studied analyzing postural and corticospinal responses. Moreover, empathy for pain is also interpreted as the anticipation of pain in ourselves (Avenanti & Aglioti, 2006; Avenanti *et al.*, 2005). Indeed, in the second part of the present thesis, we investigated if the freezing response that normally occurs in the corticospinal system during a painful stimulation, may occurs also during the anticipation of a painful stimulation

The first section of this thesis presents different theories and studies describing empathy features and approach and defensive behaviors (withdrawal and freezing). Specifically, in the first chapter, theories about empathy and in particular empathy for pain are reported. Then theories about motor behaviors (approach / avoidance and freezing behaviors) are exposed. In the second section of the present thesis, the aim of the thesis is reported and the following experimental studies are described. Finally, all results are discussed.

CHAPTER I

INTRODUCTION

1. Empathy and empathy for pain

“Empathy” is an English word derived from the ancient Greek word (ἐμπάθεια pronounce as *empathia*). The literary meaning is "physical affection" or "passion" which comes from ἐν (en), "in, at" and πάθος (pronounce as *pathos*) which means "feeling" or "suffering". Despite its linguistic roots in ancient Greek, the concept of empathy has been interpreted in different forms. Theodor Lipps has developed the concept of *Einfühlung* (i.e. empathy) from his definition of aesthetic appreciation of an object to a definition that is applicable to human sciences. Empathy in this case is defined as a meaning for recognizing each other as minded creatures (Lipps 1903, 1905). Lipps described Empathy as a psychological resonance phenomenon that triggers inner processes allowing us to recognize other persons as minded creatures (Lipps, 1905). In this context, the perception of facial or body emotional expression generates an “inner imitation” by the observer. Adam Smith (1853) already noticed this human behavior. Nowadays, neuroscience findings support the Lipps’ idea of empathy (e.g.: Preston & de Waal, 2002; Amodio & Frith, 2006). Dues to the heterogeneity of the psychological theories of empathy, it proves difficult to give a univocal definition of the concept of “empathy”. According to Preston and de Waal, empathy is ‘any process where the attended perception of the object’s state generates a state in the subject that is more applicable to the object’s state or situation than the object's own prior state or situation’ (Preston and de Waal, 2002, p.4). This definition may be quite general but gives simple meaning that can be used to describe all empathic processes.

In the literature, it is possible to find different terms that are linked to the concept of empathy such as sympathy (also referred as empathic concern; Batson *et al.*, 2007), compassion, personal/emotional/empathic distress. They are used often interchangeably with “empathy” but are slightly different and are considered as empathic vicarious responding (Eisenberg & Eggum, 2009). In particular sympathy is a moral emotional response oriented to the other person (Batson, 2009) and it comes from the ‘the apprehension of another’s emotional state or condition’ (Eisenberg & Eggum, 2009, p.71). Sympathy may originated from a cognitive process like perspective-taking (Eisenberg *et al.* 1991; Eisenberg & Eggum, 2009) and from empathetic response (Eisenberg *et al.*, 2006). It is important to note that empathy is not necessarily associated with a prosocial motivation (Singer & Lamm, 2009). Recently, the

term compassion has been preferred over sympathy because it allows a wider description of emotional states like sympathy, pity and empathic concern (for more information see review, Goetz *et al.*, 2010). Singer and Klimecki (2014) describes compassion (Figure 1) as a feeling *for* and not a feeling *with* the other and present it as the most present affective state in care giving behavior and in professions like therapist, nurse and doctor. On the contrary, sometimes empathy can lead to a self-oriented affective reaction that is called, according to different authors, personal or empathic distress (Decety & Lamm, 2009b; Eisenberg & Eggum, 2009; Singer & Klimecki, 2014; Figure 1). It corresponds to an aversive emotional state felt by the observer when he/she is faced with the observed person's negative state: the observer generally feels anxiety and discomfort (Batson, 2009). Eisenberg *et al.* (Eisenberg *et al.*, 2006) describes the individuals' process of reaction with personal distress to others' discomfort, as persons unable to regulate their own emotional response. They have an egoistic motivation to reduce their negative feelings (Batson, 1991) and prosocial motivation is reduced (Tice *et al.*, 2001). For instance, Lamm *et al.* (2007) have shown that the observation of other person's pain leads to a higher personal distress when the subject has to explicitly project him/her self into other's painful situation, underlining the influence of self-perspective in personal distress.

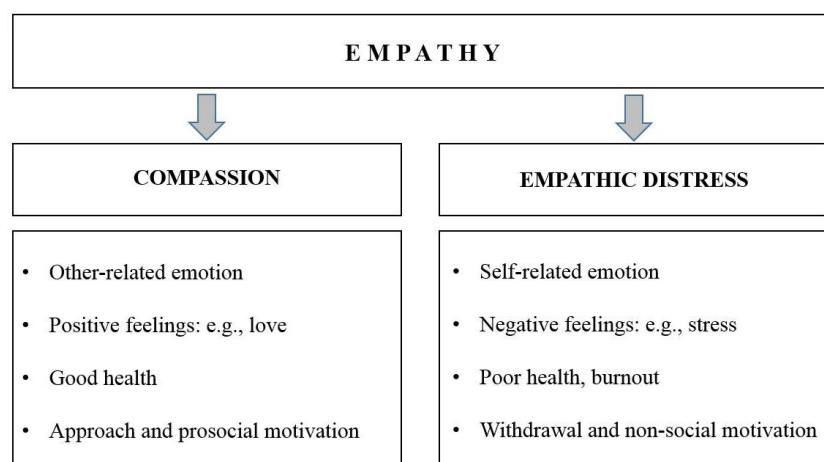


Figure 1. Model representation of two empathic reactions (empathic distress and compassion) to other's suffering (modified from Singer & Klimecki 2014)

Goubert *et al.*, (2005) as shown in Figure 2 modeled the psychological processes involved in empathy for pain model, where sympathy (or compassion; Singer & Klimecki, 2014) and personal/empathic distress were considered as affective components of the model. Both sympathy and personal/empathic distress were characterized by distinct motivational behavioral responses. This empathy for pain model incorporates different modulations of the

empathic responses leading to different behavioral outcomes: ignore other person's pain; focusing on the self-protection or taking action to reduce other person's pain and applying a prosocial behavior. Specifically, this model describes the way in which sub-processes that are necessary to empathy, or ongoing with it, allow empathy itself to occur. These processes allow to collect information about other people feelings, thoughts, intentions and emotions. Two kinds of mechanisms are associated to empathy: *bottom-up* (understanding other people affective and mental states, mimicking them) and *top-down* (making inferences about people feelings and thoughts) neural mechanisms (Decety & Lamm, 2006). Therefore, empathy is influenced by both bottom-up and top-down mechanisms (Decety & Lamm, 2006). These mechanisms could both lead to similar emotions in the observer. For example, when we look at someone cutting vegetables and suddenly she accidentally cuts her finger with a knife, we should be concerned by the simulation of her pain (mainly due by *bottom-up* influences) or in consequence of thinking about the pain experienced by the observer (mainly due by *top-down* influences). Goubert *et al.* (2005) includes in their model the influence of contextual factors such as the nature of the relationship between the observer and the victim or participant. The next section describes empathy influence on bottom-up and top-down mechanism.

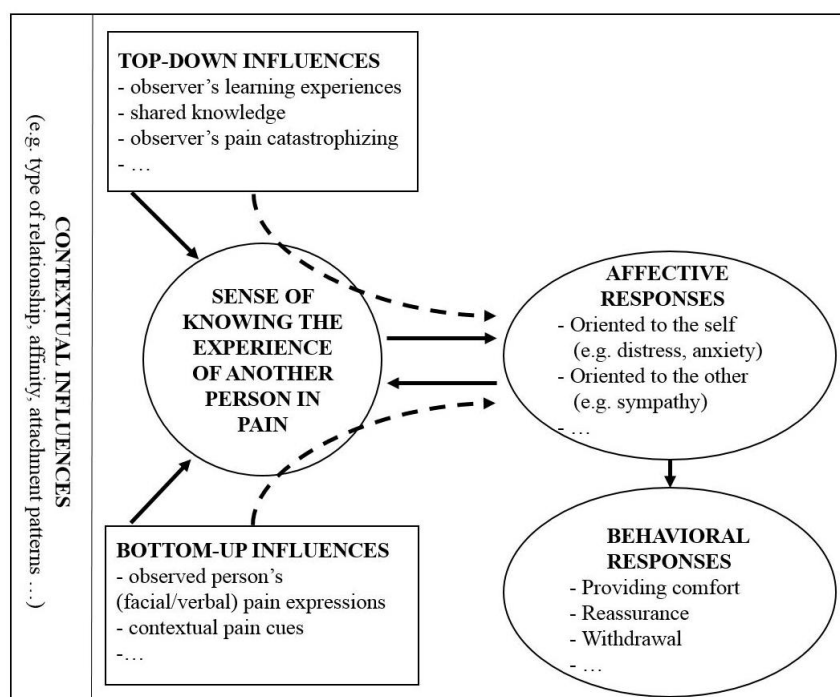


Figure 2. Goubert *et al.* (2005) in this model describe bottom-up and top-down mechanisms influencing empathy for pain and their affective and behavioral responses.

1.1. Bottom-up empathic mechanisms

“Bottom-up” influences, in the context of empathy for pain, refer to characteristics of the person in pain or to the context in which the painful stimulus occurs. Bottom-up influences include facial and verbal pain expressions and situational pain cues and affect the degree of experienced empathy. Also, distressed facial and body expressions that clearly and automatically communicate pain might elicit others’ empathic responses (Goubert *et al.*, 2005; Goubert *et al.*, 2009). In the following sections, we summarize the bottom-up processing models involved in the production of empathic responses.

1.1.1. Simulation theory

The simulation theory is based on the activation of bottom-up processes. Humans could empathize with others without the implementation of high-level cognitive mechanisms. The term simulation refers to our ability to internally simulate the emotional states of others (Carruthers & Smith, 1996; Davies & Stone, 1995a, 1995b; Goldman 2006; Preston & de Waal, 2002). This automatic process allows us to virtually experience the other people feelings, and several authors suggest that this simulation process is at the foreground of other’s comprehension (*the shared-manifold hypothesis* (Gallese, 2003); *the unmediated resonance model* (Goldman & Sripada, 2005); *the shared circuits hypothesis* (Keysers & Gazzola, 2006)). Gallese (2003) claims that shared representations between the witness and an individual action or feeling the emotion are the biological substrate of the simulation of their corresponding sensorimotor, affective, or mental states (Goldman 2006). A shared representation allows us to project ourselves imaginatively into another person’s perspective by simulating their mental activity using our own mental apparatus (Keysers & Gazzola, 2009). Simulation theory is supported by literature’s data showing that common neural regions subserve both the execution and the observation of actions or emotional states (Jackson *et al.*, 2005; Keysers & Gazzola 2009; Singer *et al.*, 2004). For example, to understand what another person is doing, we would simulate her movements by implementing our own motor programs and to understand what another individual feelings, we would simulate his emotions by implementing our own affective programs.

1.1.2. Perception-action mechanism

The covert mimicry process that catches the actions and emotions of another person (Gutsell & Inzlicht, 2010; Preston & de Waal, 2002) allows us to share feelings with others (Decety & Jackson, 2006). Simulation operates through a perception-action-coupling mechanism which is based on the fact that perception and action are subserved by partially common neural circuits: the observer can automatically mirror the representation of the observed action or emotion allowing the activation of similar autonomic and somatic responses which are activated within the observer (Decety & Jackson, 2004; Preston & de Waal, 2002; Van Baaren *et al.*, 2009). This organization is useful and adaptive because the use of a common neural pathway for both the perception and the implementation of an action (or an emotion) allows energy and time economy. The perception-action system allows a resonance with the other's emotional state (Decety & Sommerville, 2003). The scientific framework to the conceptualization of other's automatic and unconscious imitation as the foreground of a similar emotion in the observer has been formalized within the motor theory of empathy (Preston and de Waal, 2002).

However, the perception-action mechanism is the basis for the imitation and it includes components of empathy, mimicry and emotional contagion. *Mimicry* is the automatic imitation and synchronization of others' movements and affective behaviors (Hatfield *et al.* 1994). This mechanism is supported by electromyographical studies reporting a similar spontaneous facial contraction in the observer when showed with other's affective facial expression (Dimberg 1990; Dimberg & Oehman 1996). Moreover, emotional contagion seems to appear in very early developmental stages and even before the comprehension of another person affective states (Trevorthen *et al.*, 1999). For example, newborn infants are able to mimic adult's facial expressions (e.g., opening the mouth and making tongue protrusion; Meltzoff & Moore, 1977). In adults, emotional contagion is a low level mechanism that mainly precedes and supports empathy (Singer & Lamm, 2009). The distinction between the self and the other is not always present in empathy contagion because this is a subcomponent of empathy and it is present in infants. For this reason, it is often considered as a precursor of empathy (Singer & Klimecki, 2014) or a sub-component of the empathic process. In summary neither mimicry, nor emotional contagion could be sufficient to have a whole experience of empathy. In fact, these processes may partially lead to a confusion between the self and the other. Anyway, an essential component of empathy is the self / other awareness (Decety & Jackson, 2004) that helps to

perceive the self and the other representations as separated entities (i.e. to perceive that the affective experience we are about to feel is caused by someone else's affective or physical state) (Decety & Lamm, 2006; Singer and Lamm, 2009).

1.1.3. The Mirror Neuron System (MNS) in monkeys

The Mirror neurons system (MNS) supports the presence of a perception-action-coupling mechanism (for a review see Rizzolatti & Sinigaglia, 2010; Rizzolatti & Fabbri-Destro, 2008; Fabbri-Destro & Rizzolatti, 2008). Mirror neurons were first discovered in the ventral premotor area (area F5) of the macaque monkey as a particular class of visuomotor neurons increasing their firing rate both when the monkey performs a specific motor act and when it observes another monkey (or the experimenter) performing the same or a very similar action (Di Pellegrino *et al.*, 1992; Rizzolatti *et al.*, 1996a; Umiltà *et al.*, 2001). The term “mirror” derives from their physiological properties supporting the idea that the observed action is “reflected” in the observer’s motor system. Neurons with similar observation/execution matching properties have been found in: (i) the inferior parietal lobe (IPL), i.e. the prefrontal cortex (PF) and the PFG areas (e.g., Keysers & Perrett, 2004); (ii) superior temporal sulcus (STS; Rizzolatti & Fadiga, 1998; Perrett *et al.*, 1990; Figure 3). Two main functional roles are suggested for the MNS network: (i) to be the neural correlate of imitation (Jeannerod, 1994; Iacoboni 2009); (ii) to understand the goal motor acts performed by others (Rizzolatti *et al.*, 2001; Umiltà *et al.*, 2001). In this way, the matching system may offer a parsimonious explanation of how I understand the actions of others by providing a direct mapping of the visual representation of the observed action into a motor representation of the same action. According to the direct-matching hypothesis (Rizzolatti *et al.*, 2001), during the observation of an action, the monkey motor system resonates with whom performed the action, allowing the motor knowledge of the visual features of an observed motor act.

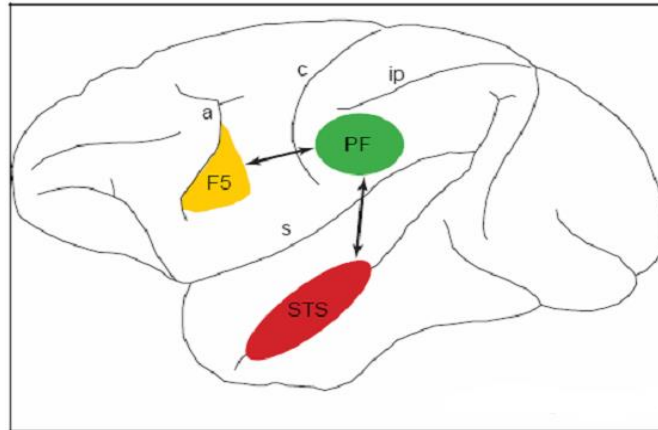


Figure 3. The MNS in monkeys.

Lateral view of the macaque brain with the location of area F5 in ventral premotor cortex, area PF of the inferior parietal lobule and the superior temporal sulcus (STS) together with their anatomical connections (arrows) shown on a lateral view of the macaque brain. Abbreviations: a, arcuate sulcus; c, central sulcus; ip, intraparietal sulcus; s, sylvian sulcus. (Modified from Gazzola *et al.*, 2007)

1.1.4. The Mirror Neurons System in humans

After the discovery of mirror neurons in monkeys, indirect evidence supporting the existence of an analogous human MNS has been raised by several neurophysiological (magnetoencephalography, MEG, e.g. Nishitani & Hari, 2000; event related potentials, ERPs, e.g. Cochin *et al.*, 1998; Transcranial Magnetic Stimulation, TMS, e.g. Fadiga *et al.*, 1995) and neuroimaging studies (positron emitting tomography, PET, e.g. Rizzolatti *et al.*, 1996b; functional magnetic resonance imaging, fMRI, e.g. Buccino *et al.*, 2001). Similar areas to monkeys are activated in human brains, allowing the understanding of others actions and their intentions (Iacoboni *et al.*, 2005). fMRI studies revealed the areas involved in the humans mirror neurons system. These areas include the ventral (Buccino *et al.*, 2004; Aziz-Zadeh *et al.*, 2006) and dorsal premotor cortex (Gazzola *et al.*, 2006; Gazzola *et al.*, 2007), inferior frontal gyrus (IFG), Broadman area (BA 44; Iacoboni *et al.*, 1999), which is considered as the human homologous of monkey area F5 (Di Pellegrino *et al.*, 1992) and the rostral inferior parietal lobule (homologous of monkey area PF; Buccino *et al.*, 2004; Aziz-Zadeh *et al.*, 2006). In summary, these studies revealed regions in humans which have similar anatomical and functional network as those described in monkeys. Furthermore, it was found that the observation of body part movements (transitive actions, i.e. with an interaction between a biological effector and an object; and intransitive actions, i.e. without an interaction between a

biological effector and an object actions) activated the premotor cortex following the classical motor organization of Penfield's Homunculus's somatotopy (Buccino *et al.*, 2001). The MNS in humans has some peculiarity as compared to the one of monkeys as some of the humans mirror neurons also become active during the observation of intransitive movements (Fadiga *et al.*, 1995; Iacoboni *et al.*, 1999).

1.1.5. Specific properties of the mirror response

fMRI studies are useful because they reveal the identification of the brain areas involved in action execution responding to action observation. This technique is limited to study the primary motor cortex (M1) activity of both the participant and the observer. It is possible using others methods like single pulse TMS applied on M1, coupled with the registration of motor-evoked potentials (MEPs; a detailed description of this technique is described in chapter 5).

Action observation induces changes in MEP size that are specific to the muscle that would be involved in the observed action (Fadiga *et al.*, 1995). Fadiga *et al.* (1995) first provided evidence that excitability of M1 is modulated during action observation. After this study, motor facilitation during action observation has been replicated in several other studies (e.g.: Alaerts *et al.*, 2009; Urgesi *et al.*, 2010; Catmur *et al.*, 2007). The motor facilitation during actions observation has been studied by means of MEPs recording. For instance, with this technique, it has been shown that the corticospinal (CS) system activity is specifically modulated by the muscles involved in the observed actions (for contralateral hand actions; Alaerts *et al.*, 2009) and the CS activity seems to closely simulate time-course of activation during movement execution (e.g. Gangitano *et al.*, 2001). TMS and MEPs characteristics and functioning are described in later part of this chapter.

1.1.6. Mirroring emotions and sensations

Several studies show that the production and the imagination of an action are subserved by partially covering neural networks. Ehrsson *et al.*, (2003) showed that imaging voluntary actions activates motor and somatosensory areas that are specifically involved during the execution of that movement. Along these lines, it has been found that the somatosensory cortex is also involved in the mirroring activity. During the observation of other's body parts directly

touched or touched using tools, activation of the secondary somatosensory cortex is recorded and the same brain activity is recorded during the direct sensation of touch in the involved body parts (Keysers *et al.*, 2004). After the first studies about mirror properties for motor and sensory behaviors, recent studies investigated the involvement of mirroring processes in emotional and empathic experiences. Indeed, the sensorimotor system seems to be involved in understanding others emotions via the simulation of the other's body state (Gallese *et al.*, 2007). Mirror properties have been found for the experience of disgust. The direct exposure to disgusting odorants, but also the visual perception of facial expression of disgust activated the same portion of anterior insula (Wicker *et al.*, 2003). Moreover, the observation of others social or affective cutaneous experiences might activate the posterior insula (a brain region that processes the interoceptive information of the body; Ebisch *et al.*, 2011). In addition, activation in the MNS (in the ventral premotor cortex, orbital part of inferior frontal gyrus) and in the amygdala and insula (involved in emotional process) has been reported for both the imitation and observation of other people's emotional facial expressions (Carr *et al.*, 2003; Schulte-Rüther *et al.*, 2007).

All these findings support the models of simulations (see section 1.1.1.). An implicit and bottom-up action, sensation and emotion understanding is due to the effect that common cortical areas are activated during first person body experiences and during third person observation of the same experience (Decety & Grèzes, 2006; Gallese 2003). The MNS, i.e., the network that is active both when people observe someone else's actions, feelings and emotions and when they live those experiences in a first person perspective, seems to be the biological substrate for others emotions' understanding and is the main candidate for the simulation account of empathy (Iacoboni & Dapretto, 2006; Agnew *et al.*, 2007; Enticott *et al.*, 2008). Moreover, it seems that the simulation process may be dependent on the level of shared characteristics between the empathizer and the target (Preston & de Waal, 2002; Gutsell & Inzlicht, 2010).

For now, there are still some debates if the sensorimotor resonance is the mechanism at the foreground of emotional contagion. For instance, Cheng *et al.*, (2012), studied empathy in juvenile psychopaths, i.e., persons characterized by enduring antisocial behaviors; besides they have a diminished affective empathy and affective resonance with others. The authors showed greater suppression of mu rhythm (i.e., greater sensorimotor resonance and no deficit in MNS) when these juvenile psychopaths were exposed to the sight of other injured individuals, even if they have an atypical affective arousal development. Indeed, although psychopaths are

characterized by lack of empathy they seems do not have deficit in sensorimotor resonance (Blair, 2011).

1.1.7. Empathy studied through the lens of the working model of empathy for pain

The motor theory of Preston and de Waal (2002) described a scientific frame to the idea that a mechanism of other's automatic and unconscious imitation exists and allows the activation of an emotion in ourselves that is similar to that felt by observing other. Empathy is the affective response to the direct perception or to the imagination of the other's affective state (Singer & Lamm, 2009). In a broad sense, many authors described this experience as an *emotional contagion* between two subjects (Levenson & Ruef, 1992; Hatfield *et al.*, 2009). Nowadays, this term recalls to a precursor and a sub-component of empathy. Functional neuroimaging and neurophysiological findings showed that similar areas of the brain are activated for both experiencing and witnessing a painful event, suggesting that the experience of feeling pain is neurobiological similar to witnessing another in pain. It is possible to elicit this type of empathic neural activation using different stimuli such as viewing animated visual images of hands and feet in blocks depicting painful and non-painful situations (e.g., Jackson *et al.*, 2005), observing pain facial expression (e.g., Lamm *et al.*, 2007), hearing human voices in pain and suffering (Lang *et al.*, 2011) and even by receiving an arbitrary cue signaling that the other person was receiving a painful stimulus (e.g., Singer *et al.*, 2004). fMRI studies showed that during empathy for pain paradigms, there is a constant activation of brain regions involved in the first-hand experience of physical pain. These include the bilateral anterior insula (AI), the anterior medial cingulate cortex (amCC) and the posterior anterior cingulate cortex (pACC). These regions are associated with the affective-motivational processing of painful stimuli (i.e., impulses and desires in ending and avoiding the noxious stimulation; Decety & Jackson, 2004; Decety & Lamm, 2006; Hein & Singer, 2008). These areas are more activated when perceiving or imaging another in physical pain but also for social distress (e.g. when participant are excluded from a ball-tossing game: the more they subjectively feel rejected, the greater was the activation in ACC and in AIC, as a consequence of social pain (for a review see Eisenberger, 2011) and important sadness (Beauregard *et al.*, 2006; Eisenberger *et al.*, 2003; Meerwijk, Ford & Weiss, 2013). However, there is an overlap of activation of amCC / pACC, in left fronto-insular cortex and in the precuneus both when someone undergone a painful event

and / or observe someone else in painful situation (for a review: Lamm *et al.*, 2011). This provides support to the concept of shared representations permitting the understanding of other's pain. In addition, the periaqueductal gray area (PAG) involves in this network of first/third hand experience of pain. Moreover, the thalamus and the central nucleus of the amygdala send projections to the AI which is indirectly connected with the secondary somatosensory cortex (SII). Some studies support the implication of bilateral primary and secondary somatosensory cortices (SI and SII) in pain perception of visual stimuli (Bufalari *et al.*, 2007; Moriguchi *et al.*, 2007; Decety *et al.*, 2008). In a recent meta-analysis, Lamm *et al.* (2011) suggests that a generalized somatosensory process is activated when we perceive picture stimuli representing body parts and this is amplified when these limbs undergo painful stimulation. In a similar vein, a transcranial magnetic stimulation (TMS) study by Avenanti and colleagues (e.g.: Avenanti, Minio-Paluello, Bufalari, & Aglioti, 2006; for more details about TMS studies on empathy for pain see chapter 5) showed that motor-evoked potentials that quantify corticospinal excitability (Bestmann & Krakauer, 2015), are modulated when participants observe a needle penetrate deeply the observed hand, but not when they observed the hand pinpricked. This may suggest that the somatosensory quality of pain (i.e., the intensity of the observed pain) plays an important role in determining whether somatosensory areas are or not involved during empathy for pain process (Singer & Lamm, 2009). On one hand, the ACC dorsal portion (its "cognitive" portion) is connected to the prefrontal cortex, the parietal cortex and the motor system: it process top-down and bottom up stimuli (Bush *et al.*, 2000; Figure 4). On the other hand, the ACC ventral part (its "emotional" portion) is connected with the amygdala, the anterior insula, the ventral striatum and the hypothalamus (Bush *et al.*, 2000). Singer and colleagues (Singer *et al.*, 2004) also showed that ACC and AI activity significantly covariated with subjects' scores in Balanced Emotional Empathy Scale (Mehrabian & Epstein, 1972) and the Empathic Concern Scale (a subscale of the Interpersonal Reactivity Index; Davis, 1983), supporting the empathic involvement of these brain areas. On the other hand, Decety (2011) underlies that we have to carefully consider these as the neural correlates of empathy. Empathy is a complex phenomenon to be studied and these neural activations may better describe the awareness for aversive stimuli.

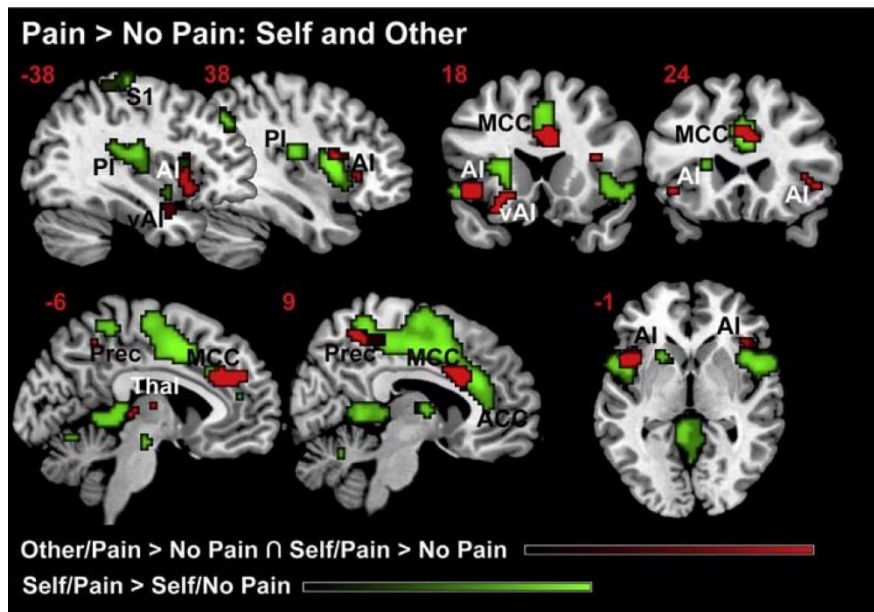


Figure 4. Common and distinct activation during direct experience of pain and in observation of other's pain. AI: anterior insula; PI: posterior insula; MCC: medial cingulate cortex; ACC: anterior cingulate cortex; SI: primary somatosensory cortex; Thal: Thalamus. In red: conjunction of self/other activations; In green: self-related activation (directly adapted from Lamm *et al.*, 2011).

Fox and colleagues (Fox *et al.*, 2013) have recently found greater activation within the ACC, insula, somatosensory cortex and dorsal striatum when Jewish subjects observed anti-Semitic persons in pain compared to likable tolerant person in pain. These areas are functionally connected with the amygdala, frontal regions and reward regions. Since this network is involved in the emotional regulation process, these authors argue that seeing individuals in pain is more likely to be explained by the effect to be exposed to a dangerous situation rather than an empathic response (Fox *et al.*, 2013). These findings support the evidence that shared representations of affective-motivational processing of painful stimuli may be more representative of general self-protective mechanisms of avoiding threat rather than describing the sensory characteristics of pain (Iannetti & Mouraux, 2010; Decety, 2015). Moreover, studies on patients (mostly on psychopaths) have recorded a normal activation of the sensorimotor system, even with absence of affective empathy in these patients (Blair 2011; Decety 2011). Furthermore, recent meta-analysis (Lindquist *et al.* 2012; Wager *et al.* 2008) on emotion showed a little overlap between first-hand emotions regions and the perception of emotions.

1.1.8. Conclusions

Evidence supporting the idea of shared representation in social interaction has emerged from perception-action study. Observing an action evokes an identical response to the execution of the same action suggesting the existence of a mirror system in the frame of social interaction (Stamenov and Gallese, 2002). Mirroring social behaviors is the basis of simulation (Gallese, 2001). Simulation suggests that perceiving the state of another individual evokes a representation of the observed feelings and emotions in the observer (Decety and Lamm, 2007) and it is based on the idea that perception and action shared some similar properties. fMRI and neurophysiological studies revealed evidence that the modulation of central processes by pain observation is associated with the emotional and sensory feeling of physical pain experienced by the observer (Lamm *et al.*, 2011; Avenanti *et al.*, 2005). These findings support the simulation theory and the shared representations for emotions and feelings of others. Meanwhile, there is an ongoing debate if empathic neurobiological mechanism is represented by the MNS. Studies about psychopaths (Blair 2011; Decety 2011) and recent meta-analysis (Lindquist *et al.* 2012; Wager *et al.* 2008) challenge these theories. It is also important to consider that research on the involvement of the MNS in emotion remains recent (Agnew *et al.*, 2007), and further studies may better clarify if there is an effective involvement of MNS in the emotional and empathic domain.

1.2. Top-down empathic mechanisms

Simulation and bottom-up processing system are not sufficient to explain all the processes involved in other's feelings and sensations understanding. Top-down are control mechanisms that permit a voluntary cognitive understanding of other's mind allowing both generation of empathic response and modulation of already existing empathic response (Singer & Lamm, 2009). Pain catastrophizing, learning experiences (e.g., habituation or pain avoidance) and attentional processes are mainly modulated by top-down influences and they are important in modulating feelings of empathy for pain (Goubert *et al.*, 2005; Goubert *et al.*, 2009; Hein & Singer, 2008). Decety and Lamm (2006) highlight the regulation role of top-down mechanisms. It is possible to deregulate and reduce empathic emotions like personal distress, or to promote an empathic response and prosocial behaviors. The cognitive inference

of other's experience is done by a voluntary evaluation of their emotional and mental state. Adam Smith suggested that imagination could promote the projection in someone else's shoes (Smith, 1976). In fact, mimicry is a useful mechanism that permits automatic synchronization with other person's feelings, but sometimes can be confusing in emotions' attribution between the self and the other (Jankowiak-Siuda *et al.*, 2011). Top-down control processes include attention and self-regulation (Decety and Lamm, 2006) and these cognitive mechanisms are underpinned by executive functions (De Vignemont & Singer, 2006; Hein & Singer, 2008). Top-down attentional mechanisms can act by increasing or decreasing the focus on emotion-eliciting stimulus like the external cue saliency or the empathizer internal state (De Vignemont & Singer, 2006). This top-down cognitive modulation may intervene quite earlier (*early appraisal model*: after the perception of emotional cue which is modulated by internal and external contextual factors), or in a later process (*late appraisal model*: the empathic response is automatically activated by the perception of an emotional cue and this response is modulated only in a second time by the context; De Vignemont & Singer, 2006). In the next section, main theories and concepts linked to a top-down elaboration of the empathic stimuli are discussed.

1.2.1. Theory - Theory

Theory-theory proposes an explanation about how we understand the external world (Ratcliffe, 2006). It specifically suggests that individuals make inferences about how others think and feel using their own mental models of the world (Carruthers & Smith, 1996; Gopnik *et al.*, 1997). This way of understanding the others is differentiated from simulation theory, i.e. automatically understand other's feelings by directly experiencing the same feelings and emotions. An example of inference might be - if we see an individual observing a snake we can infer that he is probably feeling fear because people are generally afraid of snakes. According to theory-theory, it is possible to make inferences about the observed person that are not similar or also incongruent to our feelings. This is not possible for the simulation theory. Carruthers and Smith (1996) reported that when a person gains while playing chess against his adversary, he feels emotions of happiness and at the same time he recognizes negative feelings of the person he is playing against. In theory-theory, people take into account their personal experience, their beliefs and contextual factors for understanding other's feelings and emotions (Leiberg & Anders, 2006). Theory-theory is closely related to another concept for understanding others, i.e., theory of mind and it is explained in the next section.

1.2.2. Theory of Mind (TOM)

Another theory that supports the cognitive side of empathy is Theory of Mind (TOM). It is necessary to differentiate our emotions from those of others and to understand that other's minds can be different from ours (Decety & Jackson, 2004; Decety & Grèzes, 2006; Brass *et al.*, 2009) in order to infer the contents of other individual's minds. Hoffman (2000) proposed that the simulation of other's emotions (simulation theory) and sharing the emotions with others might lead to a self/others confusion followed by a personal distress (i.e., an aversive response focused on the self, in response to other's emotions; Batson *et al.*, 1997). Neuroimaging studies have reported that the self/other confusion is only partial because AI and ACC areas are activated both for the imagination of self and other's pain, but some parts of these brain regions are selectively activated for the self-perspective only (Jackson *et al.*, 2006; Lamm *et al.*, 2011). Theory of Mind (TOM) refers to the ability to make this distinction in the observer between his point of view and the other's perspective (Premack & Woodruff, 1978). TOM ability includes *mentalizing* which means to infer other's mental states (Gallagher and Frith, 2003). A paradigm used in order to assess TOM abilities is based on the false-beliefs task. For succeeding in these tasks, subjects have to recognize that they might have beliefs inconsistent with what other participants/actors know about the situation (Flavell & Miller, 1998; Wellman *et al.*, 2001) otherwise a false belief in regard to the reality might be attributed to others (Wimmer & Perner, 1983). False belief tasks are widely used in child development research (Flavell, J. H., 2000). An example of a sample story proposed to children is the "Sally-Anne' task". "Sally hides a marble in a basket and then leaves, in her absence, Ann moves the marble to a nearby box" (Baillargeon *et al.*, 2010). When Sally returns, the children were asked where Sally will look for the marble. The correct response "Sally will look in the basket, where she put the marble" is normally given by children at the age of four (Baron-Cohen *et al.*, 1985), children under the age of three and people with autism fail the task (Wellman *et al.*, 2001; Baron-Cohen, 1995) (i.e., they reply that Sally will look in the box, where the child knows the marble is actually hidden).

1.2.2.1. Neural correlates of TOM

The important role of different brain regions and structures in inferring the mental states of others has emerged from recent neuroimaging studies. These techniques used either PET (Fletcher *et al.*, 1995; Goel *et al.*, 1995) or fMRI (Gallagher *et al.*, 2000; Vollm *et al.*, 2006)

and evidenced the role of brain regions in TOM like the medial prefrontal cortex (mPFC) is involved when we think about our own and others mental states (Amodio & Frith, 2006; Gallagher & Frith, 2003; Macrae *et al.* 2004). Also, the posterior part of the superior temporal sulcus (pSTS) and the temporo-parietal junction (TPJ) and temporal lobes are activated during TOM and perspective-taking paradigms (Frith & Frith, 2003; Brass *et al.*, 2009).

The mPFC is activated both in verbal (Fletcher *et al.*, 1995; Vogeley *et al.*, 2001) and imagination tasks (Brunet *et al.*, 2000; Gallagher *et al.*, 2000). This region is activated when subjects have to think about the emotions that they are experiencing and in the rest condition of tasks that analyze cognitive processes (Gusnard *et al.*, 2001). This region is also activated when we have to make a distinction between an action implemented by the self or by others (Farrer *et al.*, 2003), during self-regulation (Cheng *et al.*, 2007) and for the imagination of living a painful experience for the self or for another individual (Jackson *et al.*, 2006). Ruby and Decety (2004) studied the role of emotion in perspective-taking, brain regions involved in the distinction between first and third person perspective (i.e., their mother perspective) include the mPFC, frontopolar gyrus, right TPJ and left temporal lobe (Ruby & Decety, 2001, 2003; David *et al.*, 2006). Studies show that dorsal mPFC (dmPFC) may represent the neural correlate of cognitive TOM (i.e., beliefs reasoning and false belief tasks) otherwise the rostral part of mPFC (rmPFC), may be better activated during TOM affective tasks (i.e., desire reasoning; Abu-Akel & Shamay-Tsoory, 2011; Carrington & Bailey, 2009; Hartwright *et al.*, 2014). In a recent study, (Hartwright *et al.*, 2014) suggested that dmPFC was more activated for cognitively effortful situations with no regard to the quality of mental state, rmPFC seemed to be more activated by the reasoning, mentalizing on desires especially in more complex tasks.

TPJ is the region where the temporal and parietal lobes intersect at the end of Sylvian fissure and plays a central role in the self/other distinction (Saxe & Kanwisher, 2003). This region appears to be bilaterally implicated in attributing the agency between the self and the other (David *et al.*, 2008). The left TPJ seems to be necessary to attribute false beliefs to others (Apperly *et al.*, 2004; Samson *et al.*, 2005) and during the other's imitation (Decety *et al.*, 2002). The right TPJ is activated when the subject has to imagine how someone else is feeling during emotional (Ruby & Decety, 2004) or painful (Jackson *et al.*, 2005; 2006) situations and not when they imagine these situations in a self-perspective. Young *et al.* (2010) used rTMS on the right TPJ (rTPJ) to mimic a temporal virtual lesion of this region. They found that interfering on rTPJ activity leads to judge attempted harm (i.e., the agent intent to harm another individual

but he / she fails) as more morally permissible compared to what they think with a normal rTPJ activity. Thus, rTPJ seems to be involved in the capacity to use mental states (like attempted harm) in moral judgement (Young *et al.* 2010).

Temporal poles (TP) are also implicated in the TOM neural network, which is suggested by its function in semantic and episodic memories (Simmons & Martin, 2009). This region performs important functions such as helping us to make an association between an observed behavior and a particular mental of the past episode memory (Gallagher & Frith, 2003). Along these lines, Frith and Frith (2003) argued that TP boost mentalizing. From the elaboration of a past episode, this region allows the creation of a semantic and emotional context to the actual situation. Moriguchi *et al.* (2006) have found a positive correlation between TP activity and the interpersonal reactivity index score (IRI; Davis, 1996; Aketa 1999) in personal distress. This region seems to be more activated when subjects felt distress and negative feelings as a consequence of empathizing with others. This behavior may be particularly evident in the case of empathy for pain such as when to empathize with negative feelings and sensations of others.

The right hemisphere seems to have a more important role in TOM functions (Weed *et al.*, 2010), lesions on right frontal regions (Stuss *et al.*, 2001) have severe deficits in TOM tasks. Moreover, Shamay-Tsoory *et al.* (2005) have shown that patients with lesions in the ventromedial prefrontal cortex have more severe impaired performances in affective TOM tasks and Brunet *et al.*, (2000) found a greater activation in right mPFC compared to left mPFC for non-verbal attribution of intentions TOM tasks. Right frontal activity compared to the left one is associated with withdrawal motivation (Harmon-Jones, 2004; Tullett *et al.*, 2012; van Honk & Schutter, 2006).

1.2.2.2. Empathy for pain TOM

Circuits involved in TOM neural network (TPJ, temporal poles, ventral part of mPFC) and top-down mechanisms are mainly activated by cue-based studies (the subject can see an actor physically present in the same room, when abstract visual symbols appearing on a screen, indicates to him/her that the actor is going to receive a painful or non-painful stimuli) instead of picture-based paradigm (Lamm *et al.*, 2011). Mentalizing network and top-down control give opportunity to make social inferences about other's mental states (Van Overwalle and Baetens, 2009) basing these inferences on our knowledge, previous experiences and beliefs (Mitchell,

2009). This means that these mechanisms may modulate the quality of empathy for pain on the basis of our experiences. Decety *et al.* (2008) in their study analyzed aspects linked to TOM in the context of empathy for pain in healthy children by investigating the intentions of making pain to others. The neural network of empathic pain matrix (aMCC, ACC, PAG, Insula) is activated in adults, and interestingly, when children observed an individual intentionally inflicting pain to a person, the authors reported a greater activation in regions involved in social interaction, moral behavior, TOM regions which include TPJ, medial orbitofrontal cortex, paracingulate cortex and the amygdala. Gu and Han (2007) analyzed the role of top-down attentional processes on empathy for pain. In the fMRI scanner, participants rated pain intensity of pictures representing painful or neutral situations or to count the number of hands depicted in the visual stimuli. Pain rating task needed to pay attention to other's pain and the authors showed that only this task led to the ACC / paracingulate, insula, and frontal cortex activations in empathic responses to pain. This study highlighted the complexity of the interpretation of painful stimuli. Bottom-up processing is adaptive which permit faster reaction to possible dangerous stimuli. Whereas, top-down influences are essential to better understand the observed scene in a social fashion.

1.3. Top-Down and Bottom-Up Interplay

Leiberg and Anders (2006) discussed creation of an “artificial dichotomy” (Mitchell, 2005) between the researchers that support Simulation theory (e.g., Gallese, 2003) and those that support theory-theory (e.g., Saxe, 2005). They argued that not all the aspects of empathy could be totally explained by an isolated theory that a conjunction model that takes into account both theories may better explain how we can understand others (Leiberg & Anders, 2006). Albeit different, top-down and bottom-up processes of empathy are somehow overlapping and reciprocal and both of these mechanisms are helpful in empathy to understand others.

In their model, Decety and Lamm (2006; Figure 5) suggest that bottom-up processes (based on shared representations) and top-down processes (control and regulation) are interrelated and both play a fundamental role in the generation and modulation of empathic responses (Singer & Lamm, 2009). Bottom-up processes in the lower level of the model include automatic emotional sharing to a continuous update of perceptual inputs. Top-down processes give flexibility to the model, and regulate cognition and emotion by means of selective attention

and self-regulation through executive functions (activation of prefrontal and cingulate cortex; Decety & Lamm, 2006; Hein & Singer, 2008; Singer & Lamm, 2009). Indeed, the implicit information continuously updates the explicit controlled information and the latter will modulate the first one in a flexible way. This meta-cognitive loop gives us the possibility to react (or not) to other's personal state. Indeed, during the process of other's understanding, bottom-up automatic empathizing processes acting through autonomic and somatic ascending neural pathways may be inhibited by top-down descending neural pathways coming from prefrontal regions (Jankowiak-Siuda *et al.*, 2011).

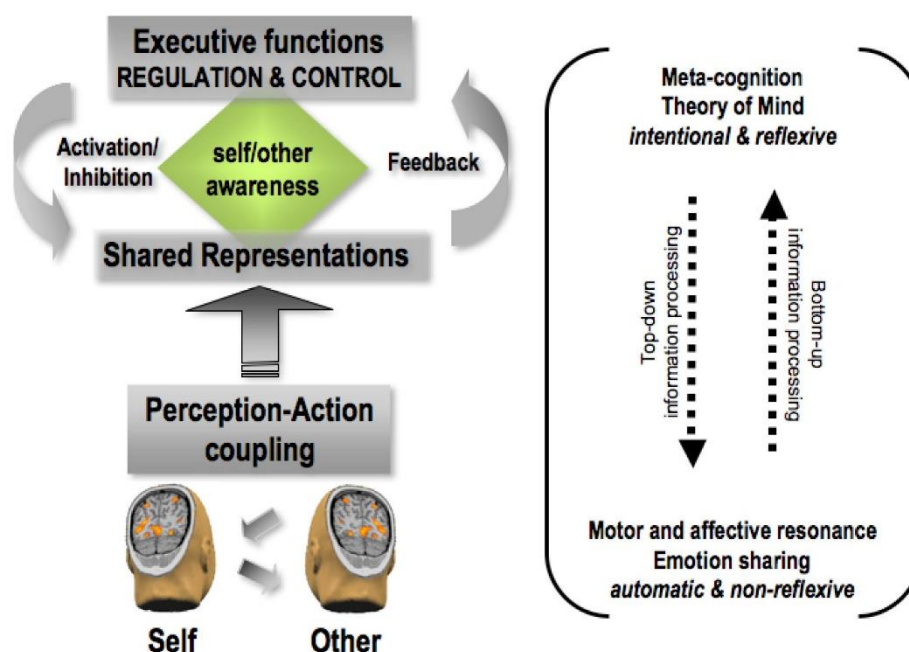


Figure 5. Schematic representation of the model proposed by Decety and Lamm (2006). Bottom-up and top-down mechanisms of empathy: Bottom-up mechanisms are automatically activated/inhibited by perceptual inputs; top-down control, exerted by the executive functions acting through attention and self-regulation, control the lower levels and add flexibility to the model (Directly adapted from Decety & Lamm, 2006).

1.4. The role of “Self” and “Other” concepts in empathy

Shared brain representations of affective elaboration of painful stimuli support that during the observation of other's pain and there is a prevalence in activation of bottom-up circuits, there is a partial overlap between self-representations and other-representations. As described above, emotional contagion, the precursor and sub-component of empathy, is based on the emotional sharing process without making a distinction between self and other. For instance, it is common to say that the cry of a baby is “contagious” for other babies that hear

it and they start crying without being aware of the cause of their emotional state (De Vignemont & Singer, 2006). A complete overlap between self and other's pain representations may lead to a self-oriented aversive response consisting of emotional/empathic distress (Decety & Grèzes, 2006). However, it is important to underline that in the case of empathy for pain, we don't feel the pain of others in all situation (Decety & Lamm, 2006). Self and other's representations may be differentiated even if it is possible that the self/other confusion can occur (Decety & Grèzes, 2006). Jackson *et al.*, (2006) suggested that self versus other distinction in observing other's pain might be the two extremities of a continuum. It is possible to identify different shades of pain representations in this circumstance. The authors argue that these different representations of pain may be influenced by the proximity, tangibility and self-embodiment of the pain.

Cognitive TOM perspective-taking paradigm asks directly to overtly adopt others' perspective during empathic paradigms. Jankowiak-Siuda *et al.* (2011) suggested that the first step of this paradigm is to make a distinction between the other and the self. Secondly, it is possible to think about the way in which the other individual may think, feel, desire and believe. The self / other distinction in perspective taking is confirmed by the fact that when we make inferences about other's the *mentalizing*, TOM network is activated and the automatic mimicry network should be inhibited (Jankowiak-Siuda *et al.*, 2011). Talking about the neural basis, one hypothesis predicted that the frontal lobes act in keeping the self and the other perspective respectfully separated or at least in inhibiting the self-perspective interference (Decety & Jackson, 2004; Decety and Lamm, 2006). Moreover, there are evidences that the development of self/other distinction is tied to the functional development of executive functions and prefrontal cortex (Chakrabarti & Baron-Cohen, 2006; Decety and Lamm, 2006; Zelazo, 2004). Along these lines, the right inferior frontal gyrus is involved in cognitive flexibility and in the ability to inhibit the egocentric response (i.e., attributing our intentions, emotions and beliefs to someone else) that are essential for the attribution of a specific mental state to others that might be different from our own mental state (Samson *et al.*, 2005; Moriguchi *et al.*, 2007; Lamm, Meltzoff & Decety, 2010).

In order to infer other's mental states, it is also necessary to have an intact self-awareness. Moriguchi *et al.* (2006) recorded in people with alexithymia (i.e., a deficit in self-awareness), a reduction in TOM mentalizing, perspective taking and empathic concern. Indeed, self-awareness that is developed thanks to the sense of agency (Jeannerod, 2003) is necessary if we want to make inferences about others (Decety & Lamm, 2006). "Agency" allows the

differentiation between ours and others' behaviors (David *et al.*, 2008). Humphrey (1990) reported that self-recognition permits the awareness of our mental states and this ability permits us to infer other's mental states. It has been shown that TPJ has a fundamental role in agency (Jackson & Decety, 2004). In addition, Uddin *et al.* (2006) have shown a reduction in self / other discrimination after the stimulation of the right inferior parietal lobule with repetitive TMS during a task in which subjects have to make a distinction between their face and other-familiar faces.

1.5. Factors modulating empathy

Several factors can modulate the level of empathy felt by the observer. The *intensity of stimulation* and the *involvement of emotions* may play role on the empathic modulation (De Vignemont & Singer, 2006). In empathy for pain, it is possible to record significant inhibition of corticospinal excitability only with an observation of a deep penetration of a hand by a needle rather than observing a needle only pinpricking the hand (Avenanti *et al.*, 2006). Pain intensity stimulation is an important factor for recording an empathic response and the observation of pictures of hands in painful situations compared to the observation of the same situation. Meanwhile, in a cartoon version modulate the empathic response of the observer showing a greater ACC activity during the observation of the more realistic pictures (Gu & Han, 2007). Also, observing facial expressions of chronic and acute pain patients changes the intensity of activation of the ACC and the AI in the observer (Saarela *et al.*, 2007). In addition, the observer's level of distress might be modulated by the *social relationship* that we have with others. We can be more or less empathize with the observed person depending on how much we perceive the observed person similar to us (Batson *et al.*, 1997) and if we consider him/her as part of our group (Yabar *et al.*, 2006). For instance, Cheng *et al.* (2010) found that the activity in ACC and AI painful empathic areas was increased in adopting the loved-one perspective compared to a stranger perspective during the observation of painful visual stimuli. Concerning the in-group/out-group differences modulating empathy for pain responses, Montalan *et al.* (2012) found that the observer tended to rate painful visual stimuli as more painful when it was adopted an in-group perspective compared to a self-perspective. Moreover, Xu *et al.* (2009) found a greater activation of ACC during the observation of faces of the same racial group of the subject (in-group member) being pricked by a needle as compared to the observation of racial out-group members. In addition, Avenanti *et al.*, (2010) in their TMS experiment found

a greater corticospinal inhibition (i.e., a greater empathic response) for the observation of a hand belonging to the same racial in-group that was deeply penetrated by a needle and this response was greater for who had a stronger implicit racial bias.

In addition, *the situation* can modulate the intensity of empathy for pain felt for another individual (Jankowiak-Siuda *et al.*, 2011). Lamm *et al.*, (2007) showed that cognitive and motivational factors can modulate the intensity of the observer's empathic response: having information about the context in which the painful situation happens. In simpler form, a therapeutic setting, induce an activation of a top-down cognitive control that, in this case, brings to a reduction of the empathic painful response (focusing on long-term benefits of treatment reduce the automatic empathic response for the temporary pain induced by this treatment). In addition, individual's personal context and his characteristics, like the expertise, may modulate the empathic arousal. For example, Cheng *et al.* (2007) have shown that when physicians practicing acupuncture look at visual stimuli depicting needle inserted in different body parts, the activation of their pain matrix wasn't relevant compared to the control group, in which the automatic empathic pain network (ACC, AI, SI and periaqueductal cortex) was activated. Instead, regions involve in emotion regulation and TOM (mPFC, TPJ) mainly activated in physicians. Another factor influencing the empathic response has been demonstrated to be the gender of the empathizer. Yang *et al.* (2009), in their experiment found differences between males and females mu suppression, a biomarker of empathic mimicry. The authors reported stronger mu suppression in females when they looked at painful and non-painful images. Moreover, voxel based morphometry (VBM) study revealed that the pars opercularis of prefrontal cortex and inferior parietal lobule, i.e. where humans mirror neurons are situated, have a larger volume of gray matter in females compared to males (Cheng, Lee *et al.*, 2008). These results suggest that there are neuroanatomical gender differences in the human mirror neurons system (Cheng, Lee *et al.*, 2008) and that females might be more adapted to empathize with others, suggesting they have a more efficient bottom-up system and a less active prefrontal area involvement in inhibition of automatic empathic response (Jankowiak-Siuda *et al.*, 2011).

1.6. The adaptive role of empathy and empathy for pain

Empathy yields to adaptive information about potentially dangerous events and facilitates compassion and caring responses among people (Goubert *et al.*, 2009). Empathy,

specifically empathy for pain is considered to have an adaptive value and essential for human moral development (Hoffman, 2000), the facilitation of relationships (Van Baaren *et al.*, 2009) and for survival. It has different functions permitting affective sharing, empathic concern and perspective taking (Decety, 2015), as explained in previous section. In addition, the communication of pain and empathy demands attention and focuses on escape and healing and may facilitate survival (Plutchik, 1987; Williams, 2002). Indeed, the capability to understand what it is happening to other people, especially in the context of pain may generate different reactions in the observer ranging from feelings of distress and fear for personal safety, to compassion and willingness in helping the individual in pain (Goubert *et al.*, 2009). In this section, it is aimed to firstly address how the empathic adaptive behaviors survived across different species, behavioral and neural aspects of empathy and its related altruistic behaviors are described. Besides behavioral and emotional reactions of empathy are analyzed under the light of motivated behaviors, which are better described in chapter two.

1.6.1. Evolutionary continuity of the mechanisms of empathy across mammalians

According to Thompson (1987), humans are empathetic to other individuals who communicate negative feelings from their infancy and argues that this behavior is ethologically relevant. It signals a presence of threat in the environment allowing the implementation of self-protection mechanisms. In addition, pain is adaptive and has an evolutionary role, indicating the presence of a potential threat (Akitsuki & Decety, 2009). Porges (2003) defined as “neuroception” the neural circuits that are specific in assess safety risk in the environment. After this risk evaluation, the neural system allows to organize the most appropriate defensive response in the frightful environment-to fight, to freeze or to flight (Akitsuki & Decety, 2009). According to theories about the evolution of social behaviors, their phylogenetic origin depends on the evolution of sympathetic and parasympathetic systems permitting a fast and unawareness evaluation of external stimuli and a consequently rapid motor response while maintaining visceral homeostatic states (Porges, 2001). Adolphs (2006) affirmed that all mammalians have neural circuits that allow fast perceiving and recognizing others facial and vocal expressions. These capabilities to quick react to external stimuli rely on a neural system, which is the same system suitable for monitoring homeostatic balance and survival responses in human beings and others mammalians. After the risk evaluation, defensive behaviors should be inhibited to social behaviors occurrence (Carter *et al.*, 2011) allowing interactions and even prosocial

behaviors. Neural circuits involved in risk detection in others movements, face expressions and communication include the temporal cortex, which is connected to amygdala, in a top-down control system allowing the inhibition of defensive responses when others' expressions are analyzed (Adolphs, 2006; Carter *et al.*, 2011). Mammalians have a common neural network activated in empathic behaviors that includes amygdala, hypothalamus, brainstem, basal ganglia and orbitofrontal cortex (Decety *et al.* 2012; Decety, 2015). Parasympathetic and sympathetic systems represent the two antagonist branches of the autonomic neural system and their activity is also involved in the empathic network; moreover neuroendocrine/hormones slowly regulate social behaviors (Decety 2015; Figure 6).

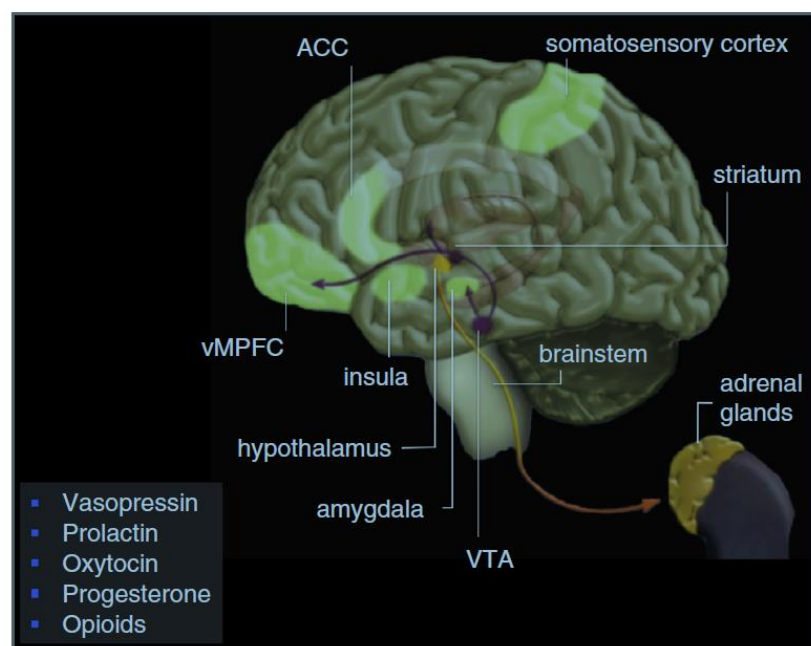


Figure 6. The complex neural network of empathy includes amygdala, hypothalamus, brainstem, striatum, orbitofrontal cortex and for empathy for pain is included the activation of insula and anterior cingulate cortex (as reported in section 1.1.7.). Moreover the autonomic nervous system and neuroendocrine system are involved in the empathic network (directly adapted from Decety, 2015)

Emotional contagion, a basic form of empathy, is present in infants when they are exposed to another's infant cry (Sagi & Hoffman, 1976), has an adaptive value. Rats (Church, 1959) and pigeons (Watanabe & Ono, 1986) express similar responses of emotional distress to the pain of conspecifics. In fact, previous researches suggest that signals from conspecifics may influence the animal's behavior. Warning of pain or fear lead to feelings of distress in the witness (Edgar *et al.*, 2011), instead, a pleasant state due to a playful situation may induce

positive sensations in the observer (Held & Špinka, 2011). As reported in section 1.5, empathy is modulated by the effect that we perceive within the observed individual as part of our social group and this behavior is common also in animals. For instance, Langford *et al.* (2006) recorded an increase of pain response in mice only observing their cagemates conspecifics experiencing pain while this response is not present observing foreign conspecifics. In addition, Jeon *et al.* (2010) recorded higher fear response, represented by a freezing behavior, in mice observing conspecifics related to them receiving painful stimuli. Studies on non-human primates showed that hungry monkeys prefer to obtain no food if it led to an administration of electric shock to another conspecific (Masserman *et al.*, 1964). Moreover, this response was stronger if the observed monkey was familiar to the witness monkey (Masserman *et al.*, 1964). Along these lines, Hamilton (1964), in his “inclusive fitness theory” (better known as “kin selection”) affirmed altruistic behaviors are influenced by the relatedness between individuals and the rapport of cost and benefits in helping others (Foster *et al.*, 2006). Similarly, the idea of reciprocal altruism supports individuals tend to be helpful and cooperative with those they expect to return the favor (Trivers, 1971). These mutually beneficial exchanges are common in apes because non-human primates tend to render a service to those they have positive relationships (de Waal, 2008; de Waal & Brosnan, 2006; de Waal & Luttrell 1988). Kin selection and reciprocal altruism support that empathy evolved in animals as a proximate mechanism of an ultimate benefit for the self and the proliferation of shared genes (de Waal, 2008). In humans, empathy is often associated to altruism and prosocial behaviors (Batson, Ahmad, & Tsang, 2002) and may leads to helping behaviors. On the other hand, empathy may have direct benefits on the self, for self-protection, especially when there is an exposure to threatening stimuli. Batson (Batson *et al.* 1997), citing Wegner (Wegner, 1980 (p. 131)) quoted “Empathy is one way in which positive forms of social behavior may be motivated by selfish desires”. In this vision of empathy, the other is included in the self as an extension of it (Batson *et al.* 1997). Finally, Kin selection and reciprocal altruism suggest that empathy boost us to help others when there is a beneficial repayment for the self with reciprocity or for increasing one’s representation in the gene pool (Batson et. al, 2005; de Waal, 2008). In conclusion, there are many evidences considering empathy as a phylogenetic ancient mechanism (de Waal, 2008). Empathy is present in different species, it seems to be linked to the ability to recognize social signals and to adopt adaptive behaviors, approach or avoid other individuals (Decety, 2015).

1.6.2. Behavioral and emotional reactions to others pain

Recent studies evidence emotional contagion and other forms of empathy present in infancy are not only automatic as it was thought previously but also modulated by the context (Decety, 2015). In adult, the automatic reactions to others' accidentally rapid pain are still present and their neural correlates are mainly represented by the limbic system that is activated by emotional processes (Jackson *et al.*, 2006). This limbic system is more activated than higher cognitive functions and learning mechanisms permit to modulate the empathic response (Decety & Lamm, 2006). This implied that both the person in pain and the witness might have automatic and deliberative reactions to the first-hand experience of pain and to the observation of a painful event (Goubert, *et al.*, 2009). For instance, Chiesa *et al.* (2015) reported behavioral and physiological evidences in favor of an implicit empathic reactivity. The subliminal observation of an individual being touched in a painful, neutral or pleasant way lead to a congruent affective reaction in the witness and the physiological response correlate with the observer's empathic trait. Automatic reactions to others unexpected pain might occur along with a withdrawal response, even if to understand completely the other's feelings top-down influences like perspective taking or the memory of past painful events should intervene (Jackson *et al.*, 2005;). First-hand automatic reactions to pain in acute like crying, reflexive withdrawal and facial expressions (Craig, 2009) tend to induce automatic empathic reactions to others pain in the observer (Lamm *et al.*, 2011). Deliberative reactions to others pain mainly occur when pain persists after the automatic reactions and generally include more complex behaviors like verbalization of pain for the person in pain and the observer generally produces judgments (Table 1; Goubert *et al.*, 2009).

		Person in pain's expression	
Observer's reactions		Automatic reactions (e.g., reflexive escape, facial grimaces, cry)	Deliberate behavior (e.g., self-report, purposive actions)
	Automatic reactions (e.g., involuntary, viscerally experienced, motor preparation)	The uncontrolled response to acute pain is more likely to instigate involuntary empathic responses	Less likely to activate spontaneous empathy
	Deliberate behavior (e.g., contemplation or active decision making)	Reflective or contemplative consideration follows temporally	Likely to instigate reflection, questions about credibility

Table 1: Interactions between automatic and deliberative reactions of the person in pain and the empathic reaction of the observer (directly adapted from Goubert et al., 2009)

Automatic reactions to other's pain has been recently suggested that shared circuits activated in first-hand experience of pain (see chapter 1, section 1.1.7) but also in observing someone else in pain or in feeling social pain are not those involved in somatosensory and nociception characteristics of pain. Meanwhile, the activity of these circuits is promoted by potentially dangerous events, fostering defensive mechanisms like avoidance and withdrawal behaviors (Decety, 2010; Decety & Christen, 2013). Indeed, the bottom-up automatic process of empathy for pain underlines the functional and adaptive role of these mechanisms. The perception and immediate empathic reaction to other's pain may promote self-protection inducing avoidance of threat and withdrawal behavior in order to protect ourselves (Yamada & Decety 2009). This automatic reaction promotes self survival, but shared representations of pain may also promote prosocial behaviors between parents and offspring (Preston and de Waal, 2002), including compassion, caring responses among people living in a community (Goubert *et al.*, 2009) and promote social ties in the context of social pain (Decety & Christen, 2013).

Vicariously adopting the emotional state of another individual prepares the observer to react and interact with the environment and eventually prepares the bases of social bonding (Plutchik, 1990). According to the literature, it is possible to empathize with the others in positive and negative context. Concerning the empathic painful situation, three main opposed responses are associated with empathy: (i) *approach* associated with the elicitation of caretaking responses, (ii) *withdrawal* elicited by stimulus avoidance and self-protection (Goubert *et al.*, 2005; Singer & Klimecki, 2014); (iii) *freezing* behavior (e.g. Avenanti *et al.* 2005; 2009). Individuals empathic with others are sensitive and they are likely to help them, even if being empathic with others is not sufficient to boost helping behaviors (Eisenberg *et al.*, 2001). Compassion is an emotion coming from the observation of another individual physically or psychologically suffering and it is accompanied with an impulse to approach and to heal the target individual (Dewar *et al.*, 2011). This other-related feeling is involved especially when the observed person is self-relevant to the observer experiencing compassion (i.e., those important for the well-being like relatives, partners and friends; Goetz *et al.*, 2010). Moreover, altruistic behavior tends to be activated when we faced other altruistic and cooperative peers (Henrich, 2004).

Meanwhile, personal distress like compassion is another emotional state linked to empathy. Personal distress tends to be self-directed (Goubert *et al.*, 2005; Singer & Klimecki, 2014) and lead the observer to ignore other people distress. More specifically, this distressed

empathic response demands physiological and cognitive resources to the onlooker. This might be inhibiting the resources for the observer's potentially helpful response (Decety & Lamm, 2009b). Indeed, personal distress is negatively related to prosocial and helping behaviors (Eisenberg *et al.*, 1989; Eisenberg *et al.*, 2006). An important factor that can modulate helping behaviors is to or not to have the possibility to easily escape dangerous situation. Batson *et al.* (1983; 1987; 1991) have shown that individuals with a higher level of self-reported compassion tend to adopt helping behaviors even if there is a possibility to easily escape from the situation. However, individual that reported higher personal distress tends to help less the others when there is the possibility to leave the situation. In addition, automatic freezing behaviors may be adopted during the observation of visual painful stimuli. This kind of response is normally recorded in first-hand experience of pain: an inhibition of motor response is recorded when painful stimuli are delivered on subjects' hand (Farina *et al.*, 2003; Urban *et al.*, 2004). Avenanti *et al.* (e.g. Avenanti *et al.*, 2006) have evidenced this response also in observation of painful stimuli. The authors interpreted this response as an empathic response to others pain. Further description of this behavior is provided in section 3.

In the next section (section 2), behavioral mechanisms at the foreground of the empathic (prosocial and avoidance) responses are described. This includes the approach / withdrawal and freezing responses. Specifically, the chapter starts with a description of the evolutionary role of these behaviors. The neurobiological and neurophysiological bases of approach / avoidance and freezing behaviors, and the operating principles of these behaviors will be explained. The next section helps to understand the motor, postural and physiological behavior of individuals faced to motivational emotional stimuli. In the experimental part of this thesis, the motor, postural and physiological behaviors associated with empathic painful emotions and pain anticipation are explained.

2. Approach / avoidance and freezing behaviors

Regulation of body functions by keeping a homeostatic balance in physiological functions allow the survival of the individual and develop a dynamically implemented appropriate reaction to an eventual variation in body's homeostasis (Cannon, 1939). These changes in body state are caused by the individual internal needs or by external stimuli. They induced physiological responses and mental states like pain and fear (Damasio & Carvalho, 2013). Zajonc (1998) suggested that each living being is provided from his birth moment with the ability to implement approach and avoidance behaviors to specific classes of stimuli. Carver (2006) defines approach / avoidance responses as behavioral "building blocks". These two tendencies are involved in the organism regulatory activity. Moreover, it has been shown that potentially threatening situations can also induced freezing behaviors especially when escaping from the situation that was perceived to be impossible (Blanchard *et al.*, 2001; Campbell *et al.*, 1997). William James (1890) already described in his work "Principles of Psychology", a link between sensation and action. Pain and pleasure are considered as "spring of action". Pain is an action inhibitor while pleasure is an action strengthening (William James (1890), pp. 549-559). For example, quickly escaping from dangerous situations or reaching and grasping appetitive food when we are hangry allows the survival of the individual and the achievement of short-term objectives. Automatic mechanisms widely trigger these fast and non-conscious responses (Chen & Bargh, 1999; Neumann *et al.*, 2003). These automatic responses like reflexes are firstly implemented in reaction to external stimuli and these responses have a preparatory function in organizing more complex behaviors (Ferguson & Barg, 2008). Moreover, different neural circuits subtend this basic automatic evaluation of stimuli and more complex mechanisms are involved in the conscious elaboration of the stimuli (LeDoux, 2003; Ruiz-Padial, *et al.*, 2011). More specifically, somatic and physiologic circuits are activated by an automatic evaluation of the stimuli. These automatic neurological responses are functional to prepare the organism to produce an adaptive behavioral response. Damasio (1999) suggests that the emotional and motivational states and their relative processes are distinguished by a series of physiological markers such as heart rate, blood pressure, respiration rate (Lazarus, 2006) and muscular activation (Coombes *et al.*, 2007). In the following sections, the operation mode of approach / avoidance and freezing behaviors will be described.

2.1. The adaptive and evolutionary role of approach / avoidance and freezing behaviors

Fundamental survival value of these basic behaviors make it possible to find approach / avoidance and freezing-like behaviors in different living species. For example in flatworms, the basic behaviors of approach (to appetitive stimuli) and withdrawal (from aversive stimuli; e.g., Wisenden & Millard, 2001) are observed. Also in mollusks like the *Aplysia Californica*, it is possible to find these two behaviors (Leonard & Lukowiak, 1986). Lang and Bradley (2008) suggested that mammals have the same basic approach / avoidance behaviors as the simplest species has but their responses seem to be more complex and not always oriented in one specific direction. Indeed, in evolved organisms with a cerebral cortex, the initial automatic approach / avoidance responses might be modulated by top-down and external factors such as cognition, individual differences and interactions with the context (e.g., social interactions and cultural norms) (Lang & Bradley, 2008). Concerning freezing behavior, Bolles (1970) has shown that among different species that there are some more inclined freezing reactions and other in which this kind of response is rarely found. For instance, rats, in specific settings can manifest approach / avoidance (Olds & Olds, 1963) or freezing behaviors (Blanchard & Blanchard, 1977; LeDoux *et al.*, 1984).

Automatic reflexes are also important for the species survival and can activate and drive the organism to action. Specifically, reflexes are automatic reactions (i.e., not voluntarily) driven to a goal (Grewe, 2001). Konorski (1967) proposed a dichotomist organization of unconditioned reflexes. Specifically, preservative reflexes (e.g. ingestion, copulation) are essential for the organism preservation and also protective reflexes (e.g. avoidance of noxious stimuli) bringing to action in case of emergency like nurturance and sexual passion. Talking about startle reflex (a defensive reflex that takes part of the orienting response). Greenwald *et al.* (1998) have recorded a potentiation of this response in humans when they are exposed to a shock and unpleasant visual stimuli. The authors suggest the modulation of startle response in humans is consistent with animal's response. Bradley *et al.*, (1990) have shown also a modulation of the EMG amplitude of the orbiculari oculi induced by startle probes. They showed that negative valence stimuli elicited higher response and positive stimuli reduced the amplitude of this response. Talking about the impulses, Radel *et al.* (2011) have shown that if

environmental stimuli threaten the autonomy of an individual, he automatically implements a defensive response. All these automatic behaviors are functional for the species survivals and it has been suggested that animals and humans may share the same neural basis for defensive reflexes (Davis, 1992).

2.1.1. Defensive behaviors in animals and humans

Defense behaviors can be activated by potential or actual threatening situations like pain and induce stimulus avoidance in animals and humans. Defensive behaviors can be divided in anticipatory and immediate defense behaviors (Misslin, 2003).

The *anticipatory defensive behaviors* prepare the individuals to act. This behavior is manifested for the novelty of the presented stimulus or situation. For instance, the “new object reaction” (Misslin & Ropartz, 1981) is a consistent defensive response present in rats as they avoid any new object presented in their environment. Moreover, Blanchard and Blanchard (1989) described the “risk assessment behavior” as a combination of approach and avoidance movements that different animals species do when they have to leave their environment in order to look for food or coupling. In humans, the anticipatory defensive behavior is present for example in infants. Fagan (1976) showed that infants responded to a negative expression like fear with a defensive response. This infant reaction has been interpreted as a lack of familiarity for the fear stimulus.

The *immediate defensive behaviors* consist of all responses implemented against a potential or actual predator, including fight, flight and freeze responses. Usually, when any of this kind of defensive behaviors is implemented, non-defensive responses like eating and drinking are inhibited. Fleeing behaviors are applied when the prey sees the predator and wants to escape (King, 1985). During freezing behavior, the individual inhibits all body movements except for eyes movements and respiration (Misslin, 2003). Freezing is a state of attentive immobility that helps animals in hiding by predators (Lang and Davis, 2006; Öhman and Wiens, 2002) and it is considered as a part of the defensive cascade (Hermans *et al.*, 2013; Lang *et al.*, 1997; Lang *et al.*, 2000). Fighting response is adopted only when the prey has no possibility to freeze or flee and it has to go towards the predator (Misslin, 2003). Indeed, primarily, the prey tries to adopt one of these last two responses. It is not possible to freeze or flee at the same time, but they can occur in succession in the same individual (Eilam, 2005). For instance, when fear

is perceived, an initial sensation of immobility is followed by an ardent desire to escape from the fear-inducing situation (Öhman & Arne, 2000). Eilam (2005) posits that three factors can modulate the prey's decision to initially adopt a freezing or a fleeing behavior, which include individual differences, distance from the predator and facility to reach the refuge. Freezing behaviors are mainly applied when the predator is perceived far away and before the predator recognizes the prey. This is in agreement with the “distance-dependent defense hierarchy” (Gallup, 1974), far away the animal freeze, when he is detected by the predator flee and when the distance is still closer the prey fight (Figure 7). In addition, Fanselow (1994) described the predatory imminence continuum in the defensive behavior system, and highlighted the physical distance between the prey and the predator as a key variable. The closeness of a refuge may induce the animal to flee more than to freeze (Blanchard & Blanchard, 1989); otherwise freezing response will be applied with a greater probability. Personal differences like gender and age may also play a role in the freezing / fleeing choice (Blanchard *et al.*, 1991; Borowski, 2002). It is important not to confuse tonic immobility with freezing response because tonic immobility mainly implemented when the prey is very close or it is already taken from the predator. This kind of response represents a last essay to survive in pretending to be dead (Gallup, 1974).

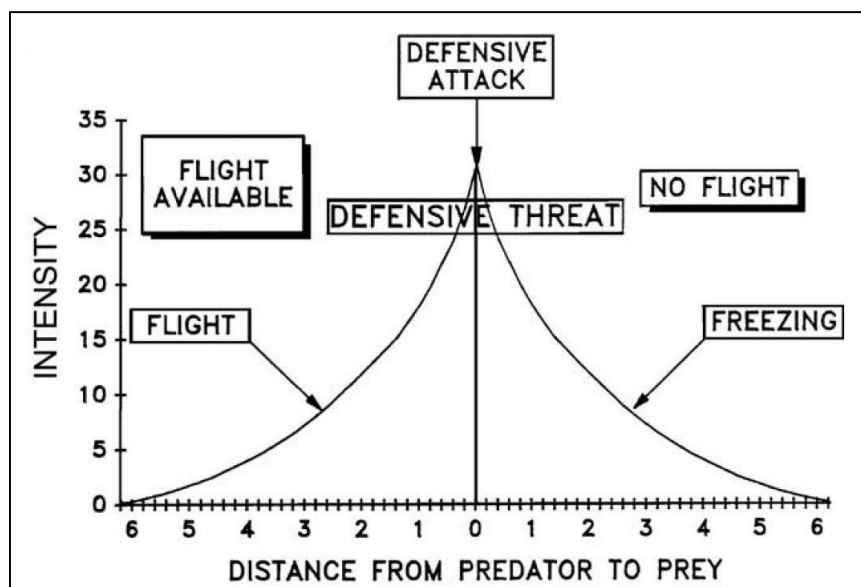


Figure 7. Defensive distance and avoidance behavior: when the prey is closer to the predator defensive response is represented by a defensive attack; instead, flight and freezing responses are implemented when an intermediate defensive distance is established between the prey and the predator (directly adapted from McNaughton & Corr 2004).

Other three behaviors are considered defensive behaviors even if they do not belong to the fight, flight and freeze main defense response system. Misslin (2003) includes in his description of defensive systems the Hypoalgesia (i.e., the absence in sensitivity to pain for preventing tissue damage caused by defensive responses (Bolles & Fanselow, 1980), the submissive postures (i.e., behaviors whose aim is to avoid or stop conflicts in conspecifics with behaviors such as lowered eyes in humans) and the autonomic arousal (i.e., all the sympathetic and parasympathetic changes that go with the sensorimotor changes during the fight-flight-freeze response). For instance, in animal models, slowing of the heart rate (HR; i.e. fear bradycardia) is recorded during freezing behavior - even though skin conductance (a marker of sympathetic activity) continues to increase (Lang *et al.*, 1998), these imply that both parasympathetic and sympathetic systems are activated during freezing. The HR begins to increase when the body engages in an avoidance response pattern - indicating the preparation of body action to avoid the stimulus (Fanselow, 1994). Talking about the fear bradycardia, Campbell *et al.*, (1997) say that this state is experienced when a prey perceives a predator. A defensive behavior is implemented through the activation of the limbic system inducing deceleration of HR and a generalized motor inhibition and an augmentation in focused attention on threat. When fear is perceived, an initial sensation of immobility is followed by an ardent desire to escape from the fear-inducing situation (Öhman and Arne, 2000). When the individual is preparing for action, HR and peripheral conductance are increase, and it is recorded a vasodilatation in peripheral muscles (Misslin, 2003), preparing the body to a defensive action (Lang *et al.*, 1997).

2.1.2. Neural correlates of approach / avoidance and freezing behavior in animals

Delgado *et al.*, (1954) reported avoidance behavior in cats when they received electrical stimulation in hypothalamus-thalamus areas. In addition, Cominski *et al.*, (2014) examined the impact of damaged hippocampal in rats' may have in avoidance learning. Authors suggest a dysfunction in hippocampus might induce persistent avoidance responding and this condition might rend the individual more vulnerable in developing anxiety. Moreover, Hoebel *et al.* (2008) explained that the *in vivo* release of acetylcholine in the nucleus accumbens lead to a behavioral inhibition and negative stimuli avoidance. The authors suggested that the

dopaminergic system and the relative quantity of released dopamine (DA) depend on a cholinergic “stop” system that opposes its function. In fact, the release of DA in the nucleus accumbens in ventral striatum and its relative dopaminergic pathways are involved in appetitive motivation and approach behaviors. The ventral tegmental area (VTA) promotes appetitive-approach behaviors. VTA dopaminergic activity can induce an approach to a place where they have been previously conditioned to receive a positive reward (Fields *et al.*, 2007).

2.1.3. The reinforcement sensitivity theory: approach / avoidance and freezing behaviors in humans

In humans approach / avoidance theories describe how behaviors are motivated by unconditioned appetitive and aversive stimuli (Lang *et al.*, 1998). It is important to specify that even in literature, there is a divergence in the definition of these concepts, approach / avoidance, approach / withdrawal and appetitive / aversive concepts are often interchanged. This indicates the levels of complexity of motivational processes as a function of their positive or negative valence (Elliot & Church, 1997; Elliot *et al.*, 2013). The reinforcement sensitivity theory (Corr, 2008; Gray, 1973; Gray & McNaughton, 2000) supports the evidence of rewarding and punishing value of stimuli on emotion and on motivated approach and avoidance responses. There are three neuropsychological systems.

The first one is the *Behavioural Approach System* (BAS) that is associated with positive, appetitive motivations and approach behaviors (Figure 8). Carver and White (1994) subdivided this system in three sub-scales: (i) BAS Reward Responsiveness, responsive to reward; (ii) BAS Fun Seeking that looks for new rewards; (iii) BAS Drive that is goal-oriented and, for the authors, is the most important and useful of the three dimensions. The other two systems are associated with avoidance responses.

The second system is the *Fight-Flight-Freeze System* (FFFS; Gray & McNaughton, 2000). It is activated by unconditioned aversive punishment stimuli (for the animals these stimuli are represented by predators) inducing fear and consequent avoidance defensive responses (McNaughton & Corr, 2004). Fight, flight and freezing responses are described before (see the section describing *the immediate defensive behaviors*) and are similar in humans and animals (Figure 8). Moreover, this system is described in animals as a system of active

avoidance because it directly removes the prey from the danger and it is accompanied by emotions of fear or even panic (Corr *et al.*, 2013).

The third system described in the reinforcement sensitivity theory is the *Behavioral Inhibition System* (BIS). As the FFFS, the BIS motivate avoidance behaviors but in this case these responses are produced by conflicting stimuli (Figure 8). These kinds of stimuli have blended valence and opposite goals. For instance, this happens when there is a coactivation of two conflicting approach / avoidance tendencies like when a threatening social situation is approach (Corr, 2013). This system is associated to a passive avoidance and leads to anxiety (Corr *et al.*, 2013), a threat-related emotion that is characterized by sustained, abnormally high levels of muscle tension (Lang *et al.*, 2000).

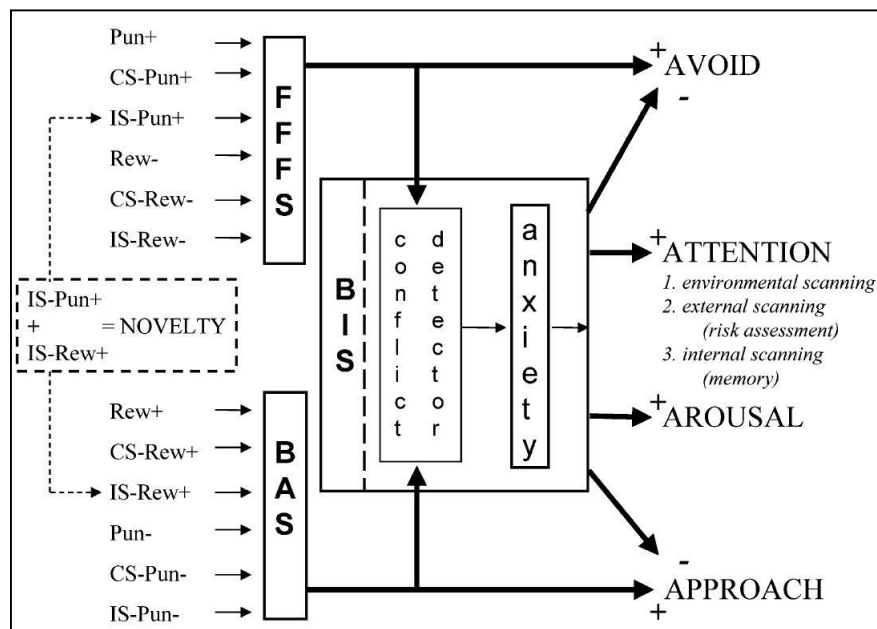


Figure 8. Interactions between the three systems (FFFS, BAS, BIS), their inputs, i.e. punishments (Pun), rewards (Rew), innate stimuli (IS) and conditioned stimuli (CS) and their final behaviors (avoid or approach). Pun and Rew might be presented (+) or omitted (-). When a stimulus activate both FFFS and BAS an approach-avoidance conflict is detected and the BIS is activated. (Directly adapted from Corr *et al.*, 2013).

The reinforcement sensitivity theory is used to describe the biological antecedent of personality and temperament (Smillie, 2008). A major activity in the BAS system tends to manifest extraversion (or positive emotionality) in the individual and it is generally associated with an approach motivation (Elliot & Thrash, 2002). On the contrary, a greater activity of BIS

system leads to neuroticism (or negative emotionality) and is associated with avoidance-withdrawal motivation (Elliot & Thrash, 2002). For instance, to be more sensitive to avoidance motivation and to have higher BIS scores, it may predict an enhanced defensive reflex response (Cook *et al.*, 1991; Corr, 2001).

Indeed, the reinforcement sensitivity theory highlights the role of inter-individual differences in the BAS, the BIS and the FFFS systems activity. These differences might lead to the expression of distinct personality traits in individuals (Smillie, 2008). Moreover, personality traits may modulate the effects of affective stimuli (Stemmler & Wacker, 2010) and this might be evidenced recording biological markers of motivation like the heart rate variability (Thayer & Lane, 2009).

Neural correlates of the reinforcement sensitivity theory systems

The neural mechanisms at the base of the approach / avoidance motivation rely on basic, distinct and interconnected neural structures (Bernston *et al.*, 1997; Gray, 1990). Concerning the neurobiology of BAS system, Gray and McNaughton (1996) originally suggested areas involved in the realization of the objectives are located in the limbic system and basal ganglia. As described before in animals, humans DA pathways from the VTA towards the ventral striatum (where the nucleus accumbens is located) play an important role in supporting appetitive-approach behavioral responses. Moreover, the DA projections to the prefrontal cortex are very important for the reward processing (Knutson & Cooper, 2005; McClure *et al.*, 2004; Pickering & Gray, 1999). The basal ganglia are essential to implement and control the motor response.

Talking about the neurophysiological basis of the defensive system, the FFFS activation is mainly related to the activity of the periaqueducal gray matter for a situation of intense or proximal threat, the medial hypothalamus, the amygdala and the anterior cingulate cortex (more distal threat) (Smillie, 2008). All these regions are associated with primary defensive reactions (Gray & McNaughton, 1996). The other defensive system, the BIS, consists of the neurophysiological point of view in the septo-hippocampal system (i.e., hippocampal formation and the medio-lateral septal area) and the Papez circuit, especially the amygdala (Gray, 1982). Regarding the neurobiological neurotransmitters involved in FFFS and BIF modulation, when a threatening stimulus is detected, serotonin (5HT) and noradrenaline (NA) are released and

their projections reach all levels of FFFS and BIF inhibiting or potentiating the behavioral response (Corr *et al.*, 2013).

2.1.4. Conclusions

In order to maintain an internal homeostasis, all organisms have to control their internal needs and to interact with the environmental stimuli (Damasio & Carvalho, 2013). This equilibrium is guaranteed thanks to two main systems (including their subsystems) giving birth to the different kinds of reflexes and responses described in the previous sections - the approach-appetitive system and the avoidance-defensive system (Elliot *et al.*, 2013, Lang & Bradley, 2008). Schneirla (1959) asserts that for all animal species there is a common biphasic system inducing the individuals to approach or avoid different situations. He suggests that this behavior is evolved with the ontogeny producing more complex responses to the environmental stimuli. In addition, Masterson and Crawford (1982) suggested that humans and animals might have the same defense system. Moreover, Lang and Davis (2006) support the evidence that the defensive behaviors in animals and humans might share common neural basis and especially the amygdala seems to be the core neural structure (Davis, 1992; LeDoux, 1995). Studies further supported the hypothesis of a fundamental evolutionary role of the approach / avoidance and freezing behaviors in humans and animals showing that the appetitive and avoidance systems share similar neurophysiological and neurobiological basis in humans and animals (Delgado *et al.*, 1954; Gray & McNaughton, 1996; Hoebel *et al.*, 2008). Obviously, mechanisms at the foreground of these behaviors are more complex in the human beings with reference to those found in the simplest organisms. Indeed, they are not automatic or inevitably consequent (Lang & Bradley, 2008) but their basic functions remain the same in all individuals. Successful reaction to external stimuli and internal needs in order to accomplish the final goal that are necessary for the individual survival. Indeed, approach, avoidance and freezing behaviors are among the most useful actions for animals, ancestors and humans in supporting survival in life-or-death situations (Fredrickson, 2001; Tooby & Cosmides, 1990).

2.2. Emotion, motivation and approach /avoidance and freezing like behaviors

The observation of others states especially when they are related to negative feelings like pain induces the production of empathic emotional states in the observer. Scherer (2005) described emotion as a condition inducing reactions at different levels including neurophysiological variations, motor response, cognition, motivation and subjective sensations. Lang (1995) defined emotions as action disposition. They quickly prepare the organism to potentiate the appropriate response to emotional stimuli. Different theories support the functional adaptive value of emotions (Darwin 1872, Keltner & Gross, 1999) and they are considered as a part of motivational approach / avoidance behaviors (Frijda *et al.*, 1989). This vision suggests that emotional and motivational systems facilitate adaptive survival behaviors like avoidance of threats (Löw *et al.*, 2008), forming attachments (Niedenthal *et al.*, 2002), creating and maintaining cooperative relationships (Fessler, & Haley, 2003). Scherer (1994, p. 127) defined this type of emotions as an “intelligent interface” between external environmental input and functional beneficial output. This general description about emotions and their effects on the motivational behaviors might fit also with the specific case of empathic emotions. This implies personal empathic distress and compassion. In this context, empathic emotions could cooccur with behavioral approach / withdrawal responses to external stimuli (Goubert *et al.*, 2005). Indeed, it seems that there are close links between the approach / avoidance motivation and the positive / negative empathic emotions.

2.2.1. The dimensions of valence and arousal

The adaptation of an individual to his environment is based on the evaluation of the valence of external stimuli (Berntson & Cacioppo, 2008; Ferguson & Barg, 2008). Dominant emotion theories argue that individuals tend to implement motivated approaches associated to positive states or conversely they execute withdrawal behavior co-occurring with the exposure of negative states (Cacioppo *et al.*, 1997; Cacioppo *et al.*, 1999; Davidson *et al.*, 1990; Elliot & Covington, 2001; Lang, 1995; Watson, 2000). These theories are framed in the valence categorization process. The difference between positive and negative feelings influences the

cognitive and behavioral aspects of emotions (Lang, 1995; Watson 2000). In his biphasic emotion theory, Lang defined the dimension of valence as “strategic” that influence the driving behaviors (Lang *et al.*,1990; Lang, 1995).

The valence parameter is important in the context of learning theories. Behaviors with a positive outcome are reinforced and behaviors leading to a negative outcome are generally inhibited (Rotter, 1973). Several studies confirmed the approach-avoidance / positive-negative emotions coupling (Rotteveel & Phaf, 2004; Seidel *et al.*, 2010; Stins, *et al.*, 2011; Marsh, *et al.*, 2005). Otherwise, it seems that this dichotomy is not always respected. It seems that approach behavioral response is not solely attributed to positive emotions (Harmon-Jones & Gable, 2008). For instance, anger has a negative valence but drives the individual to approach the others (Carver & Harmon-Jones, 2009; Ekman & Friesen, 1975; Plutchik, 1990; Wilkowski & Meier, 2010); fear also may induce approach behaviors (Marsh *et al.*, 2005).

Arousal is another emotional dimension and it represents the intensity of emotional activation switching from excited to calm (Lane *et al.*, 1999). This concept is separated from the dimension of valence and refers to the energy mobilized in the motivational processes and ensures that the energy resources to realize the behavior (Hanoch & Vitouch, 2004, Lang *et al.*, 1990).

2.2.2. Motivational intensity and direction

Davidson (1993) argues the main function of emotions is to motivate an approach or an avoidance behavior through a stimulus. The approach / avoidance model helps to define and understand motivated behaviors in humans and animals (Elliot and Church, 1997; Elliot *et al.*, 2006). As described earlier, the motivational function of the emotion may influence the approach / avoidance tendencies (Harmon-Jones & Gable, 2008). Behaviors implemented in response to emotions may be described in terms of their motivational intensity and direction. These dimensions of motivational intensity and direction might be used to categorize emotions according to their functional outcomes (Harmon-Jones & Allen, 1998; Harmon-Jones & Gable, 2008). Moreover, it is possible to categorize different behaviors according to their associated positive or negative emotional valence and according to their motivational intensity and direction. Gable and Harmon-Jones (2008) showed differences in the level of positive affect. Approach motivation associated with low positive affect leads to broadened attention instead

high positive affect might be associated with the breadth of attention, approach desired objects compared to neutral stimuli.

2.2.3. Automatic and controlled approach / avoidance processing

In order to react quickly to the environmental stimuli, individuals have to rapidly determine whether these stimuli are bad or beneficial for their survival (Chen & Barg, 1999). Barg (1994) explained that “automatic” process made of four components: unintentionality (the automatic process might begin without control), efficiency (it requires few resources), unawareness (the automatic process is mainly implemented without consciousness) and uncontrollability (it is not possible to stop the automatic process once it started). As described earlier, reflexes allow automatic evaluation and automatic responses (i.e., hormonal, neuronal, autonomic and somatic) preparing the organism to action (Zajonc, 1998). Indeed, these physiological and somatic responses allow the individual to produce a behavior adapted to the external context (Berntson & Cacioppo, 2008; Lang & Bradley, 2008). Emotions quickly prepare the organism to the action (Lang, 1995) and motivations are defined as energization of behaviors (Elliot, 2006), contributing to the action preparation. Chen and Barg (1999) have shown that the automatic elaboration of stimuli influences the motor response. Negative valence stimuli promote the activation of the negative avoidance motivational system favoring a withdrawal behavior (i.e., pushing faster the lever away from the body) while positive valence stimuli activate the positive approach motivational system inducing an approach behavior (i.e., pulling faster the lever toward the body).

Automatic reactions are useful to implement a quick and adaptive response but human beings compared to simpler organisms have also the possibility to act voluntarily: planned responses might inhibit automatic behaviors (Bargh & Ferguson, 2000; Smith & Neumann, 2005). Neumann *et al.*, (2003) also support the idea that the approach / avoidance motivated behaviors start in an automatic fashion. Moreover, human beings have the possibility to regulate and inhibit this automatic response if it is not adequate to the context. According to the authors, most of the basic evaluative processes are not sufficient to evoke the final emotional response. Cognitive processes allow completion of emotional and behavioral processes implementing an alternative response to the automatic one. In their dual-process model, Strack and Deutsch (2004) suggested that human social behavior is controlled by two interacting systems. These

represent the *reflective system* that controls behaviors with a decision process and it is restricted to situations where cognitive ability is available and the *impulsive system*, which is always involved in information processing and activates behavioral responses from the elaboration of perceptual inputs or from reflective processes. Generally, in healthy individuals, the reflective system controls the impulsive system through cognitive (a decision-making process drives the behavior making choices and predictions about the consequence of actions and the influence of emotion; Strack & Deutsch, 2004) and behavioral controls (impulse control mechanisms act inhibiting motor responses and reorient the attention; Bechara *et al.*, 2006). These systems integrate also motivational components of the behavioral response and the dimension of valence is associated to approach and avoidance behaviors (Cacioppo *et al.*, 1993). In the impulsive system, approach / avoidance motivational directions influence the information process and its consequent behavioral response (Cacioppo *et al.*, 1993). This means that, when the impulsive system is in avoidance (or approach) mode, this supports the processing of negative (or positive) external stimuli and affects the implementation of the final coherent avoidance (or approach) behavior (Strack & Deutsch, 2004). Indeed the valence of stimuli or the valence of affect and the approach or avoidance directions are facilitated if they are compatible with the direction of the motivation. Moreover, all these components are bidirectionally oriented and cognitive, affective and behavioral mechanisms might orient the individual motivation (Strack & Deutsch, 2004).

The neural level of automatic and controlled approach / avoidance behaviors: bottom-up and top-down influences.

In their somatic marker hypothesis, Bechara and Damasio (2005) focused on the role of emotions that drawing the attention quickly to possible action outcomes. This helps to choose the appropriate reaction. In fact, when emotions are elicited by the observation of emotional stimuli, the central nervous system and the peripheral somatic and autonomic nervous systems are activated allowing the implementation of survival motor reflexes (Lang & Bradley, 2008). As said earlier, the homeostatic balance and the formation of emotions are dynamically regulated by brain functions in connection with the visceral and muscular effector organs (Lang *et al.*, 1998). These physiological modifications in the body are connected to the somatosensory regions (primary and secondary somatosensory cortex, insula, cingulate cortex) and brainstem nuclei (Bechara & Damasio, 2005). The authors called this anatomical system “body loop”

because it includes the body and brains regions that linked to the body through different connections such as the spinal cord, the vagus nerve and humoral signals.

It has been suggested that when the affective components disturb or even interrupt the cognitive control, bottom-up circuits are activated through amygdala and striatum to the prefrontal cortex (PFC; Dolcos & McCarthy, 2006). This region is the main top-down control region (Miller & D'Esposito, 2005). As described earlier, in the section illustrating the neural correlation of the reinforcement sensitivity theory, neuroimaging studies report that amygdala and insula are mainly activated for automatic avoidance reactions to aversive stimuli (Bechara *et al.*, 2006; Cunningham *et al.*, 2010) and the ventral striatum is activated during automatic approach reactions towards positive stimuli. This is also associated with reward processing (Bichot *et al.*, 2011; Delgado *et al.*, 2000). Indeed, these results support the idea that automatic processes are subtended by subcortical region. The limbic system is the neuro-anatomic model of emotions (Lang & Bradley, 2008), the septal area is associated with processing pleasurable stimuli (Berridge & Kringelbach, 2013) and the amygdala is more activated (Hermans *et al.*, 2008) when an individual is afraid (fleeing behavior) or aggressive (fighting behavior). At the neuroanatomical level, the core structure of the limbic system involved in approach / avoidance motivated behavior is the amygdala, which projects to the paralimbic structures (i.e., the septal region; Mogenson *et al.*, 1980). The hypothalamus is also connected to the amygdala, and its function is to activate different vegetative expressions (i.e., heart rate, breath rate and blood pressure variations; sweat glands activity; digestion; muscular activity) associated with the related emotional reactions (Critchley & Harrison, 2013). As mentioned earlier, the limbic system is connected with prefrontal areas. PFC is activated during the intentional control of emotions, in the behavioral control and its regulation (Miller, 2000; Koechlin *et al.*, 2003). At functional level, the orbitofrontal cortex (OFC) activation represents the affective valence of an external stimulus (Sescousse *et al.*, 2010). Moreover this region allows making a relation between a situation and its corresponding somatic states (Bechara & Damasio, 2005). The dorsolateral prefrontal cortex (DLPFC) activation permits higher behavioral control (Duncan, 2001; Miller & Cohen, 2001). Inhibition and decision making processes are mediated by medial prefrontal cortex (mPFC; Damasio *et al.*, 1991). Davidson (1998) supports the idea that a system of emotional hemispheric lateralization exists with (i) the right PFC representing the substrate of an avoidance system that allows fighting or withdrawing aversive situations and (ii) the left PFC associated to positives emotions and appetitive behaviors supporting the

approach system. More recently, Harmon-Jones *et al.* (2004; 2010) confirmed this asymmetric activity in prefrontal cortices for motivational approach / withdrawal behaviors. In conclusion, it is important to point out the automatic and controlled systems interaction allowing the execution of fast and adaptive motivated behaviors and their eventual inhibitions if they are not adapted to the situation. Moreover, the top-down controlled influences allow reflecting on the observed behavior. Instead, the PFC is mainly responsible for control.

In this section approach / avoidance and freezing behaviors, their neural underpinnings and different theories that may explain these behaviors have been described. Moreover, the evolutionary and adaptive value of these behaviors has been highlighted. In the next section, techniques and empirical studies using these techniques for studying approach / avoidance / freezing behaviors and empathy for pain are described.

3. Overview of research methods applied in the study of in approach / avoidance / freezing behaviors and empathy for pain

Different techniques are used to elicit and measure approach / avoidance behaviors and empathy for pain reactions. Different methods and techniques mostly used to study approach / avoidance / freezing and empathy behaviors are described in this section. Some of these techniques have been applied in the present thesis. The principles of these methods are more deeply described and examples of studies using methods applied in the present thesis are also presented. In the second part of this chapter, parameters of the techniques used in this thesis are described.

3.1. Visual stimuli

The presentation of affective pictures may induce physiological and approach / avoidance and freezing behavioral reactions (Lang & Bradley, 2008). The International Affective Picture System (IAPS) is a set of colored pictures made up of neutral (e.g.: household objects; neutral faces), pleasant (e.g.: erotic; families; sport) and unpleasant (e.g.: mutilated and disfigured bodies; attacking animals and humans) normative emotional stimuli (Lang *et al.*, 2008). This battery may be used to induce approach /avoidance and freezing behaviors. In addition, video stimuli with different valence categories may be used to induce changes in subjects' behavior (e.g. Hagenars *et al.*, 2014).

In the context of empathy for pain, pictures are often shown to the participants like images representing right hands and feet executing familiar actions with painful (e.g., to shut one's finger in a door) or non-painful consequences (e.g., to close a door without any painful consequence) (e.g., Jackson *et al.*, 2005; 2006). The type of pain may be different (thermal, mechanic, pressure) and also the intensity of pain may vary (Jackson *et al.*, 2006). These stimuli were validated demanding to the participants to assess the level of pain for each condition. Pictures with suffering facial expression (e.g., Lamm *et al.*, 2007) are also used. Showing video stimuli of painful (e.g.: a needle penetrating a hand) and non-painful events (e.g.: a Q-tip touching a hand, or a needle penetrating a tomato) represent another possibility to study empathy for pain (e.g., Avenanti *et al.*, 2005).

3.2. Reactions times (RTs) and accuracy

The presentation of emotional / empathic visual stimuli is often associated with the registration of reaction times (RTs) recorded starting from the onset of the stimuli presentation. This is a simple technique that permits to define the timing necessary to reply to emotional and empathic stimuli. For example, it has been shown that RTs tend to be longer when participants have to reply to high arousing unpleasant images compared to those less arousing (Bradley *et al.*, 1992; Lang *et al.*, 1997). It has been suggested that the augmentation of RTs might be due to the effect that are more attentive resources might be demanded for encoding new stimuli (Bradley *et al.*, 1992; Lang *et al.*, 1997). In empathy for pain studies, several researches recorded RTs during the presentation of painful and non-painful stimuli (e.g.: Jackson *et al.*, 2005; 2006). RTs and accuracy were recorded in these experiments, when participants had to

rate the intensity of pain assigned to the situation displayed in the presented image. Participants are generally instructed to reply as quickly as possible. To rate the level of pain different experiments generally use a point visual scale demanding participants to rate the intensity of pain for each image ranging from “no pain” to “worst pain possible”. For instance, Jackson *et al.*, (2006) demanded to the participants to adopt different perspectives looking at visual stimuli (“Self”, i.e., the subject’s own perspective; “Other”, i.e., a specific unfamiliar person perspective; “Artificial”, i.e., a plastic limb perspective) and rate their pain as fast as possible. Results showed participants differentiated the artificial from both the two human perspectives (“Self” and “Other”). Moreover, they rated the images as significantly more painful for the self-perspective compared to the other one. In addition, RTs were significantly faster when participants adopted the “Self” perspective. In another study, a difference has been reported between the “Other unfamiliar” and the “Other loved”. In fact, Cheng *et al.*, (2010) reported higher ratings of pain intensity and unpleasantness for the “Self” and “Other loved” perspectives compared to the “Other unfamiliar” perspective. These examples of studies evidenced for instance the influence of perspective on rating the pain caused by an action represented in a picture. Moreover, RTs are also influenced. This technique is used in the first empirical study conducted in this thesis described in section 1 of Chapter 3.

3.3. Approach-Avoidance Task (AAT)

The Approach-Avoidance Task (AAT) is an implicit task. This technique permits to evaluate automatic responses and controlled responses (Krieglmeyer & Deutsch, 2010). The AAT requires participants to have either approach or avoid stimuli presented on a computer screen. There are three possibilities to implement this paradigm. The first one is called stimulus-response compatibility task (SRC) which required participants to touch a key on a computer keyboard for moving a manikin on the computer screen towards or away an image (De Houwer *et al.*, 2001). The second one is the joystick version that record the participant’s choice reaction time in pulling a lever (i.e. arm flexion = approach) or pushing a lever (i.e. arm extension = avoidance) through the stimuli (Rinck & Becker, 2007). The third type is Selective Attention / Action Tendency Task (SA/ATT) consists in reaching and touching stimuli on a touch screen and to measure the velocity of reaching or avoiding the stimuli (Sharbanee *et al.*, 2013). The

main measured outcome consists in recording reaction times (RTs) in both compatible (approach positive stimuli and avoiding negative stimuli) and incompatible conditions (approach negative stimuli and avoiding positive stimuli). RTs tend to be longer in incompatible conditions and shorter in compatible conditions.

The AAT task, is a useful technique to study approach / avoidance tendency, but especially with the SCR task it has a limited ecological validity because the instructions tasks concerning the arm movements may be inverted (i.e. arm flexion = avoid) or pushing a lever (i.e. arm extension = approach). Indeed, it is also possible to reverse these movements by giving the opposite instruction to the subject. This means to extend the arm when reaching for a desirable object and to flex the arm when withdrawing from an aversive stimulus (Eder and Rothermund, 2008; Van Dantzig *et al.*, 2008). This AAT technique has not been used in the experiments of the present thesis.

3.4. Neuroimaging and electroencephalography

Neuroimaging technique includes different structural and functional imaging techniques permitting to study which brain regions are more involved during the execution of a task and most neuroimaging technique includes functional magnetic resonance imaging (fMRI), magnetoencephalography (MEG) and positron emission tomography (PET). The use of these techniques permitted to highlight the neural correlates of approach / avoidance and freezing behaviors (e.g. Taylor *et al.*, 2004). These techniques are also useful to study the regions involved during empathy for pain tasks (Cheng, Yang, *et al.*, 2008; Lamm *et al.*, 2011). The electroencephalography (EEG) and event-related potentials (ERPs) are non-invasive techniques with a good temporal resolution. Tullett *et al.*, (2012) for instance, recorded EEG to study the right frontal cortical asymmetry in withdrawal behavior and empathy.

3.5. Psychophysiological measures

The modifications of nervous system activity during appetitive / aversive behaviors are distinguished by a series of physiological markers (Blair *et al.*, 2004). The rapid and automatic

evaluation of a stimulus prepares the organism to execute adapted behavior. A possibility to study these changes during the action preparation is to use psychophysiology which is a technique to measure peripheral manifestations of the emotional states linked to the autonomous and somatic systems activation in a non-invasive manner (Lang and Bradley, 2008). The most used electrical autonomic measures are the muscular activity (recorded with electromyography, EMG), the cardiac activity (measured with the electrocardiogram, ECG and heart rate, HR), the electrodermal activity (recorded with the skin conductance response, SCR) and oculometry. The principal disadvantage to record these markers is that they give only indirect information about brain activity (Sequeira *et al.*, 2009). These indexes characteristics and their techniques of registration are described in the next section.

3.5.1. The muscular activity and the electromyography

The variation of muscular activity is a peripheral index of the mental and behavioral activity and can be measured by the Electromyogram (EMG) (Lang *et al.*, 1998). This technique may be used to examine the mimicking of other's expressions. A previous study (Lundquist & Dimberg, 1995) has shown that concordant facial muscular contractions both in the model's and in the witness facial expression (zygomaticus major is mainly contracted in the observer during the observation of smiling faces and the corrugator supercilli is mainly contracted during the observation of angry faces). In order to study approach / avoidance and freezing behaviors, it might be useful to measure the activity of two antagonist muscles - the soleus and tibialis anterior. The main activity of the soleus is a plantar flexion of the foot (Elder *et al.*, 2003), increasing the angle between the foot and the leg. In the studies about the human natural standing, it has been evidenced the continuous activity of the calf muscles, especially of the soleus, pulling the body backwards against gravity (Portnoy & Morin, 1956). Soleus muscle activity gives important information about body proprioception during the natural standing and its contraction signal changes in body position (Di Giulio *et al.*, 2009). The tibialis anterior is antagonist to the activity of the soleus muscle. Tibialis muscle is mainly involved in the dorsiflexion of the foot (Elder *et al.*, 2003). The activity of these two muscles might be monitored by recording the electromyographic (EMG) signal, applying surface or intramuscular electrodes on the muscle belly.

3.5.2. The cardiac activity and the electrocardiogram

The cardiac activity is measured by an electrocardiograph, which produces a graph of the heart electric activity called electrocardiogram (ECG). The measure of cardiovascular reactivity corresponds to the responsiveness of the cardiovascular system to react to stress and it has been used for a long time as an indicator of motivation intensity (Brehm and Self, 1989), task engagement (Maier *et al.*, 2003) and attention (Ruiz-Padial *et al.*, 2011). Moreover, cardiovascular reactivity might signal anxiety (Friedman 2007), defensive reaction (Lang *et al.*, 1997) but also rewarding responses (Fowles, 1980). Concerning defensive reactions and the cardiac activity, Lang *et al.* (Lang & Bradley 2008; Lang *et al.*, 1998) reported that in animal studies when an aversive stimulus is presented, an augmentation of heart rate (HR) is recorded, when the animal has the possibility to escape in order to prepare the avoidance behavior. Otherwise, a reduced HR, called fear bradycardia is recorded when the animal freezes. HR is the number of beats per minute (bpm). Cardiovascular activity is regulated by the sympathetic and parasympathetic antagonist actions. From this point of view, an increase of HR may due to a reduction of parasympathetic activity and an increase of sympathetic activity (or to a co-activation of sympathetic and parasympathetic systems; Friedman, 2007). Cardiovascular activity is also involved during motor and postural control. For example, Azevedo *et al.* (2005) studied the effect that images of mutilation have on postural control and on heart rate and found a significant reduction in heart rate and body sway during the exposure to unpleasant pictures.

3.5.3. Electrodermal activity (EDA)

The electrodermal activity (EDA) is a measure of the electrical property of human body skin surface. Skin resistance changes as a function of sweat glands activity and this is controlled by the sympathetic nervous system. For example, a painful stimulus elicits a sympathetic response and an augmentation of electrodermal activity (a reduction of the electrical resistance of the skin) (e.g. Dube *et al.*, 2009). The EDA is used in psychophysiology to quantify the activation level of emotional stimuli (Sequeira *et al.*, 2009). The skin conductance method is the technique mainly used to record the EDA (Fowles *et al.*, 1981): a constant current flow (0.5 volt) is applied across the electrodes and the signal is amplified.

3.5.4. Oculometry and eye-tracking

The emotional valence of stimuli and their arousal may influence the size of the pupil diameter (Prehn *et al.*, 2011) and the sight direction in the observer (Price, 2000). The eye-tracking and the pupilometer permit to register the viewing time, the pupil size variation and the sight direction. For example, participants tend to watch affective (pleasant and unpleasant) pictures longer than neutral ones (Hamm *et al.*, 1997). For instance Chiesa *et al.* (2015), studying the subliminal empathic processing and found a significant regression analysis between pupil dilatation and empathic trait. They suggest the empathic trait might predict the pupil dilatation during the presentation of painful / pleasant stimuli.

3.6. Posturography

3.6.1. Step initiation

Another method for studying approach / avoidance, freezing behaviors is the step initiation task. This is applied to measure approach / avoidance movements of the whole-body toward a desired stimulus or away from an aversive stimulus. This behavior is typically applied in everyday life, for this reason, Koch *et al.* (2009) proposed for the study of step initiation an ecological method to study approach and avoidance behaviors. Measures recorded during the step initiation task should include reaction times (e.g., in Naugle *et al.* (2011) is calculated from the offset of the stimulus to the initiation of the motor response), postural parameters (displacement of the body's center of pressure (COP) in anteroposterior (AP) and mediolateral (ML) directions), step length and its velocity. The main lack of this technique is that the subject has still to execute an order, make a step forward or backward as soon as possible. Moreover, this technique should take in account which leg each subject prefers to make the first step.

3.6.2. Postural control

Standing seems to be a natural behavior and appears to occur without much effort for the majority of adults. However, maintaining a stable and upright position is a complex task that involves the activity of interacting sub-systems: central, motor and sensorial systems. The sensorial information comes from different sources: vestibular apparatus, vision and

proprioception. In fact, several studies have showed that stimulating the proprioceptive (Jeka *et al.* 1997; Johansson *et al.* 1988; Kavounoudias *et al.* 1999), the vestibular (Day *et al.* 1997; Johansson *et al.* 1995; Forbes *et al.*, 2015) and the visual systems (Dijkstra *et al.* 1994; Lestienne *et al.* 1977; van Asten *et al.* 1988) induce changes in postural control. The proprioceptive system detects information about the condition of the muscular system (its applied force and the orientation of body segments) and about the external environment (temperature, contact surface and external stimuli). The visual system gives us information about the external environment and the body orientation. The vestibular system gives us information about the body's acceleration in space and about its orientation to the gravitational field. The brain processes the sensorial signals to select and program the appropriate motor responses (Winter *et al.*, 1990). Processing this information allows the human balance to be kept in an upright position. Moreover, to maintain a stable postural control is necessary to adapt motor responses to the task and the environment (Van der Kooij *et al.*, 1999). Woollacott and Shumway-Cook (2002) define postural control as the control of a body segment in space that guarantees the balance and the orientation of the body. Several factors disturb postural balance. For example the morphologic and biomechanics characteristics affect balance postural control. Postural control uses a lot of resources because the majority of body mass is situated at two thirds of body height above the ground (Winter, 1995). Maintaining a stable posture is essential for the human being. Winter *et al.*, (1990) report that maintaining a stable posture is a very exigent task, it is more exigent with rapport to the human body structure.

The study of postural control, compared to the study of step initiation, appears to enable a broader analysis of stiffening, freezing and withdrawal behaviors. It can be used to monitor an overall global motor response in an upright stance and can quantify a variety of parameters describing the natural postural body sway. Postural control is mainly analyzed using a posturographic platform (Figure 9). This tool is operated by three posturographic sensors displayed at three different coins forming a triangle. These posturographic sensors transduce and compute the three-dimensional components of single equal force (sum of the three forces collected from the three captors) acting between the participant's feet and the platform surface, whose name is center of pressure (COP). According to the distribution of the force collected from the three sensors, it is possible to records the displacement of the body's COP in the anteroposterior (AP) and mediolateral (ML) directions. A decrease in COP excursions in the AP and ML directions associated with bradycardia evidences a freezing behavior whereas a backward AP shift of the COP reflects avoidance-withdrawal behavior. More specifically

different measures are usually collected in order to detect COP variation: length of sway path (i.e., the total displacement of sway), standard deviation (SD[COP]; i.e., dispersion of COP displacement from the mean position during a time interval), Root Mean Square (RMS; i.e., when the COP signal has zero mean, RMS and standard deviation provide the same result), Amplitude of COP displacement (i.e., Distance between the maximum and minimum COP displacement for AP and ML directions), Mean velocity (MV; i.e., to determine how fast were the CP displacements), COP's Area (i.e., area of displacement of the COP) and COP's mean position (i.e., the COP's mean position along the AP and ML axes).

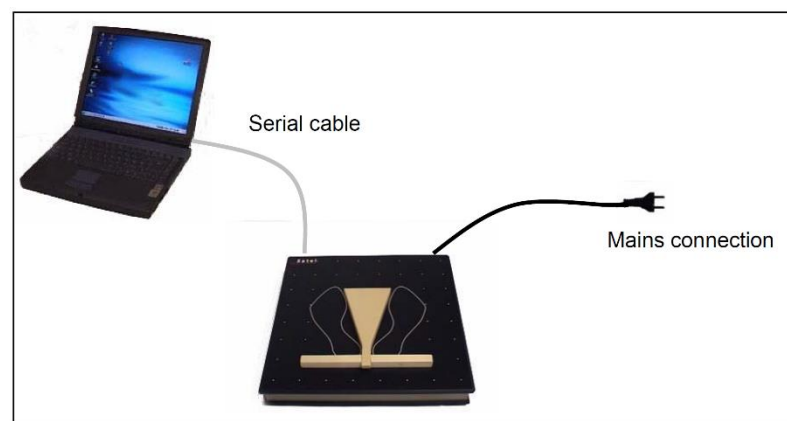


Figure 9. Posturographic platform setting. The posturographic platform is connected to a PC to quantify the COP's movements (modified from Satel User's manual).

Studies using static posturography and electrophysiology during the presentation of emotional stimuli and for recording approach / avoidance and freezing behaviors

In this section, different studies that used static posturography and electrophysiology methods associated to the presentation of affective stimuli and postural threat conditions are discussed. The main objective of these studies was to investigate approach / avoidance and freezing behaviors. Defensive reaction, i.e., avoidance or freezing behaviors, were the most adapted behaviors. The presented techniques were used in the present thesis.

Hillman *et al.*, (2004) were the first researchers to study changes in posturographic parameters upon exposure to affective pictures (pleasant/appetitive, neutral and unpleasant/aversive). They notably analyzed gender differences: compared with males, females displayed a greater rearward AP displacement of the COP when presented with unpleasant IAPS

images (relative to the presentation of pleasant and neutral images). Hence, in this study, females appeared to be more sensitive to negative arousing stimuli (for a review, see Bradley *et al.*, 2001). After this first study which combined the presentation of affective pictures and the registration of postural and physiological parameters in order to monitor approach / avoidance and freezing behaviors, many others studies were subsequently performed with similar modalities. For example, Azevedo *et al.* (2005) evidenced a freezing behavior, a decreased body sway (reduction in COP's area) and bradycardia when subjects were exposed to threatening visual stimuli compared to pleasant and neutral stimuli. In another study, Facchinetti *et al.* (2006) did two different comparisons, the presentation of mutilation body images versus neutral individuals and affiliative pleasant images (e.g. smiling babies) versus neutral individuals during the registration of postural and physiological parameters. The authors recorded the diminution of medio-lateral (ML) COP (i.e., a freezing behavior) and HR deceleration during the presentation of mutilation body images compared to neutral body. Moreover, a freezing behavior (i.e., significant reduction of AP COP) was also recorded for the presentation of affiliative pleasant images compared to the neutral ones. These results evidenced that not only unpleasant events may cause freezing reactions but also the authors suggested that immobility plays an essential role for settle social relations (Facchinetti *et al.*, 2006; Porges, 2003). Along these lines, Mouras *et al.* (2015) recorded a lowered value of SD [COP] in AP and ML direction (i.e. freezing behavior) for the presentation of sexual video stimuli compared to humorous and neutral videos.

Another study showed that negative life events might influence the HR (bradycardia) and body sway amplitude (reduced AP and ML COP displacements, i.e. a freezing reaction) product in response to IAPS presentation (Hagenaars *et al.*, 2012). A further study analyzed social threats as a special, freezing-inducing stimulus. Roelofs *et al.* (Roelofs *et al.*, 2010) found significant reductions in body sway and in HR. Moreover, these reductions were significantly negatively correlated with the subjective level of anxiety (measured according to the State-Trait Anxiety Inventory (STAI; Spielberger *et al.*, 1983). This study confirmed that anxiety has a role in the balance control (e.g., Bolmont *et al.*, 2002) and this seems to depend on visual information processing (Ohno *et al.*, 2004).

Images describing painful situations (validated stimuli from Jackson *et al.*, 2005) that are normally used in empathy for pain paradigms have also been used to study postural sway perturbations. Lelard *et al.* (2013) observed small anterior-posterior displacement of the COP whenever participants had to imagine they were performing a painful action similar to those

presented on the screen in front of them (Figure 10 A). Moreover, in this research electromyogram of the tibialis anterior (TA) muscle was measured and it has been found an increase in muscle contraction, reflecting the stiffening strategy adopted by subjects and confirming the presence of freezing behavior. Recently, Hagedaars *et al.*, (2014) showed that only unpleasant films (nor the neutral, neither the pleasant films) were associated with reductions in both HR and body sway, indicating the presence of freezing behavior.

Another way to induce freezing / avoidance behavior is to place subjects in a postural threat condition, so that they truly experience fear of falling (Figure 10 B). A recent study combined the presentation of visual emotional stimuli from the IAPS with a postural threat condition (these results are reported in the appendix of the present thesis, Lelard *et al.*, 2014). The results showed a greater withdrawal along AP axe for aversive stimuli both in presence and in absence of postural threat. A time-course analysis revealed that postural differences during the presentation of aversive stimuli (relative to natural stimuli) appeared 3 seconds after the stimulus onset. This is in agreement with animal studies (i.e. freezing that lasts for at least 3 seconds (Kalin *et al.*, 1998; Rogers *et al.*, 2008) and the above-mentioned study in humans by Hagedaars *et al.*, (2014).

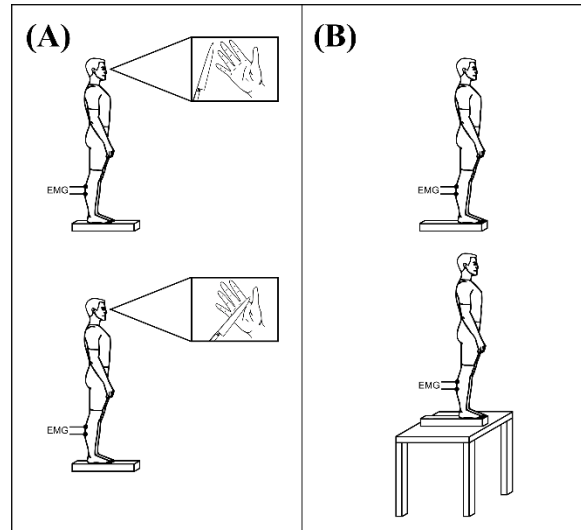


Figure 10. Postural control paradigms and EMG recording: (A) Visual stimuli observation during a postural control; (B) Postural threat paradigm (on the table) and control condition (on the ground).

Postural control and physiological measures permit to study the human natural behavior

faced to affective visual stimuli and in postural threat conditions. Unpleasant stimuli mainly elicit freezing responses or withdrawal behaviors, compared to neutral and pleasant visual stimuli. When pleasant stimuli are compared to neutral ones (Facchinetti *et al.*, 2006), a freezing behavior is also recorded during the exposure of pleasant stimuli. Moreover when two different kinds of pleasant stimuli are compared, like sexual and humorous stimuli (Mouras *et al.*, 2015), the highly arousal stimuli (i.e., sexual stimuli) induce freezing behaviors in the observer.

Postural control and physiological recordings are the techniques used in the study described in Chapter 3 section 2.

3.7. Transcranial magnetic stimulation (TMS) and motor-evoked potentials (MEPs): tools to measure the corticospinal excitability

Transcranial magnetic stimulation (TMS) is a non-invasive brain magnetic neurostimulation and neuromodulation technique based on the Faraday principle of electromagnetic induction. The magnetic field pulse range between 1 and 2 Tesla (T). TMS technique requires the use of a coil and its most common shape is a figure-of-eight coil, which permits a more focal stimulation in a region of 3cm long by, 2cm wide and at a depth of 1,5 – 3cm under the scalp (Rossi *et al.*, 2009). The pulse induced by the electric generator reaches the brain after 200ms and lasts 1ms. Different protocols of magnetic stimulation exist. For instance, the combined use of electromyography (EMG) and single-pulse TMS (spTMS) applied over the primary motor cortex (M1) representation of a muscle permits to generate a twitch called Motor Evoked Potential (MEP), in that muscle (Figure 11). spTMS has a good temporal and spatial resolution, the pulse is administered one per time and the temporal interval between each pulse is at least 3 seconds and the effect lasts 200ms (Hallett, 2000).

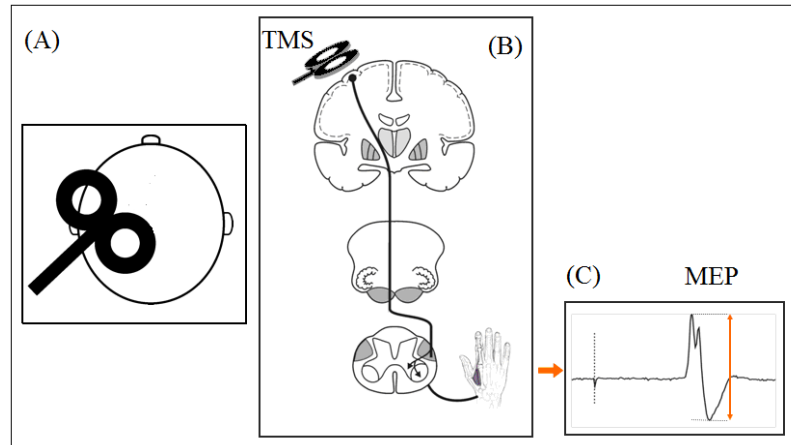


Figure 11. Schematic representation of TMS stimulation and MEPs recording. (A) TMS coil is placed over the left primary motor cortex (M1) in the area of hand's representation; (B) The electric signal generated by the magnetic field induces a depolarization along the corticospinal neuron and triggers an action potential. (C) Then a twitch called MEP is generated in the contralateral hand muscle; this activity may be recorded using EMG (modified from Rizzo et al., 2003).

The primary motor cortex (M1) is responsible for generation of the last impulse that controls the execution of a movement (Figure 12 A). It is located in the caudal portion of the frontal lobe along the precentral gyrus. These signals descend to the spinal cord and have a synapse with the spinal α motoneurons (Stinear *et al.*, 2009). This monosynaptic pathway represents the corticospinal (CS) tract. In the M1, a precise somatotopic organization of the body parts is present and specifically the representations of the digits are widely distributed in M1 (Schieber, 1999; 2001) (Figure 12 C). The lateral corticospinal path controls voluntary movements of the contralateral body part (Figure 12 B). For this reason, in order to record MEPs from the right hand muscles, the coil is placed over the left M1. When spTMS is applied tangentially to the scalp, it causes a depolarization of the CS neuron and triggers an action potential at the end of the spinal axon. Finally, this induced muscle activity is recorded with EMG and represents the MEP. The excitability of the CS tract is often evaluated recording MEPs amplitude variation.

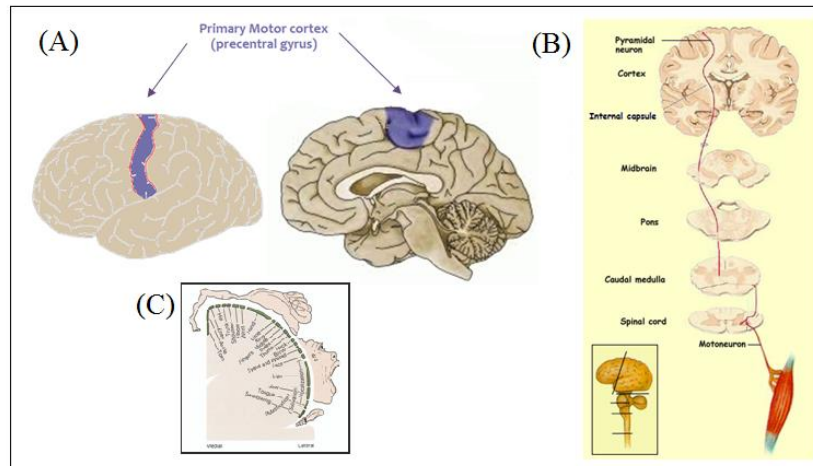


Figure 12. Motor system. (A) The primary motor cortex (M1) lies in the frontal lobe, along the precentral gyrus. (B) The corticospinal tract consists in the long axons of the pyramidal neurons; their nuclei are located in M1. (C) Homunculus representing the somatotopic organizations of the body parts.

3.7.1. Pain and motor system: spTMS and EMG recording during painful stimulation

Concerning the pain physiology, there are separate types of peripheral afferent fibers transmitting messages about the painful stimulus nature. These fibers include the A δ fibers that are sensitive to temperature variation and mechanical stimuli. The A δ are subdivided in type I (fast adapting) and type II (slow adapting). Then, the amielinic C-fibers are mainly activated by mechano-thermics stimuli. These ascending pain C-fibers reach the dorsal horn of the spinal cord in the substantia gelatinosa (SG) from the periphery (Figure 13). Here a spinal gate mechanism may act (Melzack & Wall, 1967): small fibers inhibit SG, open the gate allowing the transmission of the cells, instead large fibers activate SG closing the gate and inhibiting the transmission cells. The gate may also be closed by a central brain control: periaqueductal gray area (PAG) projects descending axons to the median raphe nuclei and from here axons are projected to the spinal cord, inhibiting the nociceptor neurons activity.

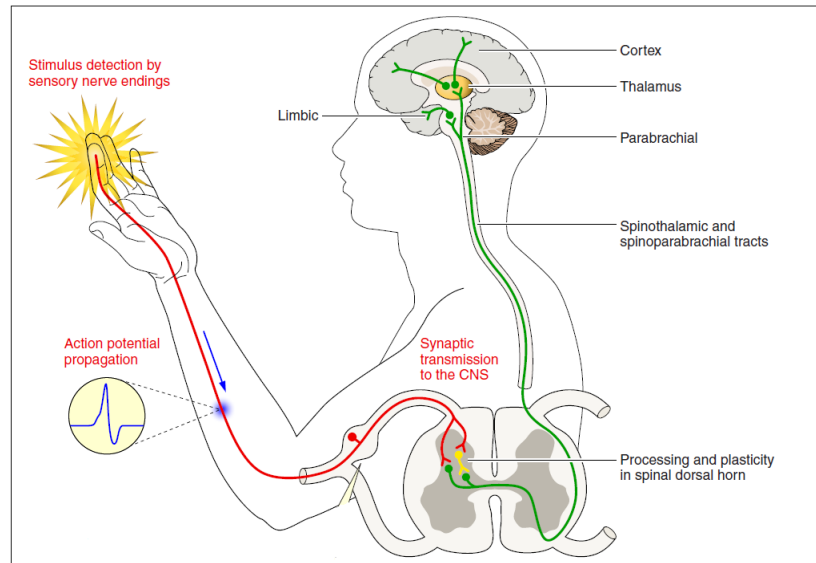


Figure 13. Pain ascending pathways. Noxious stimuli are detected peripherally by nociceptors whose soma is located in the dorsal horn ganglia and in the trigeminal ganglia. These neurons: i) project to the peripheral receptive fields; ii) allow the propagation of actions potentials along the axons directed to the central nervous system (modified from Bourinet *et al.*, 2014).

Pain is closely linked to the motor system activity (Sailer, *et al.*, 2004). For instance, when a hand touch hot object, the heat-pain activates a reflex of withdrawal response (Dworkin, 2007). This adaptive unconscious behavior prevents the hand to further damage, facilitating escaping. Moreover, the magnetic and electric stimulations of the primary motor cortex (M1) help in fight against the adverse effects of pain, especially when other therapies are ineffective (Crucchi *et al.*, 2007). This suggests that motor system might influence somatosensory sensations (Peyron *et al.*, 2000). spTMS and MEPs recording permit to highlight the inhibition of CS motor system excitability due to different kind of painful stimulation (Farina *et al.*, 2001; Farina *et al.*, 2003; Le Pera *et al.*, 2001; Svensson *et al.*, 2003; Urban *et al.*, 2004). These techniques are described in the following lines. Farina *et al.* (2001) were about the first to study the effect of tonic cutaneous pain on CS system. They applied capsaicin on the skin overlying the right first dorsal interosseous (FDI) and flexor carpi radialis (FCR) muscles; the authors stimulated with the spTMS the left motor cortex and recorded reduced MEPs amplitude in these two muscles. This is a fine-grained pain-specific corticospinal inhibition: MEPs were specifically inhibited in the two muscles that underwent painful cutaneous stimulation. The authors attributed this corticospinal inhibition to the activation of C-fibers, mediating slow nociception. Along these lines, stimulating with spTMS the left M1, Le Pera *et al.* (2001) recorded a pain-specific MEPs inhibition in the right abductor digiti minimi (ADM) and FDI

muscles, after having injected a hypertonic saline solution in these muscles. Urban *et al.* (2004) obtained a similar effect: electrically stimulating the right V finger and applying spTMS over the left M1 they recorded a significant MEPs inhibition in the abductor pollicis brevis (APB) and in ADM. Moreover, the authors found that proximal upper limb muscles were less involved in corticospinal inhibition, suggesting that this response pattern corresponds to the withdrawal reflex. In conclusion, it seems the corticospinal excitability during painful peripheral stimulation to reflect a freezing reaction (i.e., inhibition of the corticospinal system) in muscles located in the region near the painful stimulation and a withdrawal behavior (i.e., moderate inhibition and delayed facilitation of corticospinal excitability) in more distal muscles.

3.7.2. spTMS and MEPs to investigate the corticospinal system during empathy for pain

As reported in the previous section, spTMS combined with the MEPs registration is a valuable tool to measure the corticospinal excitability during the first-hand sensation of pain (Farina *et al.*, 2003; Urban *et al.*, 2004). Moreover, in the last decade, it has been found that the mere observation of painful stimuli delivered to a model's body produced a fine-grained modulation of the observer's CS system (e.g., Avenanti *et al.*, 2005) as it was happened during a painful stimulation. Avenanti *et al.* (2005) were the first to discover this effect and they assigned the freezing effect recorded in the observer to an empathy for pain reaction. In their study, compared to the observation of a Q-tip touching the right FDI muscle, to watch a needle penetrating the model's right hand FDI muscle induced in the observer a fine-grained MEPs amplitude reduction in the same right hand FDI muscle like when the hand is really penetrated by a needle. This CS inhibition is specifically recorded in the onlooker's muscle involved in the observed painful stimulation (i.e., the right FDI). The following experiments further support these findings. For instance, according to Avenanti *et al.*, (2006), the intensity of the painful stimulation is also important. The authors, using the same technique, recorded a significant MEPs amplitude reduction specifically when the observed right FDI muscle was deeply penetrated by a needle compared to the condition in which the observed person hand was only pinpricked. This result suggests that the painfulness of the observed stimulus is also elaborated at the corticospinal level. Moreover, the authors evidenced that to give different instructions to the participant had no effect in modulating the CS excitability. Indeed, to adopt first or third

person perspective or to make a passive observation did not influence the CS excitability; rather this seemed to be influenced only by the observation of the stimulus (Avenanti *et al.*, 2006). Interestingly, Avenanti *et al.* in 2009 (Avenanti, Minio-Paluello, Sforza & Aglioti, 2009) presented the model's left or right hand penetrated by a needle or touched by a Q-tip at the FDI muscle level. MEPs were recorded in FDI and thenar eminence (TE) of the left hand (TMS over the right M1) for half of the participants and MEPs were recorded in the same muscles of the right hand (TMS over the left M1) for the other half of the sample. Results evidenced a pain-specific MEPs amplitude reduction in FDI muscle during the observation of the same hand penetrated by a needle (e.g.: observation of the right hand and MEPs recorded from the right hand). Whereas, when the opposite hand is observed (e.g.: observation of the right hand and MEPs recorded from the left hand), a generalized increasing in CS excitability is recorded. The authors suggest that during the observation of painful stimuli, a freezing effect (i.e., CS inhibition) recorded in the same observed hand might be due to an embodied defensive reaction to pain in the onlooker, instead the CS facilitation recorded in the opposite hand might be due to another embodied defensive reaction: to escape or to help the other hand in removing the painful stimuli. In 2010, Avenanti *et al.* (2010) recorded a specific CS inhibition during the observation of both an in-group model's hand and an unfamiliar violet hand penetrated by a needle. In contrast, no reduction of MEPs amplitude was recorded during the observation of outgroup (e.g.: when a white subject observed a black hand). These studies examined the role of CS system during the observation of painful stimuli. Results suggested that the observer's CS system specifically recognizes the noxious stimulation in the observed hand and produces a freezing reaction in the same muscle involved in the observed model. This reaction is similar to the one recorded during first hand pain (Farina *et al.*, 2001; Le Pera *et al.*, 2001; Urban *et al.*, 2004) and it is influenced by different parameters like the race of the observed hand model (Avenanti *et al.*, 2010), the recorded muscle (Avenanti *et al.*, 2005), the observed limb (Avenanti *et al.*, 2005), the congruency with the observed hand (Avenanti, Minio-Paluello, Sforza & Aglioti, 2009) and the intensity of painful stimulation (Avenanti *et al.*, 2006).

In this introductory part of the present thesis, different theories, techniques and findings concerning empathy, emotion and motivation have been presented. In the following part the objective, methods and results of the present thesis will be illustrated. Finally, all the results will be discussed.

4. The present research

In the introduction presented earlier, there are exposed theories and recent studies about empathy, particularly empathy for pain and approach / avoidance and freezing behaviors. The main aim of this research project was to study approach / avoidance and freezing behavioral manifestations co-occurring both along with the observation of others pain and during the anticipation of pain. Another related topic, whose results will be reported in the appendix of this thesis, is the effect of emotional stimuli on approach / avoidance behavior. In this thesis, the reactions times, ratings and motor and postural variations as parameters to study approach / avoidance and freezing behaviors were measured.

For several years, as indicated in section 1.1.7 shared representations of others' individual pain were considered as the representation of empathic painful processes (for a review see Lamm *et al.*, 2011). Recent studies show the activation of affective motivational brain circuits during the observation of others pain is no more completely justified by the observers' empathic response; instead a more parsimonious explanation can better describe these activations. In fact, it is more likely that the activation of shared representations during the observation of others pain better reflects a mechanism of threat avoidance, supporting individual survival, instead of an empathic response to others pain (Fox *et al.*, 2013; Decety, 2015). These new interpretations induce my colleagues and I to investigate defensive (withdrawal and freezing) and approach behaviors in the context of empathy for pain and in pain anticipation.

Pain is a good model to study freezing and approach / avoidance behaviors because it can activate the defensive system both during a first-hand experience of pain and during the observation of painful events in another individual, signaling to others a dangerous situation (Decety, 2015; Avenanti *et al.*, 2005). This “unpleasant sensory and emotional experience associated with actual or potential tissue damage” (Merskey & Bogduk, 1994, p. 210), as defined by the International Association for the Study of Pain (IASP) has an evolutionary adaptive value like empathy and defensive behaviors have (chapter 1, sections 1.6.1. and 2.1.).

As described earlier, empathy for pain can be associated with self-directed withdrawal responses and other-oriented prosocial altruistic behaviors (Goubert *et al.*, 2005; Singer &

Klimecki, 2014). Moreover, several experimental studies highlighted the role of intrapersonal and interpersonal factors modulating empathy for pain, like gender and the perception to belong to a social group (section 1.5.). Indeed, in the first group of studies, the influence of the self / other perspective and the impact that different kinds of relationship representing the “other” have during an empathy for pain task have been analyzed. The impact of these factors has already been studied (Jackson *et al.*, 2005; 2006) but not clear yet how the differences in the perceived proximity to other individuals may modulate the rating of self / others pain in the witness and if this may be influenced by the observers gender. More specifically, in the first behavioral study (experiment 1; chapter 3, section 1), it was investigated the influence of perspective taking on the behavioral aspect of pain evaluation: pain ratings and reaction times were collected during the presentation of images depicting painful or non-painful common situations (Jackson *et al.*, 2005). Pain rating for each image has been expected to vary as a function of the perspective adopted by the participants. It has been suggested that the Self and Other-Most-Loved-Familiar (OMLF) perspectives would be associated with quicker reaction times and higher pain ratings than Other-Stranger (OS) and Other-Most-Hated-Familiar (OMHF) perspectives.

Reactions time and pain ratings can give an initial overview about the time course and intensity of rating of the empathy reaction to other’s familiar / unfamiliar pain, but it remains unclear yet the direction of this reaction. Indeed, this technique does not permit to evidence if in a context of empathy for pain, it is more probably to approach / avoid or freeze faced to other’s more or less familiar pain. In the second study (experiment 2; chapter 3, section 2), the postural parameters during the observation of painful and non-painful visual stimuli were analyzed. In addition to the role of proximity in different levels of perspective taking (Self, OMLF, OS, OMHF) was analyzed. Postural sway paradigm is not greatly influenced by the experimental instructions; for instance, the subject tends to adopt naturally a withdrawal position (avoidance) or to stay as still as possible (freezing-like behavior) during exposure to negative situations. It is expected to find differences in center of pressure in the averaged anterior / posterior position (COP-AP) among the perspectives (Self, OMLF, OS, OMHF) adopted by the participant when he is faced to images depicting painful or non-painful common situations. To our knowledge, studies using perspective-taking tasks and affective visual stimuli exposure in a postural control task have not still previously performed. According to the literature, the presentation of an aversive emotional stimulus may lead to a withdrawal (Hillman

et al., 2004; Lelard *et al.*, 2014) or a freezing behavior (Azevedo *et al.*, 2005; Facchinetti *et al.*, 2006; Hagensars *et al.*, 2012, Hagensars *et al.*, 2014;). Moreover, Lelard *et al.* (2013) found evidences about the registration of freezing behavior during the exposure to painful images. Along these lines, we hypnotize to record a withdrawal or a freezing behavior in the Self and in another perspective very relevant for the participants, i.e., the OMLF perspective.

Then the self / other perspective in empathy for pain has been analyzed in the corticospinal system (experiment 3; chapter 3, section 3). Previous studies recorded a fine-grained corticospinal inhibition (i.e., freezing effect) not only during first hand pain (Urban *et al.*, 2004), but also during the observation of the same limb penetrated by a needle presented in first-person perspective (e.g. Avenanti *et al.*, 2005; Avenanti *et al.*, 2006; Avenanti *et al.*, 2010). This effect was interpreted as the physiological basis of empathy. In recent years, the increasing interest for the concept of body-ownership (i.e., the belief that a specific body part belongs to one's own body) pays specific attention to the relation between the perspective through which a body-part is observed and the possibility for the subjects to experience it as part of their own body. In the present study, it was investigated if the empathic freezing effect recorded in the observer persists also during presentation of painful visual stimuli in a third person perspective, the one in which, in everyday life, we perceive the body parts of others (Ruby and Decety, 2001). In order to record the modulation of corticospinal excitability during the observation of painful events, transcranial magnetic stimulation (TMS) was applied over the left primary motor cortex and motor evoked potentials (MEPs) were recorded during the presentation of a hand penetrated by a needle in first and third person perspective. In this study it wasn't adopted a perspective taking paradigm (the subject did not delve into the Self, OMLF, OS, OMHF perspectives) because a previous study evidenced the inefficacy in giving different instructions on the modulation of corticospinal excitability (Avenanti *et al.*, 2006). Presenting the hand penetrated by a needle in the first and third person perspective, we aimed to define if the freezing response that occurs in the observer is not only recorded in the first-person perspective (i.e., the one in which embodiment is manifested and the perspective more relevant for the self) but also in third person perspective (i.e., the one more relevant for the interaction with others).

Finally, a study about the pain anticipation was conducted (experiment 4; chapter 3, section 4). Previous studies evidenced that the neural network activated during the observation of others pain and those circuits activated during the first hand experience of pain anticipation seems to be partially overlapped (Morrison *et al.*, 2004). Taking into account the new

interpretations about the shared representations of pain, the neural circuits activated during the observation of others pain might represent the neural basis of threat avoidance mechanism (Decety, 2015) and the activation of these circuits might co-occur with defending response for preventing dangerous events (Avenanti & Aglioti, 2006). Along these lines, a possible interpretation of empathy for pain is that it might represent the pain anticipation in oneself. For instance, Porro *et al.* (2003), have approached the study of pain expectancy using fMRI, but this technique did not permit to clarify the role of the primary motor cortex and of the corticospinal modulation during pain expectancy. Moreover, studies showed that painful stimuli induce both a strong enhancement of the skin conductance responses (SCR) and a fine-grained modulation of the corticospinal excitability, illustrating a significant inhibition of the motor evoked potential (MEP) recorded from the body district to which the stimuli were delivered (Urban *et al.*, 2004). Here, using a classical conditioning paradigm, it was investigated the modulation of corticospinal excitability, in particular the freezing effect registration during an electrical painful stimulus anticipation. It was hypothesized the mere anticipation of the stimulus delivering may induce a significant inhibition in the sensorimotor system, as it happens during the exposure to painful stimuli. The registration of the anticipatory response in the corticospinal system might represent the defensive preparation of the organism to the exposure to a dangerous event, likely during the observation of a painful event.

These last two studies were conducted in collaboration with the University of Turin during my internship in the SAMBA Laboratory. In chapter 4 all these results were discussed, giving new developments regarding empathy for pain.

Moreover, in a series of experiments, our research group has evaluated the effect of emotions on postural control. A common paradigm used to evoke automatic freezing-like and withdrawal responses to threats in controlled settings is the presentation of aversive pictures (e.g., Mogg *et al.*, 2004). These negative stimuli trigger spontaneous somatic indices of defensive activation (Lang *et al.*, 1997), and may induce freezing-like behaviors (e.g. Azevedo *et al.*, 2005). In the present study, my colleagues and I proposed to evaluate the effect of the presentation of images of mutilation with a postural threat condition on the postural control paradigm. We analyzed the influence of emotional stimuli on approach / avoidance behavior with a posturographic task. The results are reported in the appendix.

CHAPTER II
MATERIALS &
METHODS

1. Experimental subjects

The voluntary participants who took part in the following studies were students, coming from the Université de Picardie Jules Verne and from the University of Turin. The total number of participants included in the four experiments was 167 (82 F, 85M; mean age \pm s.d. = 23.53 \pm 4.74). The participants who took part in the following studies have been included in the experiments with the following different selection criteria:

- range of age: from 18 to 40 years
- gender: males and females;
- handedness: right (according to the Standard Handedness Inventory (Briggs and Nebes, 1975))
- visual acuity: normal or corrected – to – normal
- no history of motor impairment
- no prior or ongoing treatment for psychiatric or neurological disorders.

Moreover, concerning the experiment 3 (Bucchioni *et al.*, under review) and the experiment 4 in which the TMS technique was used, it was verified that each participant was free from any contraindication to TMS (Wasserman 1998; Rossi *et al.* 2009). In order to avoid eventual damages provoked by TMS to participants, the Screening questionnaire before TMS (Rossi *et al.*, 2011) was administered before the experimental session beginning. Participant were excluded to the TMS experiment if they:

- had epilepsy or previously had a convulsion or a seizure;
- had a fainting spell or syncope;
- had a head trauma associated with loss of consciousness;
- had any hearing problems;
- had cochlear implants;
- were pregnant;
- had metal in the brain, skull or elsewhere in their body;
- had an implanted neurostimulator;
- had a cardiac pacemaker;
- had a medication infusion device;

- were taking medications
- had previous problems in undergoing TMS or MRI in the past.

Moreover, different self-report measures have been administered in different studies of the present research. These includes the Beck Depression Inventory (BDI; Beck *et al.*, 1988; experiment 1), the State-Trait Anxiety Inventory (STAI; Spielberger *et al.*, 1983; experiment 1, 2, 4), the Basic Empathy Scale (BES; Jolliffe & Farrington, 2006; experiment 1, 2), the Interpersonal Reactivity Index (IRI; Davis, 1983; experiment 3), an ad hoc body-ownership questionnaire (experiment 3) and an ad-hoc measure of participant's expectancy (experiment 4). All participants gave their written informed consent prior to their inclusion on the experiment and they were naive to its purpose. Each experiment was conducted in a single experimental session, it was performed in accordance with the ethical standards of the Declaration of Helsinki and it was approved by the local investigational review board (For the Université de Picardie Jules Verne: Comité de Protection des Personnes Nord-Ouest II, Amiens, France; For the University of Turin: Ethics Committee of the University of Turin).

2. Stimuli, data collection, procedure and data analysis

In the present thesis, different types of stimuli, data collection, procedures and data analysis have been used. The description of all these different paradigms is reported below. The presentation of these stimuli was controlled and randomized by the E-prime software (version 2.0, Psychology Software Tools, Inc., Pittsburgh, PA) running on a PC.

2.1. Experiment 1

2.1.1. Stimuli

Visual stimuli used in experiments 1 and 2 were pictures depicting hands and feet in first-person or lateral perspective in a painful or a non-painful context (e.g.: the blade of a saw

placed over the hand that is holding the tree branch vs the blade of a saw over the tree branch); these pictures were chosen among those used by Jackson *et al.* (2005; 2006) in their researches (Figure 14). A total of thirty-six pictures were presented in experiment 1. The resolution of all the images was set at 500 x 375 pixels. For each participant, the pictures were presented in a random order for four times, one for each block (Self, Other-Most-Loved-Familiar (OMLF), Other-Stranger (OS) and Other-Most-Hated-Familiar (OMHF)) described below. The presentation of these stimuli was controlled and randomized by the E-prime software (version 2.0, Psychology Software Tools, Inc., Pittsburgh, PA) running on a PC.

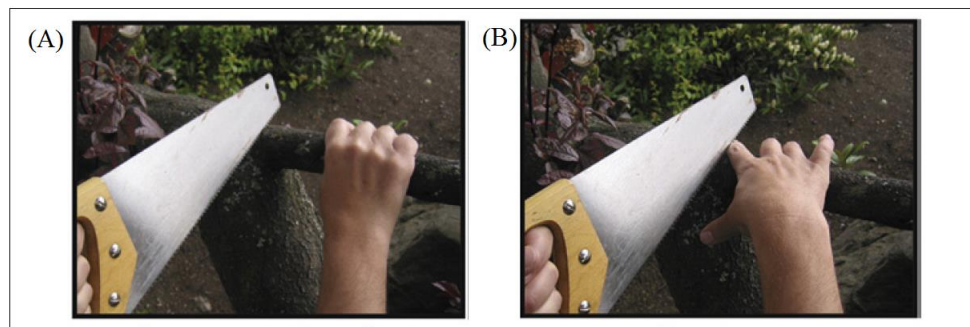


Figure 14. examples of stimuli: (A) non-painful situation (control), (B) painful situation (Modified From Cheng, Yang, *et al.*, 2008)

2.1.2. Data collection and Procedure

Before beginning the experimental session, participants were asked to filled out a series of standardized questionnaires including the French version of the Beck Depression Inventory (BDI), the STAI (Spielberger *et al.*, 2010), the French version of the Basic Empathy Scale (BES; Joliffe & Farrington, 2006; D'Ambrosio *et al.*, 2009). This last questionnaire is composed of 20-items (9 of them for the cognitive empathy component assessment; 11 of them for the affective empathy component assessment). In order to exclude participants with anxiety or depressive problems BDI and STAI questionnaires were used. BES was administered in order to verify the level of participant's empathy felt in the different conditions.

The experimental session was divided into four blocks and in each block participants have to adopt one of the four perspectives: Self, Other-Most-Loved-Familiar (OMLF), Other-Stranger (OS) and Other-Most-Hated-Familiar (OMHF). "Most loved" (OMLF) and "most hated"(OMHF) persons were divided in four categories: (i) parents and other relatives, (ii) friends and acquaintances, (iii) partners and (iv) teachers and bosses. At the

beginning of the experiment on the PC screen each participant might read: “Dear participant, you are going to see a series of images presented on the computer screen. We would like you to rate the intensity of pain that you (in the SELF block) / the most loved familiar person (in the OMLF block) / the most hated familiar person (in the OMHF block) / someone you don’t know (in the OS block) would experience in the displayed situation; please indicate your rating by selecting a number between 0 (no pain) and 9 (worst possible pain) on the keyboard using your right hand”.

The four conditions were counterbalanced across the set of the presented pictures. Participants were naïve about the final goal of the experiment. The order of presentation of the four blocks (Self, OMLF, OMHF and OS) was counterbalanced. Task consists in replying as fast as possible after the picture onset and at the same time rating the pain of the action represented in the picture by means of 10 points Likert-type scale between zero (no pain) and nine (worst pain imaginable). The beginning of each block consisted with the presentation of a fixation cross for 500 ms. One of the thirty-six pictures was presented until the participant responded. Each stimulus was presented during 1000 ms.

2.1.3. Data analysis

Two separate repeated-measures analyses of variance (ANOVAs) were performed on pain ratings and RTs, with gender as a between-subjects factor. Another repeated-measures ANOVA was then performed on pain rating data, with perspective (Self, OMLF, OMHF, OS) and stimulus value (painful, not painful) as within-subject factors and gender as a between-subjects factor. As the RT values were not normally distributed, they were log-transformed prior to the ANOVA. Paired-sample t-tests with a Bonferroni correction were used to compare the pain ratings for painful stimuli. Pearson’s correlation coefficients were also calculated for the relationship between the participants’ pain ratings for painful images and their level of empathy on the BES. The limit for statistical significance was $p < 0.05$ for all statistical analyses.

2.2. Experiment 2

2.2.1. Stimuli

Visual stimuli were chosen among Jackson *et al.* (2005; 2006) pictures (Figure 14) and were similar to those used in experiment 1. Forty-eight colored pictures depicting hands or feet in first-person or lateral perspective, in painful and non-painful situations were presented to the subjects. More specifically, twenty-four pictures represented painful situations and the other twenty-four represented the paired non-painful situations. E-prime 2.0 running on a PC did the stimuli presentation.

2.2.2. Data collection

Data collection includes posturographic and physiological measures.

2.2.2.1. Posturography

The setting conditions were defined by the French society of posturography (Normes AFP 85). Participants were instructed to stand comfortably upright on a posturographic platform (Satel, Blagnac, France) in a bipedal stance. Their fingers feet were positioned on the edge of the platform; a gap of 3 cm was displayed between the two heels and the finger feet pointed 30° outward. Participants' arms were left alongside the trunk and their task consisted in standing on the posturographic platform as immobile as possible in a bipedal stance: no voluntary movements of head, arms and legs were allowed. The Satel posturographic platform used in this experiment uses constant moment beam type sensors (approved by the French State Weights and Measures Dept.) allowing to compute the center of pressure (COP) parameters (anteroposterior (AP) and mediolateral (ML) directions). The analog values collected by three sensors located in the posturographic platform were sent to the Biopac MP150 (Biopac Systems, Inc., Santa Barbara, CA) that amplified the COP signal. The Acqknowledge software (Biopac Systems, Inc., Santa Barbara, CA) running on a PC in the data collection room, allows the digital signal COP computation. Data were stored on a PC for off-line analysis.

2.2.2.2. Physiological Measures

2.2.2.2.1. *Electromyogram (EMG) activity*

Electromyographic activity (EMG) of the tibialis and soleus muscles was recorded during posturography from the right dominant leg using bipolar Ag/AgCl surface electrodes (EL503, 8mm diameter, BiopacInc., Goleta, CA, USA) placed 2 cm apart center-to-center longitudinally. In order to maintain the inter-electrode impedance under $5k\Omega$, before the electrode positioning, the skin was abraded and cleaned with an alcohol solution. In order to guarantee a good electrical contact (no air between the skin and the electrode), an electrolyte gel was applied between the skin and the electrodes. For the soleus, electrodes were placed 2cm under the insertion of the gastrocnemii on the Achilles tendon. The other bipolar montage was applied on the tibialis muscle; the electrodes were placed over the belly of the muscle. Root mean square (RMS) of raw data over 500 ms with a sliding time window was calculated in order to quantify the muscle activation. The level of activation of soleus (SO) and tibialis muscles (TA) was represented by the RMS-SO and RMS-TA and it was expressed as a percentage of the maximal voluntary contraction (MVC).

2.2.2.2.2. *Heart rate (HR)*

Heart rate (HR), expressed in bpm, was recorded using a standard Lead II electrocardiogram. Three disposables electrodes (EL503, Biopac Inc., Goleta, CA, USA) have been placed upon the right and the left internal ankles and on the right internal wrist in order to recreate the Einthoven's triangle.

2.2.2.2.3. *Electrodermal activity (EDA)*

Electrodermal activity (EDA) was recorded with two Ag/AgCl surface electrodes, 6 mm diameter filled with isotonic paste and attached to the volar surface of the index and middle fingers of the participant's left hand. The electrodes were connected to an amplifier (GSR100C, Biopac Inc., Goleta, CA, USA). A constant-voltage device was used to apply 0.5 V across the electrodes, permitting to calculate the electric skin conductance in μ Siemens. The signal has been filtered online with a band-stop 50Hz filter. The data have been collected and stored on a PC to be analyzed later.

2.2.3. Procedure

Before starting the experimental session, participants filled a series of standardized questionnaires including the STAI (Spielberger *et al.*, 2010) and the French version of the Basic Empathy Scale (BES; Joliffe & Farrington, 2006; D'Ambrosio *et al.*, 2009) composed of 20-items (9 of them for the cognitive empathy component assessment; 11 of them for the affective empathy component assessment). This last questionnaire was administered in order to verify the level of participant's empathy felt in the different conditions. The STAI questionnaire was administered to exclude participants with anxiety problems. In fact, approach / avoidance behaviors may be influenced by anxiety, a threat-related emotion, which is characterized by sustained, abnormally high levels of muscle tension (Lang *et al.*, 2000).

2.2.3.1. Postural and physiological experimental session

Participants were tested in a dimly and quiet illuminated experimental room and the posturographic platform was positioned 2 m away from the wall where pictures stimuli were displayed using a video-projector. This was positioned in the experimental room and connected to the PC located in the data collection room where E-prime 2.0 randomized the presentation of the visual stimuli. In order to minimize the effects of the mains electricity on the registration of the physiological measures (i.e., limitation of the artifacts) all the electric wires were isolated and the disposition of electrical device in the experimental room was limited to the posturographic platform, the Biopac system and the video-projector. The experimental session was divided in four blocks each of them consisting in one of the four perspective-taking conditions. Participants in fact were instructed to imagine that: (1) themselves were experiencing the situation represented in the visual stimuli (Self condition); (2) their most-loved-familiar person was living the observed situation (Other-Most-Loved-Familiar condition, OMLF); (3) their most-hated- familiar person was doing the observed action (Other-Most-Hated-Familiar condition, OMHF); (4) a person unfamiliar to them was living the same situation depicted in the pictures (Other-Stranger condition, OS) (Figure 15). The order of presentation of each condition was counterbalanced among the subjects. After the instructions, the trial sequence of each block started with a fixation cross presentation lasting for 500 ms and then the stimulus presented for 12000ms. After the response, an inter-stimulus interval of 1000 ms was added. For each picture, a trigger corresponding to each type of emotional stimulus was sent to a Biopac MP150 system (Biopac Inc.) via E-prime software. A total of 48 trials for

experimental session were acquired: 12 trials (6 painful images and 6 non-painful images) were presented for each perspective-taking block.

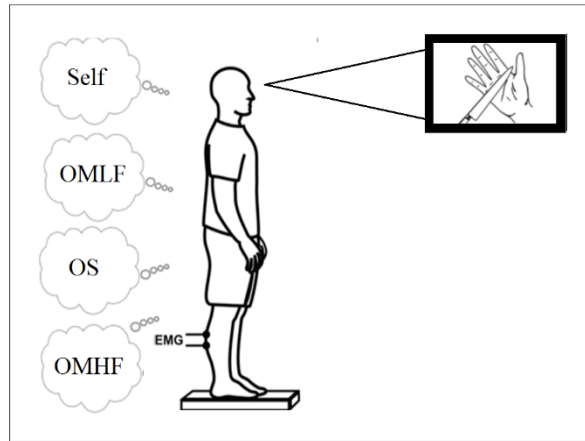


Figure 15. Experimental setting. Participant stood on the platform while he was looking at painful and non-painful visual stimuli and while he was adopting one of the four perspective-taking conditions (Self, OMLF, OS, OMHF).

2.2.3.2. Behavioral experimental session

At the end of the postural and physiological experimental session of the experiment 2, participants were asked to seat in front of a PC and rate the intensity of pain for each of the forty-eight images presented in the postural and physiological experimental session. The experimental session was divided in four blocks each of them consisting in one of the four perspective-taking conditions (Self, OMLF, OS, OMHF). The presentation of these perspective-taking conditions was counterbalanced between subjects. Instructions, data recording, procedure and data analysis were the same to those in experiment 1.

2.2.4. Data analysis

For each participant, data collected during the presentation of the two types of stimuli (painful and non-painful images) and during the four perspectives-taking conditions (Self, OMLF, OS, OMHF) were averaged for the 6 trials lasting 12 seconds (12s) each. The mean of the center of pressure (COP) displacement in the anteroposterior (AP) axis (COP-AP, in mm) was calculated for each perspective-taking condition. In order to compute the COP-AP measure,

for each subject the COP position one second before stimulus onset was subtracted from the COP position during the stimulus presentation. Moreover, the length of the COP sway path in the AP axis (path[COP]-AP, in mm) was computed. The calculation of these postural indexes may reflect an approach / withdrawal or a freezing behavior. EDA signal was analyzed offline. For each subject and each perspective-taking condition, the average peak-to-peak amplitude was extracted. A time course analysis was performed in order to highlight any temporal difference in COP-AP, HR, EMG and EDA: the data were averaged over a 1s sliding windows. In order to study the time-domain responses to painful stimuli in the four blocks, the data were entered into a 4 x 2 x 12 repeated measures ANOVA with three within-subjects factors: perspective (Self, OMLF, OS, OMHF) x valence (painful, non-painful) x time (12 seconds). *Post-hoc* test comparisons were carried out by mean of the Duncan-Pearson correlation to investigate whether (i) self report measures of pain ratings, (ii) RTs and (iii) the self-report BES measures were significantly correlated with the COP-AP mean position. The limit for statistical significance was $p < 0.05$ for all statistical analyses.

2.3. Experiment 3

2.3.1. Stimuli

Video-clips were used in this experiment as stimuli. Like Avenanti *et al.* (2005) did, in this experiment video-clips were depicted the first-person perspective right hand in a painful (needle) or in a non-painful condition (Q-tip) as show in Figure 16A. The conditions were the following: (i) deeply penetrated by a needle in the FDI muscle (Pain first-person); (ii) touched by a Q-tip on FDI muscle (Touch first-person); (iii) statically presented (Baseline first-person). Moreover, to the Avenanti *et al.* (2005) experiment new video-clips rotated of 180° in the third-person perspective (Pain third-person; Touch third-person; Baseline third-person) were added in the experiment. The experimental session was divided into two experimental blocks separated by a break. At the beginning and at the end of each block static right hands in first (Baseline first-person) and third person perspective (Baseline third-person) were randomly presented to assess baseline measures of CS excitability. In the experimental session the four types of experimental stimuli (Pain first-person; Pain third-person; Touch first-person; Touch third-person) were randomly presented (Figure 16).

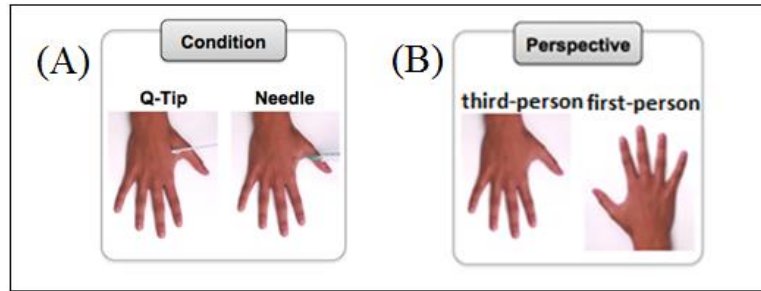


Figure 16. Experimental visual stimuli. (A) Two conditions: video-clips representing a right hand touched by a Q-tip/penetrated by a needle in FDI area; (B) Two perspectives: video-clips showing a right hand in third-person/first-person perspective.

2.3.2. Data collection

In the present study, Transcranial Magnetic Stimulation (TMS; Magstim, Whitlan, Dyfed, Wales, UK) tool and electromyogram (EMG) were used in order to record motor-evoked potentials (MEPs) from the participant's right hand. The experimental setting was so arranged: the 70 mm figure-of-eight coil was statically held on the participant's head on the area corresponding to the left motor cortex. The coil position was tangential to the scalp with the handle pointing backwards and laterally with a 45° angle to the midline. In order to record the electromyographic (EMG) graph, a pair of Ag-AgCl 11cm surface electrodes (EL503) was placed on the first dorsal interosseous (FDI) muscle of the right hand (contralateral to the hemisphere stimulated by the magnetic pulse). The registration of this muscle was selected because it was the same muscle penetrated by a needle or touched by a Q-tip in the presented video-clips. The electrodes were placed in a classical belly-tendon montage: the active electrode was placed over the muscle belly and the reference electrode over the associated joint or tendon. The ground was placed over the participant's left wrist. This setting allowed monitoring the muscular activity variation depending on the magnetic stimulation, i.e., MEPs. E-prime software running on a PC (which was connected to the magnetic stimulator) controlled the temporal scanning of the events including the magnetic stimulation and the stimuli presentation to the participant. At the same time electrodes placed over the participant's FDI muscle recorded the EMG activity; these electrodes were connected to the Biopac system (Biopac Systems Inc., Santa Barbara, CA), which was connected in its turn to another PC, where the Acqknowledge software was running (Biopac Systems Inc., Santa Barbara, CA). EMG signal was sampled (10Hz), amplified filtered with a 50Hz notch filter and stored on a PC for off-line analysis. Before the recording session, it was necessary to identify the hotspot, that is the optimal scalp

position (OSP): in this point of stimulation MEPs have the greatest amplitude. In other words, this OSP is the point on the scalp, corresponding to the representation of FDI in the motor cortex, producing the best motor answer for FDI muscle (Borckardt *et al.*, 2006). The method used to locate the area corresponding to participant's representation of FDI in the motor cortex on the scalp is scheduled as following: (i) to apply the international 10-20 system measuring two distances among participant's four reference points: *nasion-inion* distance (between the delve at the top of the nose and the bony lump at the base of the skull in the nape), the distance between the pre auricular points anterior to the right and left ear; the vertex (Cz) (i.e., the point half-way between the two crossed distances) was so indicated on the scalp with a colored marker; (ii) left motor cortex is individuated ahead 1 cm from the vertex; (iii) finally the OSP was found by moving in steps of 1 cm over the left motor cortex from which maximal MEPs amplitudes were elicited in FDI. Once the individual OSP was found, the resting motor threshold (rMT) was found too. The rMT is defined as the lowest stimulation intensity by means of which 5 MEPs (at least 50 μ V peak-to-peak amplitude) are obtained by 10 consecutives TMS pulses (Rossini *et al.*, 1994; 2015). The rMT represents the excitability motor degree of the central nervous system. This threshold value is rather constant in a single individual (Mills & Nithi, 1997) and it is almost invariable between the two hemispheres (Cicinelli *et al.*, 1997). Mean rMT was 58% (ranging from 41% to 78%) of maximum stimulator intensity. During the recording stimulation, the intensity was fixed at 115% of the rMT.

2.3.3. Procedure

Participants were tested in a single experimental session lasting approximately 1 hour and 45 minutes; each session was divided in two blocks. Participants were seated in a comfortable chair with a fixed chinrest in a dimly illuminate room. Stimuli were displayed on a 17-inch monitor (resolution 1280 x 780 pixels, background luminance of 0.5 cd/ m², refresh frequency 60 Hz) at a viewing distance of 80 cm. Participants were instructed to pay attention to the displayed video stimuli while keeping their right hand in a prone position on a pillow, still and as relaxed as possible (Figure 17 A). Each video-clip lasted 1800ms. MEPs recorded from the FDI right muscle, were acquired once for each video presentation. Two series of 12 MEPs were acquired for each experimental block. One was recorded at the beginning of the experimental block, whereas the second was recorded at the end of the same block. In conformity with the procedure baseline measures of the corticospinal excitability were assessed.

A static hand was randomly presented 6 times for each of the two perspectives (first-person and third-person). TMS stimulation was delivered 1424 ms after stimulus onset. Comparisons of MEP amplitudes for the two series allowed us to check for any corticospinal excitability change between the beginning and the end of each experimental block. These MEPs average amplitudes were calculated to set individual baselines for data normalization. For each block, video-clips of each condition were presented 9 times in a random order resulting in a total of 72 trials (4 video-clips x 9 repetitions x 2 time points). Each video-clip presentation was followed by 8200 ms of inter trial interval: a white fixation cross was presented for 7200 ms and it was then replaced by a green cross (1000 ms) prompting the participant to watch the new video-clip. Borgomaneri *et al.*, (2014) have recently evidenced two different phases in the motor cortex functional modulation: an earlier time of stimulation should evidence an orienting response; a later time of stimulation might represent motor resonance. In accordance with this study, we applied two stimulation timings: early timing (200 ms after needle penetration / Q-tip touch) and late timing (600 ms after needle penetration / Q-tip touch) (Figure 17B). Moreover, these stimulation timings correspond also to the earliest and latest stimulation timings used by Avenanti and colleagues in their previous experiments (Avenanti *et al.*, 2005, Avenanti, Paluello, Bufalari, & Aglioti, 2006, 2010; Avenanti, Minio-Paluello, Bufalari, *et al.*, 2009; Avenanti, Minio-Paluello, Sforza, *et al.*, 2009). In these experiments TMS pulse was randomly delivered in a range from 200 to 600 ms before the video-clip end.

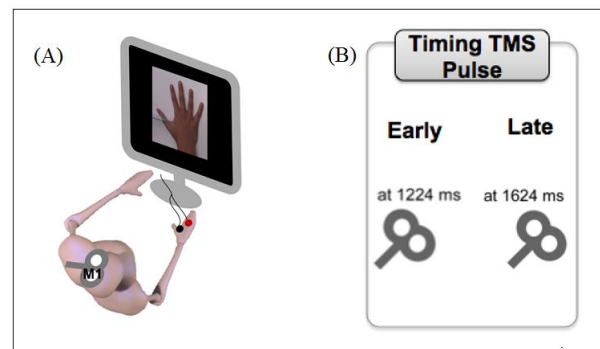


Figure 17. Experimental setting and timing of stimulation. (A) the subject is seat in front of the PC watching at the video-clips and receiving TMS stimulation on his left MI and recording MEPs from his right FDI muscle. (B) Timing of TMS stimulation.

At the end of the experimental session, the Italian version (Bonino *et al.*, 2010) of the Interpersonal Reactivity Index (IRI) (Davis, 1983) was administered to all participants. Then, a body-ownership questionnaire was administered. They had to estimate the degree of

agreement of each item of the questionnaire. Each item was presented with its related image, representative of each experimental condition (Pain first-person, Pain third-person, Touch first-person, Touch third-person). Participants were asked to answer using a seven points Likert-type scale from -3 (i.e.: I don't agree at all) to 3 (i.e.: I totally agree) if they were in agreement with the presented statements. An example of item is reported in figure 18.

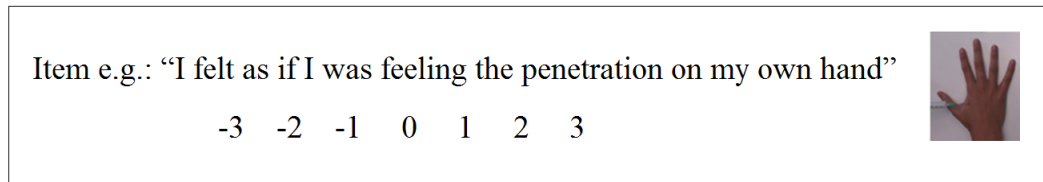


Figure 18. Example of item of body-ownership questionnaire

2.3.4. Data analysis

The MEPs values recorded in each experimental condition were averaged and normalized as percentage of the mean MEP value recorded from the baseline condition of each experimental block ($\text{MEP ratio} = \text{MEP obtained} / \text{MEP}_{\text{baseline}} \times 100$). Normalized data were entered into a 2x2x2 ANOVA with three within-subjects factors, Time (early / late), Pain (needle / Q-tip) and Perspective (first- / third-person). Furthermore, in a linear regression, the normalized MEPs values were used to predict the body-ownership questionnaire ratings. Finally, for the correlation analyses with the scores obtained at the IRI subscales, according to the Avenanti *et al.* (2005) method, we computed an index of MEPs amplitude change. For each pain condition, the obtained values were used to predict the scores obtained at the IRI subscales.

2.4. Experiment 4

2.4.1. Stimuli

Two visual stimuli were used in this experiment: a green square and a blue square (Figure 17 A and B). Both were presented paired with two sounds: a "zzz" white noise and a "bee" tone. The blue square was presented paired with the second noise (Figure 19 A) and the green square was always presented paired with the first sound (Figure 19 B). These last paired stimuli might be associated (CS+ condition) or not (CS- condition) with an electric noxious stimulus. Instead, the blue square and the "bee" tone were never associated with noxious stimuli

(Neutral condition). These stimuli were displayed in a pseudorandom order and their function was to obtain a conditioned response in participants.

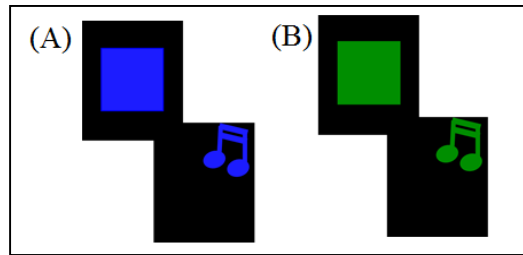


Figure 19. Visual and auditory stimuli. (A) Neutral stimuli; (B) CS+ / CS- stimuli.

2.4.2. Data collection

Experiment 4 data recordings were almost similar to those applied in experiment 3; for this reason we refer to what it has been written in experiment 3 (section 2.3.2.). Here are presented the additional and different techniques. Instead of recording FDI muscle activity, EMG of the Abductor Digiti Minimi muscle (ADM) and of the Abductor Pollicis Brevis muscle (APB) were acquired (Figure 19); the electrodes disposition and data collection were the same as those described in experiment 3. The intensity of magnetic pulses was set at 115% of the resting motor threshold (mean \pm SD $63.9\% \pm 8.24\%$, range 54-78% of the maximum stimulator output).

The two main differences between the two experiments (3 and 4) were the presence in experiment 4 of an electrical stimulation and a skin conductance response recording. Firstly, electrical stimuli consisted in constant current square-wave pulses (DS7A, Digitimer) delivered to the right digit V, using a surface bipolar electrode attached with a Velcro strap. Stimulus intensity was adjusted, in each participant, such that stimulation was deemed “painful but tolerable”, mean stimulus intensities were 34.82 ± 10.63 mA, range 20-48 mA. The stimulus duration was 200 μ s and the delivering came \sim 50 ms first the TMS pulse. Secondly, skin conductance response (SCR) was recorded using two Ag-AgCl electrodes with constant voltage (0.5 V), attached to the subject’s left hand on digit IV and III. Signal was recorded continuously (MP150, Biopac System, USA) amplified and digitalized with a sample rate of 1 kHz, band-stop filtered at 50 Hz and stored for off line analysis.

2.4.3. Procedure

Participants were seated in a comfortable chair with a fixed chinrest in a dimly illuminate room. Stimuli were displayed on a 17-inch monitor (resolution 1280 x 780 pixels, background luminance of 0.5 cd/ m², refresh frequency 60 Hz) at a viewing distance of 80 cm. The experiment consisted of two blocks separated by a 20 minutes break. In order to asses baseline of corticospinal excitability before and after each block a total of ten baselines with a fixation cross of 1050 ms in the center of the screen was presented; five out of them were paired with an electrical shock on the digit V, the other ten were unpaired and used as baseline MEP values to normalized data. After the collection of this ten stimuli, experimental trials begun.

In each block a total of 40 stimuli were presented in a pseudorandom order: 20 were neutral stimuli (N), 15 were conditioned stimuli paired with unconditioned stimuli (i.e. CS+) and 5 were conditioned stimuli unpaired with unconditioned stimuli (i.e. CS-). The pseudorandom sequence was generated firstly, so that the CS- stimuli were never presented in the first five trials in order to have no more than two equal stimuli in consecutive trials. In the N condition, a visual stimulus (i.e. a blue square) and an auditory stimulus (i.e. a tone “bee”) were presented. In the CS+ condition, a visual stimulus (i.e. green square) and an auditory stimulus (i.e. a white noise “zzz”) were presented paired with an electrical stimulus (i.e., unconditioned stimulus) on the digit V of the right hand. In the CS- a visual stimulus (i.e. green square) and an auditory stimulus (i.e. a white noise “zzz”) were not paired with the electrical stimulus to compare the physiological responses (i.e. SCR and MEP) elicited by the CS in absence of the unconditioned stimulus (Figure 20). All visual stimuli were presented for 4000 ms on a black background, attended by auditory stimuli of 1000 ms, and spaced out by a fixation cross with a variable jittering (12000-16000 ms), chosen in order to have a variable time stimuli presentation. According to the condition visual stimuli were followed (i) by a TMS pulse, in N trials; (ii) by and electric shock followed after 50 ms by a TMS pulse, in CS+ trials; (iii) by a TMS pulse, in CS- trials.

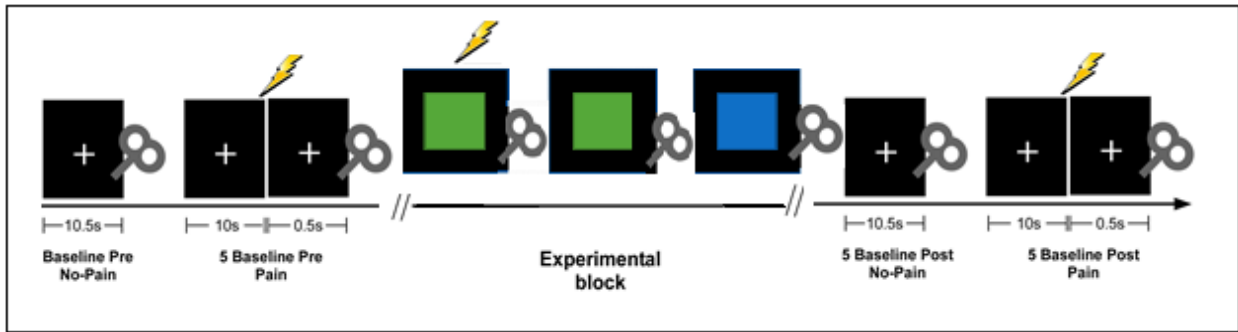


Figure 20. Schematic representation of one block of the paradigm. At the beginning of the experimental session 10 baseline (5 pain and 5 no-pain) were acquired. Then the three experimental blocks (CS+, CS-, N) was presented in a pseudorandom order. Finally the experimental session concluded with another baseline session acquisition.

At the end of experimental session each participant filled out the trait scale and the trait scale of the STAI. Moreover, an ad hoc questionnaire about the expectancy of the participant was administered. It was asked to answer by mean of a Likert-type scale from -5 (i.e. I am not agree at all) +5 (i.e. I am totally agree) about their agreement concerning different affirmations referred to the experimental condition: a) when the green square was presented I expect it would happen to me something positive; b) when the blue square was presented I expect it would happen to me something positive; c) when the “zzz” noise was presented I expect it would happen to me something negative; d) when the “bee” noise was presented I expect it would happen to me something negative.

2.4.4. Data analysis

The average peak-to-peak MEPs’ amplitude of each subject was extracted separately for ADM and APB. The absence of any voluntary contraction in the time window of 100 ms before the TMS pulse was verified by monitoring the EMG activity online and all trials with any activity greater than 50 μ V were excluded from analysis. For each muscle, all trials in which the MEP amplitude were ± 2 SD of the mean amplitude were identified as outliers and excluded from the analysis. Firstly, in order to test the effect of the electrical stimulation per-se and the TMS on the corticospinal excitability, on the MEPs amplitude of the first and the last block of baseline, a 3x2 repeated measures ANOVA was performed with ‘pain’ (two level: ‘pain’; ‘no-pain’), ‘muscle’ (two levels: ‘APB’; ‘ADM’) and ‘block’ (two levels: ‘block1’; ‘block02’) variables as within subject factors. Secondly, in order to test the effect of fear conditioning on

the corticospinal excitability, MEPs amplitude in each experimental condition expressed as percentage of the baseline, were analyzed by means of a 2x3 repeated measures ANOVA with 'muscle' (two levels: 'APB'; 'ADM') and 'condition' (three levels: 'N', 'CS+' and 'CS-') variables as within subject factors. Post hoc comparisons were carried out by means of the Duncan test.

SCR data were analyzed offline. For each subject and each experimental condition the average peak-to-peak amplitude was extracted (as a difference between the minimum and the maximum value after the trigger coding for the stimulus delivering). Then, to obtain comparable measure among subjects, the peak-to-peak responses were normalized within subject and converted into Z-scores (Garbarini *et al.*, 2014) in order to test the effect of fear conditioning on the skin conductance response a one-way repeated measure ANOVA was performed with 'condition' (three levels: 'N', 'CS+' and 'CS-') as within subject factors. Post hoc comparisons were carried out by means of the Duncan.

Pearson correlation was used to investigate whether (i) self report measure of Trait-Anxiety (STAI I) and (ii) skin conductance responses were significantly correlated with amplitude change of MEP recorded from each muscle.

CHAPTER III

RESULTS

1. Experiment 1: Do We Feel the Same Empathy for Loved and Hated Peers?

Giulia Bucchioni, Thierry Lelard, Said Ahmaidi, Olivier Godefroy, Pierre Krystkowiak, & Harold Mouras (2015). *PloS one*, 10(5), e0125871.

Empathy for pain may be associated with other-oriented prosocial behaviors and self-directed avoidances responses (Goubert et. al., 2005, Singer & Klimecki, 2014). In this research article, we focused on the study of factors modulating empathy for pain; these factors were the gender of the witness observing painful events and the perspective adopted by him/her during the experiment. These perspectives were the following: “Self”, “Other-Stranger” (OS), “Other-Most-Loved-Familiar” (OMLF) and “Other-Most-Hated-Familiar” (OMHF). The analysis of these different perspectives may allow a better comprehension of the influence of these perspectives on empathy for pain and on its motor correlates such as approach – avoidance and freezing behaviors. Previous studies focused on the influence of the link between the witness and the perspective adopted by the same witness (Jackson *et al.*, 2005; 2006; Cheng *et al.*, 2010). These studies adopted the first two or three previous presented perspectives (Self, OS and OMLF), without making use of the fourth (OMHF). Specifically, here we investigated the influence of the witness gender and the perspective taking on the behavioral aspects (pain ratings and reaction times) of the observed pain evaluation. Results showed that pain ratings attributed during the observation of painful images in the OMLF perspective were the highest as compared to the other three perspectives. Regarding the reaction times, participants rated the observed pain quicker in the OMLF and Self perspectives than in the other two. Moreover, concerning the influence of the witness gender, data suggested that female subjects were more sensitive than males in rating their pain and their most loved persons’ pain. The results of this study suggest that witness gender and the adopted perspective during the observation of painful events, influence empathy for pain and its associated correlates.

RESEARCH ARTICLE

Do We Feel the Same Empathy for Loved and Hated Peers?

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Abstract

Empathy allows us to understand and react to other people's feelings and sensations; we can more accurately judge another person's situation when we are aware of his/her emotions. Empathy for pain is a good working model of the behavioral and neural processes involved in empathy in general. Although the influence of perspective-taking processes (notably "Self" vs. "Other") on pain rating has been studied, the impact of the degree of familiarity with the person representing the "Other" perspective has not been previously addressed. In the present study, we asked participants to adopt four different perspectives: "Self", "Other-Most-Loved-Familiar", "Other-Most-Hated-Familiar" and "Other-Stranger". The results showed that higher pain ratings were attributed to the Other-Most-Loved-Familiar perspective than to the Self, Other-Stranger and Other-Most-Hated-Familiar perspectives. Moreover, participants were quicker to rate pain for the Other-Most-Loved-Familiar perspective and the Self-perspective than for the other two perspectives. These results for a perspective-taking task therefore more clearly define the role of familiarity in empathy for pain.

Introduction

Empathy is a multidimensional construct with both an emotional and a cognitive component [1]: emotional empathy is the observer's affective response to the observation, inference or imagination of another person's feelings as a consequence of emotional sharing [2], whereas cognitive empathy is the ability to adopt another person's perspective [3], [4], [5]. Empathy therefore enables us to understand another person's feelings by means of emotional simulation (an automatic tendency to mimic another person's expressions) [4], [6], [7] and to cognitive

processing (the ability to apply a perspective-taking process) [4], [8], [9], [10]. These bottom-up and top-down processes are activated at different times and in various situations during the empathic response and depending on the triggering situation [4], [9]. Moreover, cerebral networks are involved in the distinction between a situation involving the subject itself or another person [11]. In fact, the ability to infer another person's mental state requires the ability to inhibit the egocentric bias through which we attribute our own intentions, beliefs or emotions to third parties [12], [13], [14] and the frontopolar gyrus is a cerebral area which appears to play an important role in this inhibition [15]. Published data suggest that adopting another person's perspective and differentiating the agency of actions involve bilateral activation of the temporoparietal junction (TPJ) [16] and activation of the frontopolar gyrus for various tasks (whether motor-based [17] conceptual [15] or emotional [8], [18]).

Studies of pain perception have evidenced a degree of overlap between the neural network used to perceive one's own pain (posterior insula (PI), primary somatosensory cortex (S1) and large parts of medial and anterior cingulate cortices) and that used to imagine another person's pain (anterior insula (AI), anterior cingulate cortex (ACC), medial cingulate cortex (MCC) and thalamus) [7], [19], [20], [21]. Recently, an event-related potentials (ERPs) study [22] observed that the late, controlled component was less intense for evaluation of another person's pain (referred to in the literature as "the Other-perspective") than for evaluation of one's own pain (referred to as "the Self-perspective"). However, this was not the case for the early, automatic component of ERPs.

The working model of empathy for pain usually involves rating the level of pain felt in a visually depicted situation as rapidly as possible [7], [20], [23], [24]. Previous studies have demonstrated the influence of the link between the observer and the depicted character receiving painful stimulation on pain evaluation. For example, Pillai Riddell and Craig [25], showed differences in pain ratings between parents, nurses and pediatricians who observed a children's reaction to a routine injection: parents rated a higher level of pain than nurses and pediatricians. Jackson et al. [20] also recorded a significantly shorter reaction time (RT) for the Self-perspective than for the Other-perspective. Pain ratings in different racial groups have been studied, but neither Xu et al. [26] nor Azevedo et al. [27] found any significant between-group differences with respect to the racial factor. However, it is well known that, at an implicit level, the emotional response and sensorimotor resonance for pain are greater for group members than for people outside the group [28]. Gender differences in empathy for pain remains a controversial issue. For example, Yang et al. [29] reported stronger mu suppression in females than in males when they looked at painful and non-painful images, but no differences in pain ratings of painful and non-painful stimuli. In another study, Han et al. [30] did not find any difference in empathy for pain ratings according to gender. However, recent studies that applied real painful stimuli reported either a higher sensitivity to painful faces [31] or higher pain rating during the observation of another person's pain in females compared to males [32].

In a recent study [33], we demonstrated that participants tended to rate pain stimuli as more painful when they adopted an in-group member perspective rather than a self-perspective. Cheng et al. [34] asked participants to adopt three different perspectives: the participant (self), his/her partner (loved-one) or another unfamiliar person (stranger). They recorded higher pain ratings for the self and the loved-one perspectives compared to the stranger perspective, with no significant difference between self and loved-one perspectives. However, none of these behavioral studies took into account individual differences in all levels of familiarity.

In the present study, we focused on the behavioral aspect of pain evaluation and investigated the perceived intensity of pain in response to a set of previously validated pictures [7] as a function of a Self-perspective and three different Other-perspectives. We sought to determine whether the pain rating for each image varied as a function of the level of familiarity perceived

by the participant. Our starting hypothesis was that the Self and Other-Most-Loved-Familiar (OMLF) perspectives would be associated with more rapid RTs and higher pain ratings than Other-Stranger (OS) and Other-Most-Hated-Familiar (OMHF) perspectives. We also analyzed the role of gender on the perceived intensity of pain in these four perspectives.

Methods

Participants

Sixty-six right-handed healthy participants (32 males and 34 females; mean age \pm SD: 23.3 ± 5.04) took part in the study after having provided their written informed consent. Exclusion criteria were a history of visual or motor impairment and prior or ongoing treatment for psychiatric or neurological disorders. Participants were students and they received gift cards in exchange for their voluntary participation. The experiment was performed in accordance with the ethical standards of the Declaration of Helsinki and was approved by the local investigational review board (Comité de Protection des Personnes Nord-Ouest II, Amiens, France).

Stimulus material

Thirty-six pictures (depicting the hand or the foot in either painful or non-painful situations) were chosen from previously validated databases [7]. When rating the pain in the picture, participants were asked to adopt the Self-perspective or one of three different Other-perspectives (OMLF, OMHF and OS). Each condition (Self, OMLF, OMHF and OS) was presented in a separated block. For each participant, the pictures were presented in random order for four times, one for each block. The four conditions were also counterbalanced across the set of pictures. Stimuli were presented using E-Prime software (version 2.0, Psychology Software Tools, Inc., Pittsburgh, PA). The experimental paradigm is depicted on Fig 1.

Procedure

Before starting the experiment, each participant filled out a series of standardized questionnaires, including the French version of the Beck Depression Inventory (BDI) [35], the French version of State-Trait Anxiety Inventory, the subscales T (trait) and E (state) [36] and the French version of the 20-item Basic Empathy Scale (BES) [37,38], with 9 and 11 items assessing cognitive empathy and affective empathy, respectively. The BDI II and STAI were used to exclude any participants with psychiatric disorders such as moderate or severe depression and elevated levels of anxiety. A score higher than 19 on the BDI constituted an exclusion criteria (cut-off score of 20 for a diagnosis of mild depression). For the T and E subscales of the STAI questionnaire, subjects with scores more than two standard deviations higher than the median

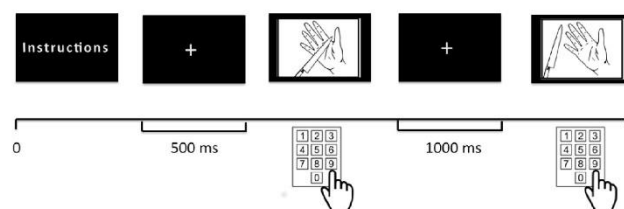


Fig 1. Schematic representation of the paradigm: the description of the task for each of the four blocks (Self, Other-Most-Loved-Familiar, Other-Most-Hated-Familiar and Other-Stranger) was presented and a fixation cross was displayed for 500 ms in the instruction part. An image of a painful or non-painful stimulus was presented until the participant had rated the imagined level of pain from 0 to 9 using a keyboard. After an interstimulus interval of 1000 ms, a new stimulus was displayed on the screen.

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French population score were excluded. Considering these criteria, two participants were excluded from this sample. The BES was used to check the level of the participant's empathy and establish correlations with the participant's responses. We chose to administer the BES because it provides better evaluation of cognitive empathy than the Interpersonal Reactivity Index [37], [39]. Behavioral variables (pain rating and reaction time, i.e. time lag between presentation of the picture and pain rating; RT) were recorded using E-Prime software (version 2.0, Psychology Software Tools, Inc.). The pain-rating task was organized as follows: at the beginning of each block (with one block for each familiarity condition), participants were instructed to imagine the pain that they (Self) or someone else (OMLF, OMHF and OS) would experience in each situation presented. More specifically, the following instructions were provided at the beginning of each block: "Dear participant, you are going to see a series of images presented on the computer screen. We would like you to rate the intensity of pain that you (in the SELF block) / the most loved familiar person (in the OMLF block) / the most hated familiar person (in the OMHF block) / someone you don't know (in the OS block) would experience in the situation displayed; please indicate your rating by selecting a number between 0 (no pain) and 9 (worst possible pain) on the keyboard using your right hand". We masked the purpose of our study, by not using the word 'empathy' in the instructions, although we did not check whether participants were aware of the purpose of the study. The order of presentation of the four blocks (Self, OMLF, OMHF and OS) was counterbalanced. Each block started with the presentation of a fixation cross for 500 ms. The stimulus was then presented until the participant responded. After the response, an interstimulus interval of 1000 ms was added. The participant's task consisted in replying as rapidly as possible after presentation onset. The participant indicated the rating on a keyboard with his/her right hand. Pain ratings were recorded on a 10-point Likert scale between 0 (no pain) and 9 (worst pain imaginable). Persons designated as "most loved" (OMLF) and "most hated" (OMHF) were also divided into four categories: (i) parents and other relatives, (ii) friends and acquaintances, (iii) partners and (iv) teachers and bosses.

Statistical analysis

All data were analyzed using the Statistical Package for the Social Sciences (version 21.0, SPSS Inc., Chicago, IL, USA). Trials with scores 2.5 SD above or below each individual mean for each condition in the pain rating task were excluded as outliers (1%). Two separate repeated-measures analyses of variance (ANOVAs) were performed on pain ratings and RTs, with gender as a between-subjects factor. Another repeated-measures ANOVA was then performed on pain rating data, with perspective (Self, OMLF, OMHF, OS) and stimulus value (painful, not painful) as within-subject factors and gender as a between-subjects factor. As the RT values were not normally distributed, they were log-transformed prior to the ANOVA. Paired-sample t-tests with a Bonferroni correction were used to compare the pain ratings for painful stimuli. Pearson's correlation coefficients were also calculated for the relationship between the participants' pain ratings for painful images and their level of empathy on the BES. The limit for statistical significance was $p < 0.05$ for all statistical analyses.

Results

Behavioral results are reported in Tables 1 (pain ratings) and 2 (reaction times) (see also Fig 2 (A) and (B)). For the OMLF perspective, 41% of the designated people were parents or relatives, 59% were partners, and none were friends, teachers or bosses. For the OMHF perspective, 1.7% of the designated people were parents or relatives, 88.1% were friends or acquaintances, 10.2% were teachers or bosses, and none were partners.

Table 1. Pain ratings (Mean values ± SD) as a function of stimuli (painful vs. non-painful) and perspective (other-negative familiar, other-unknown, self, other-positive familiar).

	OMHF	OS	Self	OMLF
Painful	4.50 ± 1.71	4.89 ± 1.55	5.42 ± 1.63	5.76 ± 1.63
Non-Painful	0.04 ± 0.21	0.05 ± 0.20	0.02 ± 0.04	0.06 ± 0.23

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Pain ratings

An ANOVA with respect to the perspective condition (Self, OMLF, OMHF, OS) revealed a significant main effect [$F(3,65) = 19.51; p < 0.0001$]. The main effect of stimulus value (painful or non-painful) was also statistically significant [$F(1,65) = 897.19; p < 0.0001$]. A significant interaction was demonstrated between the perspective and the stimulus condition [$F(3,65) = 20.65; p < 0.0001$]. The interactions between perspective and gender [$F(3,65) = 4.50; p < 0.005$], valence and perspective [$F(3,65) = 4.23; p < 0.05$], and valence and gender [$F(3,65) = 4.11; p < 0.05$] were also statistically significant.

Post hoc pairwise comparisons were finalized with *t*-tests and the Bonferroni correction was applied. Mean pain ratings were higher in the OMLF perspective compared to the OMHF perspective ($t_{65} = 6.06; p < 0.0001$), OS perspective ($t_{65} = 6.36; p < 0.0001$) and Self perspective ($t_{65} = 4.12; p < 0.0001$) (see Fig 2 panel A). The mean pain ratings (Table 1) were significantly higher for the Self-perspective than for the OS ($t_{65} = 3.03; p < 0.005$) and OMHF ($t_{65} = 3.89; p < 0.0001$) perspectives. Although pain ratings were higher for the OS perspective than for the OMHF perspective, the difference was not statistically significant. As a between-subjects variable, gender had a significant effect [$F(1,65) = 4.20; p < 0.05$]. Female participants rated the painful stimuli as more painful in the OMLF ($t_{65} = 3.38; p < 0.005$) and Self ($t_{65} = 2.98; p < 0.005$) conditions than male participants. A *t*-test was also used to compare the Other-perspectives as a whole (i.e. the pooled OMLF, OMHF and OS data) with the Self-perspectives. Painful images were rated as significantly more painful for a Self-perspective than for an Other-perspective ($t_{65} = 2.29; p < 0.05$).

Reaction times

Mean and standard deviations of RTs in the four conditions (Self, OMLF, OMHF, OS) in painful and non-painful situations are reported in Table 2. The ANOVA of the log-transformed RT data revealed a significant main effect of perspective condition [$F(3,65) = 4.76; p < 0.005$] and stimulus value [$F(1,65) = 298.89; p < 0.0001$]. A significant interaction was demonstrated between perspective and stimulus value [$F(3,65) = 64.95; p < 0.0001$]. No gender effect on RT was observed on *t*-tests with Bonferroni correction. The mean RT for painful stimuli was shorter in the OMLF condition than in the OS condition ($t_{65} = -3.43; p < 0.001$) and OMHF condition ($t_{65} = -5.37; p < 0.0001$), but was longer than in the Self-condition ($t_{65} = 4.04; p < 0.0001$) (see Fig 2, panel B). The RTs were significantly longer for the OMHF perspective than for the Self-perspective ($t_{65} = 7.80; p < 0.0001$). No significant differences in RTs were observed between females and males.

Table 2. Response time (Means values ± SD) as a function of stimuli and perspective.

	OMHF	OS	Self	OMLF
Painful	2044.4 ± 731.2	1106.2 ± 568.7	1534.1 ± 766.7	1719.2 ± 493.5
Non-Painful	1106.2 ± 316.6	1160.6 ± 456.4	1544.3 ± 851.3	1074.1 ± 357.4

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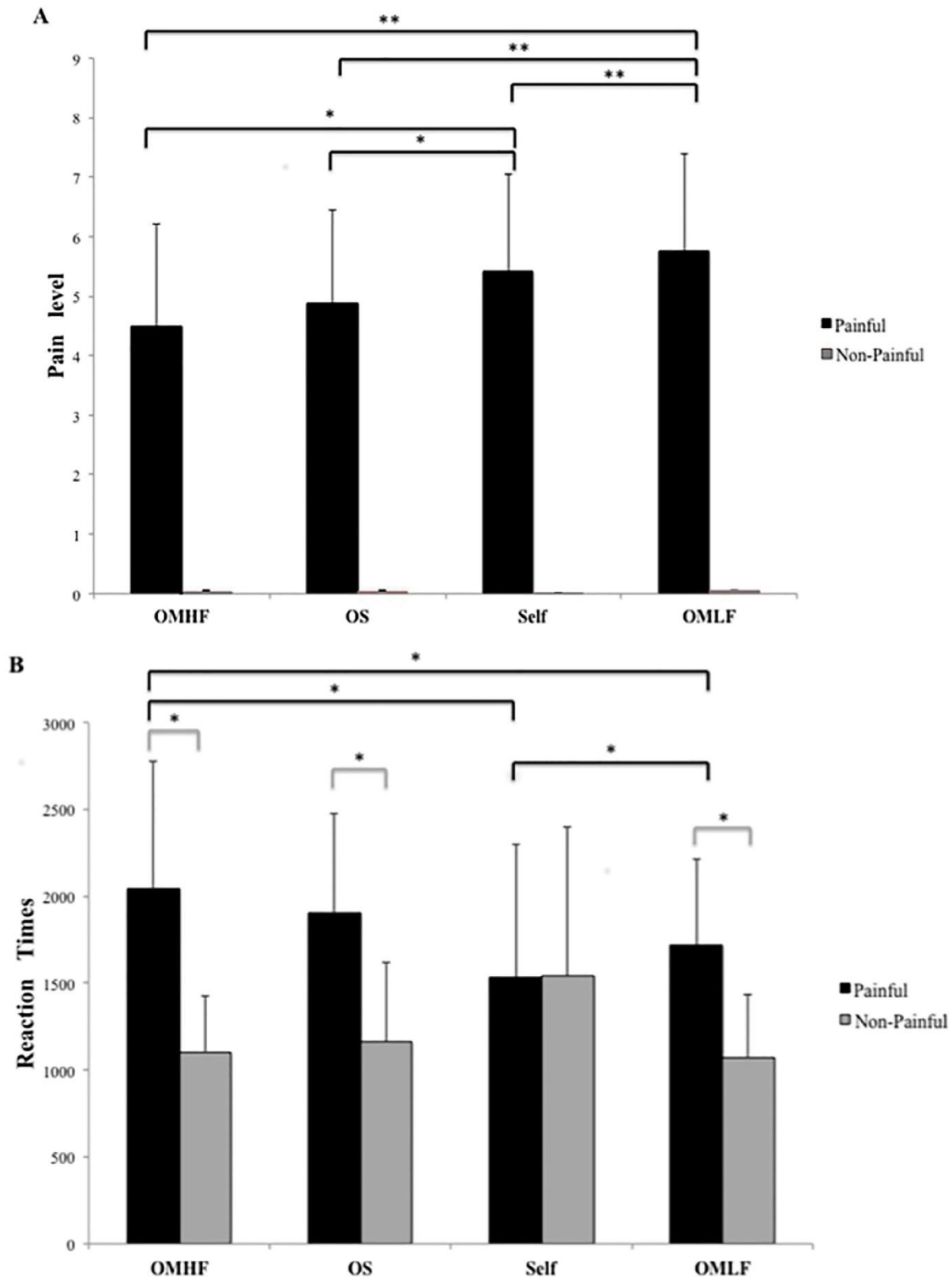


Fig 2. (A) Mean \pm SD pain ratings as a function of the stimulus (painful vs. non-painful) and the perspective (Self, OMLF, OMHF and OS). Significant differences are indicated as: * $p < 0.05$ ** $p < 0.001$ (B) Mean \pm SD RTs as a function of the stimulus and the perspective.

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Correlations

None of the correlations between pain ratings and BES scores (whether total, cognitive or emotional) were statistically significant (for example, $r = 0.212$ for the correlation between mean total BES score and the pain rating for painful situations with the Self-perspective, and $r = 0.234$ for the correlation between mean total BES score and the pain rating for painful situations with the OMLF-perspective). The RTs recorded for painful stimuli with the OMHF perspective were positively correlated with the total BES score ($r = 0.25$; $p = 0.05$). The RTs recorded for painful stimuli with the OMLF perspective were positively correlated with the BES cognitive subscore ($r = 0.29$; $p = 0.05$). The BES cognitive subscore was also positively correlated with the difference in RTs between painful and non-painful stimuli with an OMLF perspective ($r = 0.28$; $p = 0.05$).

Discussion

Empathy enables us to understand and share another person's feelings. As such, empathy plays an essential role in social interactions between humans [40]. Perception of another person in a painful situation involves much of the neural network activated during first-person experience of pain (for a meta-analysis, see Lamm et al., 2011 [21]).

In the present study, participants were asked to rate the level of pain felt from their own perspective or that of other people. Our results are in agreement with data published in the literature: the participants rated painful images as more painful when adopting a Self-perspective than when adopting an Other-perspective [20]. Moreover, our experimental design distinguished between three levels of familiarity within the Other perspective: the OMLF, OMHF and OS perspectives. The situations were rated as most painful when the participants adopted an OMLF perspective (usually that of their mother or their partner). This differentiation provides a finer judgment of the degree of painfulness attributed to each context: a painful situation is considered to be most painful when it is experienced by a loved one, then by ourselves, then by a stranger and, lastly, by someone we hate (usually a former partner or friend, or a current teacher or boss). These results are in agreement with data published in the literature [7], [20], [22] showing that painful stimuli are rated as more painful with a Self-perspective than with an Other-perspective. However, none of the previous studies focused on the level of familiarity with the "Other". Our findings remained the same when data for the Other-perspectives were pooled; the mean pain rating was significantly lower than for a Self-perspective (in line with the literature).

If we assume that the OMLF perspective is similar to an "in-group" perspective, the present results are in agreement with our previous report [33] of significantly higher pain ratings for an in-group perspective than for Self- and out-of-group perspectives. In agreement with some previous studies [20], [22] but not others [33], we found that adoption of a Self-perspective was associated with shorter pain rating RTs than Other-perspectives (OMLF, OS or OMHF). The absence of this finding in the study by Montalan et al. (2012) [33] may have been due to their smaller sample size. Furthermore, we found that the OMLF perspective was associated with the second shortest mean RTs, suggesting that the in-group perspective [33] may have been more complicated to adopt, since there was no difference in RTs between the in-group and out-of-group perspectives. Pain ratings were higher for the Self-perspective than for the Other-perspectives because the Self-perspective is more closely related to the participant's own, real

experience of pain (as reported in [20]). This finding was confirmed by the shorter mean RTs: direct experience of pain would enable more rapid rating of painful or non-painful situations. Rating pain from an Other-perspective requires more time.

Pain ratings for the OMLF perspective were even higher than for the Self- perspective. This finding confirms the hypothesis in which the level of empathy depends on affective proximity. It also agrees with previous work showing that participants produce more intense empathic responses when considering a familiar person (such as a parent, child [25] or loved one [34]) than when considering a stranger. According to Aron et al. [41], [42], the closer the relationship between two individuals, the more they are integrated into a Self-perspective. Indeed, the degree of familiarity between two people may influence the degree of empathy felt for another person [43]. Moreover, factors such as altruism (i.e.: helping others without thinking about your direct advantage) may also influence the level of empathy felt by study participants. Batson [44] proposed the empathy–altruism hypothesis, in which empathy helps to improve another person's well-being [44], [45], [46]. As previously suggested [33], we consider that this overlap between mental representations of Self and Other might explain the higher mean pain ratings for the OMLF perspective. Furthermore, our observation of significantly lower mean pain ratings for the OS and OMHF perspectives also fits with this hypothesis. The absence of a significance difference between the OS and OMHF perspectives may have been due the participants' unfamiliarity with adopting these two perspectives and the sometimes small perceived difference between them to bias. Moreover, it is noteworthy that the longest mean RTs for pain ratings were recorded for the OMHF perspective. The presence of inhibition during pain ratings for this perspective can be legitimately hypothesized.

Although previous studies conducted according to a similar methodology [29], [30] have failed to highlight a significant difference between genders in pain ratings in an empathy for pain task, pain stimulation studies [31], [32] demonstrated gender differences in rating the level of another person's pain. The influence of gender on empathy for pain remains unclear at the present time. We analyzed whether the gender variable could modulate empathy for pain ratings using self / different other perspectives. In particular, the results suggest that female subjects are more sensitive than males in rating their pain and the pain of their loved ones in line with pain stimulation studies [31], [32]. There are several examples in the literature supporting the superior empathy capacity of females [47], but they are often based on self-report questionnaires [48], as in the present study. For these reasons, these results must be interpreted cautiously and need to be reproduced by further studies.

RTs recorded during the OMHF condition were positively correlated with the total BES score: participants who spent more time rating painful conditions during the OMHF perspective probably had a higher level of empathy than other participants. Moreover, both the painful condition and the delta between painful and non-painful conditions with the OMLF perspective were positively correlated with the cognitive BES subscale, that assesses the intellectual understanding of another person's mental state [38]. Cognitive empathy is based on important cognitive functions such as executive functions and language [40]. It is important to more clearly understand the loved person's feelings, especially when the imagined person is thought to be in a dangerous situation (i.e. when the subject has to determine the level of pain that the imagined loved person would experience). Perspective-taking requires a lot of energy to maintain the interaction between limbic and high-level cognitive structures and a greater volume of information needs to be processed after a certain period of time [49]. This process then becomes less selective and slower, as it becomes painful to imagine the loved person in pain, which is probably why subjects with a higher level of empathy tend to devote more time to this task.

Zeki and Romaya [50] showed that the neural correlates of hate involve the premotor cortex, a region involved in motor planning. The authors hypothesized that seeing a hated person activates the premotor system to apply approaching / avoidance behavior. There is also evidence in favor of the existence of freezing behavior during observation of another person's pain that is specific to the muscle vicariously involved in the painful stimulation [51]. We can also hypothesize that motor response RT freezes more severely when the subject has a higher level of empathy towards his/her hated peers. Cognitive hypothesis must also be taken into account. It can be assumed that this task required more cognitive resources in relation to the OMLF condition. Subjects may feel inhibited to attribute higher pain ratings in the OMHF condition compared to the OMLF condition, which would account for the longer reaction times [52].

In conclusion, these findings confirm the hypothesis that the level of empathy depends on affective proximity [25,34]. Moreover, this study completed and clarified the behavioral results of previous studies [20], [22], [34], separating the "Other" perspective into three different levels of familiarity and analyzing the differences in pain perception with respect to these perspectives. This study showed that imagining the most loved person in a painful situation is associated with higher pain ratings and lower reactions times. On the contrary, imagining the most hated person in a painful situation is associated with lower pain ratings and higher reactions times. Overall, these results suggest that the type of relationship between the participant and the observed person in pain may modulate the way in which interpersonal factors influence pain perception.

Supporting Information

S1 File. Table of the posturographic data in the three experimental conditions. (XLSX)

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Author Contributions

Conceived and designed the experiments: GB TL HM. Performed the experiments: GB TL. Analyzed the data: GB TL HM. Contributed reagents/materials/analysis tools: TL SA. Wrote the paper: GB TL HM PK OG.

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2. Experiment 2: The postural correlates of the empathic pain response: the influence of perspective-taking.

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Experiment 1 results led us to investigate the witness' behavioral reactions to others' painful events in the four previous described perspectives (Self, Other-Most-Loved-Familiar (OMLF), Other-Stranger (OS) and Other-Most-Hated-Familiar (OMHF)). Specifically, we aimed at disentangling in which direction the observer of others' painful events was directed according to the four perspectives, i.e., if the witness approached / avoided or froze face to the observation of other's painful events. In experiment 2, we analyzed the anteroposterior direction of the center of pressure (COP-AP) and freezing behaviors (path length of the center of pressure in the anteroposterior direction; path [COP]-AP) during a postural control task when the observer looked at visual stimuli similar to those used in experiment 1. At the same time, the participants adopted one of the four perspectives (Self, OMLF, OS and OMHF). Results suggest that subjects tended to adopt a withdrawal position when they were faced to the OMLF individual suffering for a painful situation, compared to the OMHF in a painful situation.

Moreover, we computed the delta between pain and no-pain situations in the COP-AP parameter. The delta (pain – no-pain) for the perspective OMLF was significantly different from all the other perspectives at the third second of stimuli presentation: the COP-AP mean position was significantly more shifted backwards compared to all the other perspectives (Self, OS and OMHF). According to recent studies (Goubert *et al.*, 2005; Singer and Klimecki, 2014), this withdrawal reaction may be associated to self-protection and avoidance of threat response (Yamada & Decety 2009): witnessing a loved person suffering induces a stronger personal distress in the observer; in order to avoid these negative feelings and possible dangerous situations, the witness would move away from the situation. Regarding the freezing behavior, we recorded a reduction of path [COP]-AP for the OS perspective as compared to the others perspectives.

The postural correlates of the empathic pain response: influence of perspective-taking.

Giulia Bucchioni, Thierry Lelard, Olivier Godefroy, Said Ahmaidi, Pierre Krystkowiak,
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ABSTRACT

Empathy yields to adaptive information about potentially dangerous events and facilitates compassion and caring responses among people. Concerning empathy for pain, a witness looking at a painful situation may react with other-oriented and other-approach prosocial behaviors or self-oriented withdrawal responses. Moreover, it has been previously shown the level of proximity and familiarity the witness has with the target may influence the empathic response. The aim of this research was to study approach / avoidance and freezing-type responses in the witness of other's pain. Postural and physiological parameters were recorded from participants in a perspective-taking task where participants adopted four different perspectives ("Self", "Other-Stranger", "Other-Most-Loved-Familiar" and "Other-Most-Hated-Familiar") during the observation of painful and non-painful visual stimuli. The main results showed that the mean position of the center of pressure in the anteroposterior axis was more shifted backwards for the Other-Most-Loved-Familiar perspective compared to all the others perspectives during the presentation of painful visual stimuli. This result evidenced a withdrawal behavior in response to painful visual stimuli that is specific for the loved person perspective. This result suggests that witnessing a loved person suffering induces a stronger personal distress in the observer leading to a desire to move away from the observed situation, adopting self-protective withdrawal strategies.

INTRODUCTION

Empathy is a complex construct that plays an essential social role allowing us to understand and react to other individuals' inner states (Thompson, 2001). According to Lamm *et al.*, (2007), empathy has three different components: (i) the *affective* response to others and the sharing of their emotional states; (ii) the *cognitive* ability of perspective-taking, i.e. the capability to adopt other's point of view (Mead, 1934); (iii) the capacity to *control* the source of self /others experiences (Decety & Lamm, 2006). The majority of researchers working on

empathy generally recognize its cognitive and affective sub-components (Shamay-Tsoory, 2011). The affective proximity between the empathizer and the target plays an important role in modulating the empathic responses. In fact, the closeness in a social relationship that is perceived by the onlooker with the observed individual might modulate the distress intensity felt by the observer: the witnesses empathize with the observed person depending on how much he/she perceives the observed person similar to him/her (Batson et al 1997). Moreover, the feeling to belong to a group (like the same racial group) may influence the observer empathic response (Brown *et al.*, 2006; Montalan *et al.*, 2012). For instance, Xu *et al.* (2009) have found a significantly greater activation of the anterior cingulate cortex when participants observed faces of the same ethnic group of the subject (in-group member) being pricked by a needle as compared to the observation of racial out-group members.

The International Association for the Study of Pain (IASP) defines pain as an unpleasant subjective, sensory and emotional experience associated with actual or potential tissue damage (Tracey & Mantyh, 2007). Pain is a strong cue that induces sensitive reactions in whom looks at other individuals in a painful situation and helps in creating social ties (Williams, 2002). Goubert *et al.* (2005) and Singer and Klimecki (2014) in their models describe similar behavioral and affective reactions within the protagonists associated with the observation of someone else in a painful context. These authors suggest the implementation of two main groups of affective and behavioral reactions within the witness: (i) *self-oriented* responses (including distress, anxiety and withdrawal behaviors); (ii) *other-oriented* responses (like sympathy, compassion and approach towards the individual in pain that could provide reassurance and comfort. New studies on empathy for pain suggest that shared representations during affective processing of painful stimuli may be representative of general self-oriented mechanisms of threat avoidance (Decety, 2015). Motor correlates associated with empathic responses towards other's pain have been previously studied within the corticospinal (CS) system. For instance, Avenanti *et al.* (2005; 2006; 2009; 2010) conducted different experiments investigating the freezing effect (i.e., the CS inhibition) during the observation of body part penetrated by a needle. Posturography associated with the registration of electromyogram activity of leg muscles might be another mean to study the behavioral response in the observer of other's distress. For example, Lelard *et al.* (2013) found a modification of postural control (i.e. freezing effect) and higher leg muscles (tibialis anterior muscle, TA) contraction when participants looked at painful images. Moreover, physiological measures can easily detect the

emotional reactions induced by the observation of other persons undergoing to a painful stimulation (Lamm *et al.*, 2008). For instance, Vico and colleagues (2010) have found an augmentation of heart rate (HR) and skin conductance response (SCR) amplitudes when participants observed the face of loved persons as compared to babies', unknown individuals', famous characters' and neutral faces.

The aim of the present study was to analyze the approach / avoidance and freezing behavior that the witness of a painful event may adopt. In order to analyze these behaviors we recorded postural parameters of the center of pressure (COP) displacements along the anteroposterior (AP) axe were collected. Moreover, we investigated in a perspective-taking task the influence of the proximity between the witness and the observed person on the witness behavior. Specifically, we wanted to clarify the nature of the motor correlates (in term of approach / avoidance or freezing behavior) according to the perspective adopted by the witness regarding the observed character (Self, Other-Stranger, Other-Most-Loved-Familiar and Other-Most-Hated-Familiar). Postural and physiological parameters were recorded and analyzed. Moreover behavioral responses (pain ratings and reactions times) were also recorded.

METHODS

Participants

Fifty-six healthy subjects took part in the experiment (26 women; mean age \pm s.d. years = 23.77 ± 5.68 , range 18 - 40). Eleven subjects were excluded from the analysis because of technical problems during postural and physiological recording and the resulting sample included forty-five participants (19 women; mean age \pm s.d. years = 23.51 ± 5.44 , range 18 - 40) according to the following inclusion criteria: (i) right-handed lateralization (Standard Handedness Inventory; Oldfield, 1971); (ii) no previous history of visual or motor impairment; (iii) no prior or current treatment for psychiatric or neurological disorders. The procedure was approved by the ethical committee of the CPP Nord Ouest 2 (Amiens, France) and conformed to the Declaration of Helsinki of the revised Helsinki Declaration (World Medical Association General Assembly, 2008). None of the participants reported any discomfort or adverse effects during the experimental session.

Stimuli

Visual stimuli were chosen among previously validated databases (Jackson et al, 2005; 2006). Forty-eight colored pictures depicted hands or feet in first-person or lateral perspective; half of the pictures described painful situations (e.g.: a hand under a saw) and the other half represented the corresponding non-painful control situations (e.g.: a hand alongside a saw). E-prime 2.0 software (Psychology Software Tools, Inc., Pittsburgh, PA, USA) was running on a PC, controlled the randomization and the presentation of stimuli.

Data collection

Posturographic and physiological indexes were recorded.

For *posturography*, the setting conditions were defined by the French society of posturography (Normes AFP 85). Participants were instructed to stand comfortably upright on a posturographic platform (Satel, Blagnac, France) in a bipedal stance. Their fingers feet were positioned on the edge of the platform; a gap of 3 cm was displayed between the two heels and the finger feet pointed 30° outward. Participants' arms were left alongside the trunk and their task consisted in standing on the posturographic platform as immobile as possible in a bipedal stance: no voluntary movements of head, arms and legs were allowed. The Satel posturographic platform used in this experiment applies constant moment beam type sensors (approved by the French State Weights and Measures Dept.). The Biopac MP150 is connected to the posturographic platform and to a PC where the Acknowledge software is running, allowing the digitalization of the data collected by the 3 sensors present in the posturographic platform. The anteroposterior (AP) of the center of pressure (COP) is computed off-line. Data were stored on a PC for off-line analysis.

For the other *physiological indexes*, the activity of soleus and tibialis muscles has been monitored during posturography. Electromyographic activity (EMG) of the tibialis (TA) and soleus (SO) muscles was recorded from the dominant leg using bipolar Ag/AgCl surface electrodes (EL503, 35mm diameter, BiopacInc., Goleta, CA, USA) placed 2 cm apart center-to-center longitudinally. To maintain the inter-electrode impedance under 5kΩ, before positioning the electrode, the skin was abraded and cleaned with an alcohol solution. In order to guarantee a good electrical contact (no air between the skin and the electrode), an electrolyte

gel was applied between the skin and the electrodes. For the soleus, electrodes were placed 2 cm under the insertion of the gastrocnemius on the Achilles tendon. The other bipolar montage was applied on the tibialis muscle; the electrodes were placed over the belly of the muscle. Heart rate (HR), expressed in bpm, was recorded using a standard Lead II electrocardiogram. Three disposables electrodes (EL503, Biopac Inc., Goleta, CA, USA) were placed upon the right, left internal ankles and on the right internal wrist in order to recreate the Einthoven's triangle. Electrodermal activity (EDA) was recorded with two Ag/AgCl surface electrodes, 6 mm diameter filled with isotonic paste and attached to the volar surface of the index and middle fingers of the participant's left hand. The electrodes were connected to an amplifier (GSR100C, Biopac Inc., Goleta, CA, USA). A constant-voltage device was used to apply 0.5V across the electrodes to calculate the skin conductance response (SCR) in μ Simens. The signal has been filtered online with a 50Hz band-stop filter. The data have been collected and stored on a PC to be analyzed later.

Procedure

Inclusion visit.

Before starting the experimental session, participants filled a series of standardized questionnaires including the State-Trait Anxiety Inventory (STAI) (Spielberger et al 2010) and the French version of the Basic Empathy Scale (BES; Joliffe & Farrington, 2006; D'Ambrosio *et al.*, 2009) composed of 20-items (9 of them for the *cognitive* empathy component; 11 for the *affective* empathy component). This last questionnaire was administered in order to verify the level of participant's empathy felt during the four perspective-taking conditions associated to the presentation of visual stimuli with different valence (painful, non-painful). The STAI questionnaire was used to control any anxiety-bias problems (none participants were excluded for this reason). In fact, the approach / avoidance behaviors may be influenced by anxiety, a threat-related emotion, which is characterized by sustained, abnormally high levels of the muscle tension (Lang *et al.*, 2000).

Postural responses to visual stimulation

The participants were tested in a quiet and dimly illuminated experimental room and the posturographic platform was positioned 2 m away from the wall, where pictures stimuli were

displayed using a video-projector. This was positioned in the experimental room and connected to the PC located in the data collection room where E-prime 2.0 randomized the presentation of the visual stimuli. In order to minimize the effect of the mains electricity on the registration of the physiological measures (i.e., limitation of the artifacts), all the electric wires were isolated and the disposition of electrical devices in the experimental room was limited to the posturographic platform, the Biopac system and the video-projector. The task consisted in standing as firm as possible on the posturographic platform, watching at the visual stimuli. The experimental session was divided in four blocks each of them consisting in one of the four perspective-taking conditions. Participants in fact were instructed to imagine that: (1) themselves were experiencing the situation represented in the visual stimuli (Self condition); (2) their most loved familiar person was living the observed situation (Other-Most-Loved-Familiar condition, OMLF); (3) their most hated familiar person was doing the observed action (Other-Most-Hated-Familiar condition, OMHF); (4) a person unfamiliar to them was living the same situation depicted in the pictures (Other-Stranger condition, OS) (Figure 1). We asked to participants to take the perspective of a specific person that was familiar / unfamiliar to them. The order of presentation of each condition was counterbalanced among the subjects. After the instructions, the trial sequence of each block started with a fixation cross presentation lasting for 500 ms. Then the stimulus was presented for 12000 ms. After the response, an inter-stimulus interval of 1000 ms was added. For each picture, a trigger corresponding to each type of emotional stimulus was sent to a Biopac MP150 system (Biopac Inc.) via E-prime 2.0 software. A total of 48 trials for experimental session were acquired: 12 trials (6 painful images and 6 non-painful images) were presented for each of the four blocks.

Behavioral task

At the end of the postural and physiological experimental session, participants were asked to seat in front of a PC and to rate as quickly as possible the intensity of pain for each of the forty-eight images presented during the previous experimental session. We applied the same methodology as Bucchioni *et al.* (2015) study. This behavioral experimental session was divided in four blocks corresponding to the four perspective-taking conditions (Self, OMLF, OS, OMHF). The presentation of these perspective-taking conditions was counterbalanced among subjects. These instructions were given to participants at the beginning of each block: “Dear participant, you are going to see a series of images presented on the computer screen. We would like you to rate the intensity of pain that you (in the SELF block) / your most loved

familiar person (in the OMLF block) / your most hated familiar person (in the OMHF block) / someone you don't know (in the OS block) would experience in the situation displayed; please indicate your rating by selecting a number between 0 (no pain) and 9 (worst pain imaginable) on the keyboard using your right hand". After instructions were presented on the screen, the trial sequence started with a fixation cross for 500 ms. The stimulus was then presented until the participant responded. After the response, an interstimulus interval of 1000 ms was added. Immediately after the onset of visual stimuli presentation, subjects were encouraged to indicate their ratings on the PC keyboard (score ranged from 0 = no pain to 9 = worst pain imaginable).

Data analysis

For each participant, data recorded during the presentation of the two types of stimuli (painful and non-painful images) and during the four perspectives-taking conditions (Self, OMLF, OS, OMHF) were averaged for the 6 trials lasting 12 s each. The mean of the COP displacement in the anteroposterior (AP) axis (COP-AP, in mm) was calculated for each perspective-taking condition. In order to compute the COP-AP measure, for each subject the COP position one second before stimulus onset was subtracted from the COP position during the stimulus presentation. Moreover, the length of the COP sway path in the AP axis (path[COP]-AP, in mm) was computed. The calculation of these postural indexes may reflect an approach / withdrawal (for the COP-AP) or a freezing (for the path[COP]-AP) behaviors.

EDA signal was analyzed offline. For each subject and each perspective-taking condition the average peak-to-peak amplitude was extracted. EMG signal was analyzed offline. Root mean square (RMS) of raw data over 500 ms was calculated with a sliding time window in order to quantify the muscle activation. The level of activation of SO and TA muscles was represented by the RMS-SO and RMS-TA. A time course analysis was performed in order to highlight any temporal difference in COP-AP, HR, EMG and EDA: the data were averaged over a 1 s sliding windows. In order to study the time-domain responses to painful stimuli in the four blocks, the data were entered into a 4 x 2 x 12 repeated measures ANOVA with three within-subjects factors: perspective (Self, OMLF, OS, OMHF) x valence (painful, non-painful) x time (12 sec). COP-AP delta was also computed on: averaged COP-AP values during the exposition of painful stimuli were subtracted to averaged COP-AP values during the exposition of non-

painful stimuli. This data entered in a repeated measures ANOVA with two within-subjects factors: perspective (Self, OMLF, OS, OMHF) x time (12 sec).

Pain ratings and RTs were submitted in two separated ANOVAs with perspective (Self, OMLF, OMHF, OS) and valence (painful, non-painful) as within-subject factors. Post hoc comparisons were carried out for all comparisons by means of the test Duncan. Trials with measures 2 SD above or below each individual mean for each condition in the pain rating task were excluded as outliers (2%). Pearson correlation was used to investigate whether a) behavioral measures of pain ratings, b) RTs of the behavioural measures and c) the self-report BES measures were significantly correlated with the COP-AP mean position.

Pearson's correlation coefficients were also calculated for studying the relationship among the participants' anteroposterior position (COP-AP) in the different perspectives and valence and: (i) pain ratings behavioural measure; (ii) physiological measures; (iii) self-report measures (BES and STAI). The threshold for statistical significance was $p < 0.05$ for all statistical analyses.

RESULTS

Behavioral results

Repeated measures ANOVA on pain ratings revealed a significant main effect of perspective [$F(3,132) = 12.9939$, $p = 0.000001$] and valence [$F(1,44) = 752.5905$, $p = 0.000001$], indicating that mean pain ratings were higher for the painful valence in all the four perspectives. A significant interaction was found between perspective and valence [$F(3,132) = 14.5520$, $p = 0.000001$] (Figure 2). Mean pain ratings with a painful valence were higher the OMLF perspective (mean pain ratings OMLF \pm s.d.= 5.79 ± 1.37) compared to the OMHF perspective (mean pain ratings OMHF \pm s.d.= 4.50 ± 1.68 ; $p=0.000003$), OS perspective (mean pain ratings OS \pm s.d.= 5.02 ± 1.48 ; $p=0.00001$) and Self perspective (mean pain ratings Self \pm s.d.= 5.47 ± 1.48 ; $p=0.03$). Moreover, mean pain ratings with a painful valence were higher in the Self perspective compared to the OMHF perspective ($p=0.00001$), OS perspective ($p=0.002$). Finally, mean pain ratings with a painful valence were higher in the OS perspective compared to the OMHF perspective ($p=0.0004$).

The ANOVA of the averaged RTs data revealed a significant main effect of valence [$F(1,44) = 201.1014, p = 0.000001$]. A significant interaction was found between perspective and valence in RTs data [$F(3,132) = 43.7692, p = 0.000001$] (Figure 3). Averaged RTs for painful valence were shorter for the Self perspective compared to all the other perspectives: OMLF perspective (mean RTs OMLF \pm s.d. = 1719.56 ± 522.98 ; $p=0.006$); OMHF perspective (mean RTs OMHF \pm s.d. = 1798.68 ± 401.50 ; $p=0.00005$), OS perspective (mean RTs OS \pm s.d. = 1860.91 ± 477.107 ; $p=0.000005$). However, no difference was found for the Self perspective between RTs recorded during the observation of painful and non-painful stimuli (mean RTs Self painful valence \pm s.d. = 1556.495 ± 843.8 ; mean RTs Self non-painful valence \pm s.d. = 1559.78 ± 948.436 ; $p=0.954$). Moreover, averaged RTs for painful valence recorded in the OMLF perspective were shorter compared to OS perspective ($p=0.01$) but no difference was found between averaged RTs recorded in OMLF perspective and OMHF perspective ($p=0.16$). No difference was found between RTs in the OMHF and OS perspectives ($p=0.28$) during the rating of stimuli with a painful valence.

Postural results

Repeated measures ANOVA yielded a significant main effect on COP-AP measure for perspective [$F(3,132)=4.968, p=0.0026$]. These results suggest that without taking into account the valence of the stimuli on COP-AP displacement the Self perspective resulted as more shifted backward, compared to the OMLF perspective ($p=0.02$) and OMHF ($p=0.0012$). The OS perspective adopted a withdrawal position compared to the OMHF ($p=0.006$). A significant main effect on COP-AP measure for valence [$F(1,44)=7.429, p=0.009$] was also recorded due to a rearward COP-AP displacement for painful stimuli ($M \pm$ s.d. = $-0.307\text{mm} \pm 0.39$) compared to non-painful stimuli ($M \pm$ s.d. = $+0.123\text{mm} \pm 0.69$). Time main effect was presented [$F(11,484)=4.099, p=0.000009$] revealing a greater backward shift of the COP-AP from the 3rd second. Significant interactions for the COP-AP measure were found between perspective and valence [$F(3,132) = 4.279, p = 0.006$] due to a rearward COP-AP displacement for the presentation of painful visual stimuli compared to non-painful visual stimuli for the OMLF perspective (painful stimuli: $M \pm$ s.d. = $-0.5 \text{ mm} \pm 0.45$ vs non-painful stimuli: $M \pm$ s.d. = $+0.17 \text{ mm} \pm 1.58$; $p = 0.001$) and for the OMHF perspective (painful stimuli: $M \pm$ s.d. = $+0.004 \text{ mm} \pm 0.25$ vs non-painful stimuli: $M \pm$ s.d. = $+0.846\text{mm} \pm 0.19$; $p=0.01$). Significant interactions for the COP-AP measure were found also between perspective and time [$F(33,1452)= 1.623,$

p= 0.01] and among perspective, valence and time [F(3,132)= 1.544, p= 0.025] (Figure 4). COP-AP mean position during the exposition of painful visual stimuli was significantly shifted backward for the OMLF perspective compared to the OMHF perspective at the following seconds: 3rd (M ± s.d.= -1.142mm ± 3.11 vs M ± s.d.= -0.14mm ± 1.58; p=0.01), 5th (M ± s.d.= -0.909mm ± 2.63 vs M ± s.d.= +0.239mm ± 1.722; p=0.005), 7th (M ± s.d.= -0.837mm ± 2.47 vs M ± s.d.= +0.14mm ± 2.04; p=0.01) and 8th (M ± s.d.= -0.756mm ± 2.18 vs M ± s.d.= +0.16mm ± 2.02; p=0.02). COP-AP mean position during the exposition of painful visual stimuli was marginally significant shifted backward for the OMLF perspective compared to the Self perspective at the 3rd second (M ± s.d.= -1.142mm ± 3.11 vs M ± s.d.= -0.33mm ± 2.26; p=0.01). In the Self perspective COP-AP mean position during the exposition of painful visual stimuli was significantly shifted backwards compared to OMHF perspective at the at the following seconds: 7th (M ± s.d.= -0.73mm ± 2.83 vs M ± s.d.= +0.14 mm± 2.04; p=0.037) and 8th (M ± s.d.= -0.67mm ± 2.97 vs M ± s.d.= +0.16mm ± 2.02; p=0.046). A difference was found for the COP-AP averaged position during the observation of painful stimuli between OS and OMHF perspectives: the COP-AP for the OS perspective was more shifted backward compared to the COP-AP recorded for the OMHF perspective at the following seconds: for the 5th (M ± s.d.= -0.93mm ± 2.23 vs M ± s.d.= +0.239mm ± 1.722; p=0.004) and 6th (M ± s.d.= -0.82mm ± 1.88 vs M ± s.d.= -0.005mm ± 2.26; p=0.05).

A repeated measure ANOVA on COP-AP delta (painful – non-painful stimuli) was also computed. Results revealed a significant main effect of perspective [F(3,132)=4.28, p=0.006]. OMLF COP-AP delta averaged measure (M ± s.d.= -1.1mm ± 0.49) was significantly more shifted backwards compared to the Self (M ± s.d.= +0.3mm ± 0.36; p=0.002) and OS (M ± s.d.= -0.07mm ± 0.33; p=0.02) perspectives. OMHF COP-AP delta averaged measure (M ± s.d.= -0.84mm ± 0.36) was significantly shifted backwards compared to the Self perspective (p=0.01). Significant interaction for the COP-AP delta averaged measure was found between perspective and time [F(33,1452)= 1.54, p= 0.02] (Figure 5) due to a rearward COP-AP delta displacement for the OMLF perspective compared to all the others perspectives (OMHP, OS, Self) at the 3rd, 4th and 5th second of stimuli presentation p<0.01).

Regarding the path[COP]-AP averaged values, significant main effects of perspective [F(3,132)=5.371, p=0.001], valence [F(1,44)=7.289, p=0.009] and time [F(11,484)=4.495, p=0.000002] were recorded. None significant interaction was found. A reduction of path[COP]-AP was recorded for the perspective OS (M ± s.d.= 15.59mm ± 0.22) compared to the Self (M

\pm s.d.= 16.17mm \pm 0.28; $p=0.0003$) and OMLF (M \pm s.d.= 16.06mm \pm 0.22; $p=0.003$) perspectives. Moreover, path[COP]-AP was significantly lower during the presentation of non-painful visual stimuli compared to painful visual stimuli ($p=0.009$).

Physiologic measures results

A repeated measures ANOVA on mean RMS-SO values revealed a significant main effect of valence [$F(1,44)=4.399$, $p=0.04$] due to a lower RMS-SO for the presentation of painful visual stimuli (M \pm s.d.= 0.018 \pm 0.0004) compared to non-painful (M \pm s.d.= 0.019 \pm 0.0004; $p=0.04$) visual stimuli; a marginally significant main effect of time on mean RMS-SO values [$F(11,484)=1.797$, $p=0.05$] was also recorded. Repeated measures ANOVA on mean RMS-TA values revealed a significant main effect of perspective [$F(3,132)=3.273$, $p=0.02$] due to an higher RMS-TA contraction for the Self perspective (M \pm s.d.= 0.0108 \pm 0.0006) compared to the OS (M \pm s.d.= 0.009 \pm 0.0002; $p=0.01$) and OMHF (M \pm s.d.= 0.009 \pm 0.0002; $p=0.02$) perspectives; a significant main effect of valence [$F(1,44)=6.83$, $p=0.01$] was also recorded due to an higher RMS-TA for the presentation of painful visual stimuli (M \pm s.d.= 0.01 \pm 0.0006) compared to the non-painful ones (M \pm s.d.= 0.009 \pm 0.0003; $p=0.01$).

SCR averaged data revealed a significant main effect of perspective [$F(3,132)=3.24$, $p=0.02$]. Specifically, SCR recorded for the OMLF perspective resulted (M \pm s.d.= 0.3 \pm 0.04) higher compared to all the others perspectives (Self: M \pm s.d.= 0.2 \pm 0.02, $p=0.03$; OS: M \pm s.d.= 0.16 \pm 0.007, $p=0.006$; OMHF: M \pm s.d.= 0.19 \pm 0.01, $p=0.02$). A significant interaction was found for the SCR averaged values between perspective and time [$F(33,1452)=1.71$, $p=0.007$] due to a difference between OMLF perspective and all the others perspectives in seconds ranging from 1 to 11 ($p<0.05$) (Figure 6).

HR averaged values revealed a significant main effect of time [$F(11,462)=3.919$, $p=0.000019$]: at the 3rd second HR averaged measures (M \pm s.d.= 87.54 \pm 10.85) were inferior to all the other seconds of stimuli presentation except for the 2nd and 4th seconds.

Correlations

Significant correlations between the COP-AP position with pain ratings, physiological and self-report measures are reported in this section.

COP-AP and Pain ratings

Negative significant correlation between COP-AP averaged values and pain ratings behavioral measures was recorded: the more the participants COP-AP position was shifted backward, the higher were the pain ratings for the Self perspective at the 3rd time of painful stimuli presentation ($r=-0.3$; $p=0.04$).

COP-AP and physiological measure (SCR)

Negative significant correlations between COP-AP averaged values and averaged SCR data were recorded: the more the participants COP-AP averaged position was shifted backward at the 2nd second of painful visual stimuli presentation, the higher was the averaged SCR at the 1st second of painful visual stimuli presentation for the Self perspective ($r=-0.35$; $p=0.018$). Similar results were obtained in the Self perspective for the COP-AP averaged position at 9th second of painful visual stimuli presentation and the averaged SCR at the 8th second of painful visual stimuli presentation ($r=-0.29$; $p=0.049$). Instead, positive correlations were found for the OS perspective: the more the COP-AP averaged position was shifted forward at the 4th second of painful visual stimuli presentation the more the averaged SCR was higher at the 3rd second of painful visual stimuli presentation ($r=0.31$; $p=0.033$). Similar results were obtained in the OS perspective for the COP-AP averaged position at the 8th second of painful visual stimuli presentation and the averaged SCR at the 8th second of painful visual stimuli presentation ($r=-0.36$; $p=0.014$). In addition, positive correlation was found for the OMHF perspective: the more the COP-AP averaged position was shifted forward at the 6th second of painful visual stimuli presentation the more the averaged SCR was higher at the 6th second of painful visual stimuli presentation ($r=0.29$; $p=0.046$).

COP-AP and Self-report measures

Negative significant correlations between COP-AP averaged values and the BES have been found. More specifically during the observation of non-painful stimuli for the Self perspective participants tended to shift more backwards, the higher was the total BES score for the 9th ($r=-0.38$; $p=0.009$) and the 10th ($r=-0.35$; $p=0.018$) second of stimuli presentation; for the Self perspective during the observation of non-painful stimuli participants tended also to shift more backwards the higher was the BES affective sub-score for the 9th ($r=-0.30$; $p=0.043$) and the 12th ($r=-0.32$; $p=0.03$) second of stimuli presentation. For the OMLF perspective during the observation of non-painful stimuli participants tended to shift more backwards, the higher was

the total BES score for the 11th ($r=-0.35$; $p=0.018$) and the 12th ($r=-0.32$; $p=0.029$) second of stimuli presentation; finally for the OMLF perspective during the observation of non-painful stimuli participants tended also to shift more backwards, the higher was the BES affective sub-score for the 10th ($r=-0.31$; $p=0.034$) and the 11th ($r=-0.42$; $p=0.004$) and the 12th ($r=-0.41$; $p=0.005$) second of stimuli presentation.

STAI trait scale scores positively correlated with the COP-AP position during the observation of non-painful stimuli in the OMLF perspective. Specifically, the more the participants tended to stay in a forwarded position, the higher the score was at the STAI trait scale at the following seconds: 1st ($r=0.41$; $p=0.005$), 2nd ($r=0.41$; $p=0.006$), 3rd ($r=0.36$; $p=0.016$) and 4th ($r=0.3$; $p=0.044$) of stimuli presentation.

DISCUSSION

The aim of the present research was to study the behavioral reactions of the witness faced to other's painful events. In particular, we studied the approach / avoidance and freezing observer's reaction and the modulation of these responses according to the level of proximity between the empathizer and the target of the empathic response. Our results can be summed as follows: (i) we observed a modulation of the pain-level ratings by the adopted perspective, with the highest for the most loved person (OMLF); (ii) the greater postural displacement in a backward direction during the observation of painful stimuli was recorded for the most loved person perspective (OMLF) compared to the other perspectives; (iii) physiological responses like SCR still confirmed the greater observer's reaction for the most loved person perspective (OMLF) compared to the other perspectives; (iv) correlations did not permit to completely explain the postural results in term of behavioural, physiological and self-report empathy measures.

The interest to study the proximity with the empathizer rose from several studies that analyzed the factors modulating empathy. Among others, the observation of loved persons in pain seems to have an important influence on the observer's empathic responses (Cheng *et al.* 2010). Concerning the behavioral pain ratings, it is well known that pain unpleasantness ratings are influenced by dispositional empathy (Cikara *et al.*, 2014; Riečanský *et al.*, 2014). Here, we confirmed our previous results (Bucchioni *et al.*, 2015): the level of pain was rated as more painful for the loved person (OMLF) compared to the hated person (OMHF), to the stranger

(OS) and to the Self. Considering the OMLF perspective as an “in-group” perspective, Montalan *et al.* (2012) obtained similar results showing that painful situations are judged as more painful when it was imagined that an “ingroup” member was undergoing pain compared to the self and the “outgroup” member. Reaction times also partially confirmed our previous findings: RTs were faster for the Self perspective in regards to all the others perspectives (Bucchioni *et al.*, 2015; Jackson *et al.*, 2006; Li & Han, 2010). The absence of difference of valence (pain / no pain) in Self perspectives RTs was also confirmed. Moreover, as in our previous study, the OMLF perspective was associated with the second shortest averaged RTs for the painful valence stimuli as it was obtained in our previous study (Bucchioni *et al.*, 2015).

Goubert *et al.* (2005) and Singer and Klimecki (2014), described in their models two mainly responses adopted by the empathizer when he is faced to another individual’s painful situation: approach the suffering empathic target to heal him or a withdrawal response of the observer faced to a person in a painful condition in order to adopt a self-protective behavior. Moreover, it has been shown that the observer of other’s painful events may adopt a freezing behavior (Avenanti *et al.* 2005; 2006; 2009; 2010). Postural changes might put in evidence freezing-like and avoidance behavior when an individual is faced to aversive stimuli such as emotional pictures and images of mutilations (International Affective Picture System, IAPS; Lang *et al.*, 2008) as previous studies have shown (Azevedo *et al.*, 2005; Facchinetti *et al.*, 2006; Stins & Beek, 2007). The originality of this study consists in recording for the first time postural changes during a perspective-taking task while participant observed pictures of hands and feet in painful and non-painful situations. In our study, postural responses were recorded while participants stood immobile on the posturographic platform and were instructed to imagine that themselves (Self) or a person they did not know (OS), or the person they loved the most (OMLF), or the person they hated the most (OMHF) was about to live the painful or non-painful situations depicted in the observed images. In accordance with previous studies, we found a withdrawal defensive response (i.e., rearwards mean COP displacement in the AP direction) for the OMLF perspective, when the participants observed pictures with a negative valence (in this case, painful stimuli) (Eerland *et al.*, 2012; Hillman *et al.*, 2004; Lelard *et al.*, 2014). The registration of a withdrawal behavior during the presentation of aversive stimuli supports the hypothesis that the dimension of valence is associated to approach and avoidance behaviors (Cacioppo *et al.*, 1993) and that individuals tend to avoid unpleasant situations (Lelard *et al.*, 2014). Moreover, this behavior may evidence a self-protective strategy, inducing threat

avoidance and a withdrawal behavior in order to protect ourselves (Yamada & Decety, 2009). Along with Goubert *et al.* (2005) and Singer and Klimecki (2014) theories, our results evidenced a withdrawal strategy applied by the observer when he imagined that the most loved person (OMLF perspective) was about to live a painful event and this behavior might evidence the observer's self-protective strategy. In this sense, it is possible that the witness of a painful event concerning the loved person tends to adopt a rearward position in order to avoid a situation of emotional distress. Observing a loved person in pain may induce in the witness a greater empathic distress compared to the other perspective-taking conditions; in order to reduce these negative feelings the observer egoistically tends to adopt a withdrawal position. These results were further confirmed by the delta COP-AP analysis: when the averaged COP-AP during the presentation of painful stimuli is subtracted from the averaged COP-AP during the presentation of non-painful stimuli, the OMLF perspective is significantly shifted backwards compared to all the others perspectives. A time-course analysis revealed that postural differences during the presentation of painful stimuli (relative to non-painful stimuli) and for the delta COP-AP analysis appeared 3s after the stimulus onset. This timing has been evidenced to be crucial for postural changes also in previous studies (Hagenaars *et al.*, 2014; Lelard *et al.*, 2014).

An alternative possible explanation to our findings is that the COP-AP might be influenced also by the step initiation. Naugle *et al.* (2011) have found that when participants looked at pleasant images compared to unpleasant ones this led to a greater rearwards displacement of the COP, because this balance shift facilitated the following forward step. From this point of view, the greater backwards displacement of the COP can be considered as a preparation to approach the loved person in a painful state, and might lead to prosocial helpful behaviors (Singer & Klimecki, 2014). Otherwise, it is important to consider that compared to Naugle *et al.* (2011), we asked to participants to stay as firm as possible. Moreover, it is important to take into account that the withdrawal response during the exposition of painful stimuli in the OMLF perspective is stable over time between the 1st and the 9th second of picture exposure, instead the Naugle *et al.* (2011) withdrawal response is quite fast (its reaction times is of 0.3 seconds) and quickly evolve in a forward step. Indeed, it is possible that our response would represent better an avoidance response.

The only two perspectives in which the COP-AP averaged position was distinguished in term of valence (more shifted backwards for the presentation of painful stimuli and forwarded

for the presentation of non-painful stimuli) were the OMLF and OMHF perspectives. These results suggest that the effect to have adopted the perspective of a known person plays a significant role in modulating the observer approach / avoidance responses according to the valence of the presented visual stimuli. We have found that when the witness adopted the OMHF perspective during the presentation of painful stimuli the COP-AP resulted to be more forwarded compared to all the others perspectives (until the 8th second of stimuli presentation). Cikara *et al.* (2014) in a social psychology study found that the antipathy felt for the “out-group” lead to empathize less with the “out-group” itself and also to feel pleasure when the “out-group” feels pain. Along these lines, the more forwarded position for the OMHF perspective during the presentation of painful stimuli might represent the absence of empathy distress in the observer.

Talking about the other postural parameter, we found a reduction of path [COP]-AP for the OS perspective compared to the Self, OMLF and OMHF perspectives. This result is only linked to the OS perspective, not to the valence of the stimuli. Studies about children's behavior faced to a stranger found that they tend to reply with a freezing behavior in response with this threatening situation (e.g.: Buss *et al.*, 2004). Anyway, it is important to consider that it is possible that adults and children when faced to a stranger behave differently. Moreover, we did not replicated the Lelard *et al.* (2013) results (reduction of path[COP]-AP during the presentation of painful images i.e., freezing effect) for the Self perspective, probably because the number of participants that took part in our study wasn't sufficient considering the presence of several variables in the present study.

Concerning the physiological measures, in particular the EMG results, we did not found any significant interaction both for the RMS-SO and RMS-TA mean values between the valence and perspective variables over time. Anyway, a significant greater RMS-TA mean value was recorded for the Self perspective compared to all others perspectives. The increase of RMS-TA represents the adoption of a stiffening strategy and these data partially replicated the data recorded in Lelard *et al.* (2013). In their experiment, the authors used the painful and non-painful visual stimuli from Jackson *et al.*, (2005) battery and they only used the Self perspective and report a significantly activation of RMS-TA for the observation of painful images (Lelard *et al.*, 2013). Moreover, in our study, the RMS-TA mean value was higher for the observation of painful images (main effect of valence), compared to the RMS-TA mean value recorded during the observation of non-painful images. RMT-SO mean value instead, was higher for the

observation of non-painful visual stimuli compared to the painful ones, confirming that the SO and TA are two antagonist muscles. The EMG results of our study could partially confirm our previously proposed hypothesis: in our study we suggest that when participants adopt the Self perspective, if a visual painful situation is presented to them, they tend to adopt a freezing behavior like in Lelard *et al.* (2013) study; unfortunately this behavior is not completely evidenced in our study. We suggest that an increased number of participants might have evidenced this behavior.

With regard to the SCR, we obtained similar results to Vico *et al.* (2010): they found an increasing of SCR amplitude when participants observed the face of loved persons and in our study, a significant augmentation of SCR was found exactly for the OMLF perspective. No significant effect of valence was found. Previous studies recorded a significant increasing of SCR associated to the presentation of emotional visual stimuli (Horslen & Carpenter, 2011). Moreover, Lelard *et al.* (2013) did not record this SCR magnitude increase associated to painful visual stimuli and they suggested that painful visual stimuli presentation may have a lower arousal compared to the emotional stimuli used in previous studies (IAPS, images of mutilation). The HR analysis was not particularly informative: only the time variable results evidenced significant a bradycardic effect appearing at the 3rd second of stimuli presentation. These data are in partial accordance with previous results (Leard et al, 2014) results where it was evidenced that at the 3rd second of visual stimuli presentation a reduction in HR for the presentation of aversive stimuli was recorded. Indeed, this timing seems to be crucial not only for postural measures variations but also for physiological variables.

Regarding the computed correlations analyses, we found a significant correlation between pain ratings and COP-AP mean position at the 3rd second of stimuli presentation, confirming the backward displacement for the presentation of painful visual stimuli (the higher was rated the painful level of the stimuli, the greater was the backwards displacement). Unfortunately, this correlation was found only for the Self perspective. Talking about the correlations with the BES scales, we found some correlations with the OMLF perspective at different timing, but only for non-painful stimuli presentation. Indeed, an important limit to this study is the absence of correlations that can really explain our data, mostly concerning the OMLF perspective, the perspective that has given the more significant results on postural parameters.

Talking about future perspectives to this study, it would be interesting to apply our paradigm to a step initiation task in a perspective taking task during the observation of other's painful stimuli in order to completely disentangle the approach / avoidance hypothesis. Moreover, further studies analyzing whole body kinematics might better clarify the real meaning of this backwards displacement. Finally, we suggest to increase the number of participants in the study. In conclusion, the present findings shows that there is an effect of perspective-taking during the observation of painful stimuli and this influences the posture, inducing a withdrawal position for the most loved person perspective (OMLF). The concomitant greater activation of skin conductance during this perspective further highlights the importance and the involvement of the observer's postural and physiological system when he/she is projected into the loved person perspective.

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FIGURES AND CAPTIONS

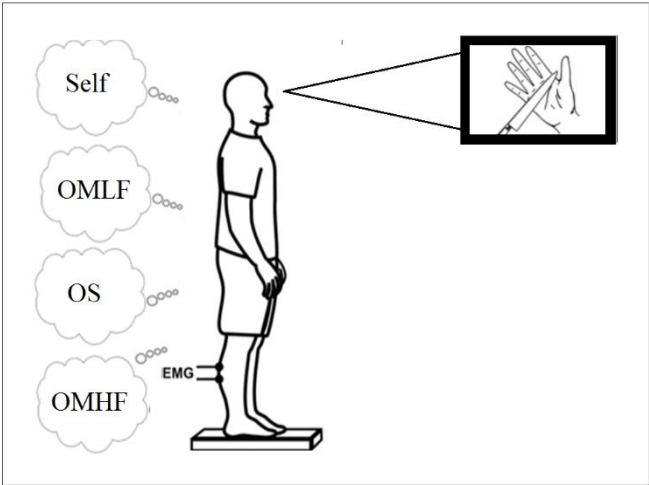


Figure 1: Experimental setting. Participant stood on the platform while he was looking at painful and non-painful visual stimuli and while he was adopting one of the four perspective-taking conditions (Self, OMLF, OS, OMHF).

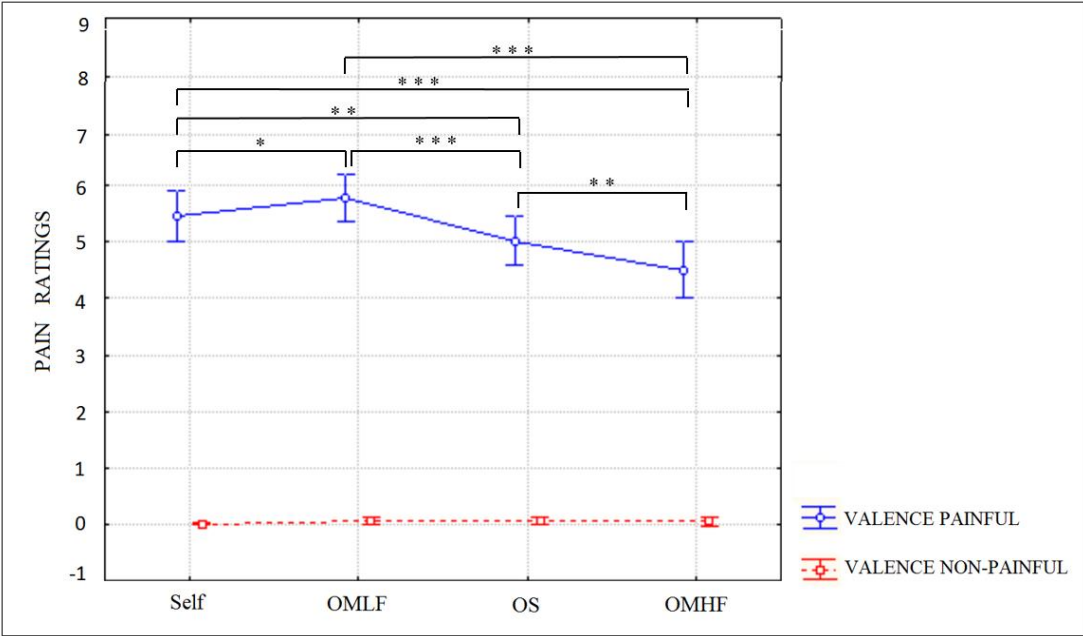


Figure 2: Pain ratings results. Significant interaction between perspective (Self, OMLF, OS, OMHF) and valence of stimuli (painful, non-painful). Vertical bars indicate the standard error of the mean (SEM). Significant differences are indicated as: *p<0.05; **p<0.01; ***p<0.0001.

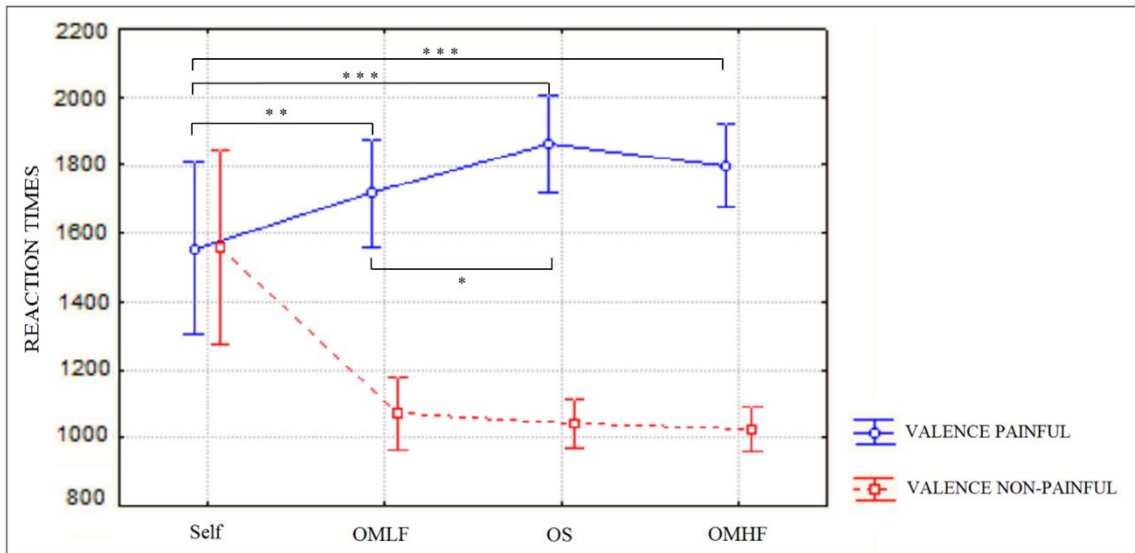


Figure 3: Reaction times (ms) results. Significant interaction between perspective (Self, OMLF, OS, OMHF) and valence of stimuli (painful, non-painful). Vertical bars indicate the standard error of the mean (SEM). Significant differences are indicated as: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$.

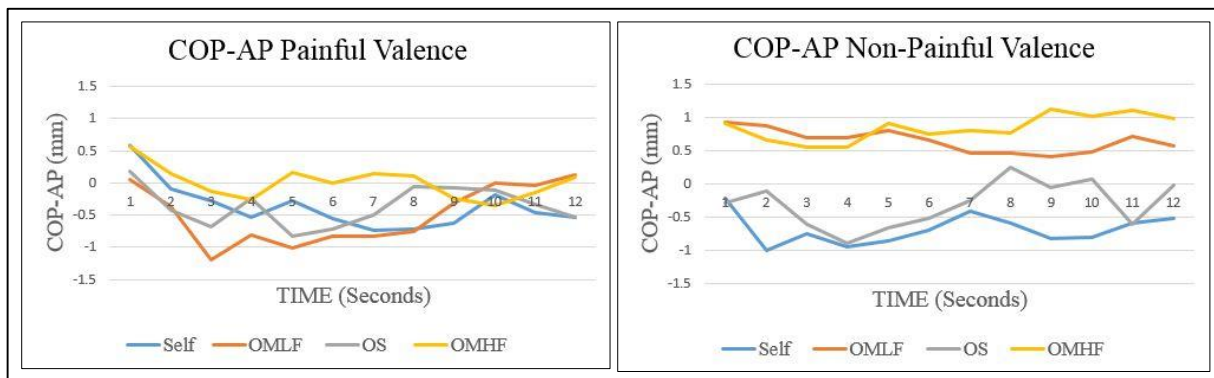


Figure 4: COP-AP results. Significant interaction between perspective (Self, OMLF, OS, OMHF) valence of stimuli (painful, non-painful) and time (1-12 sec).

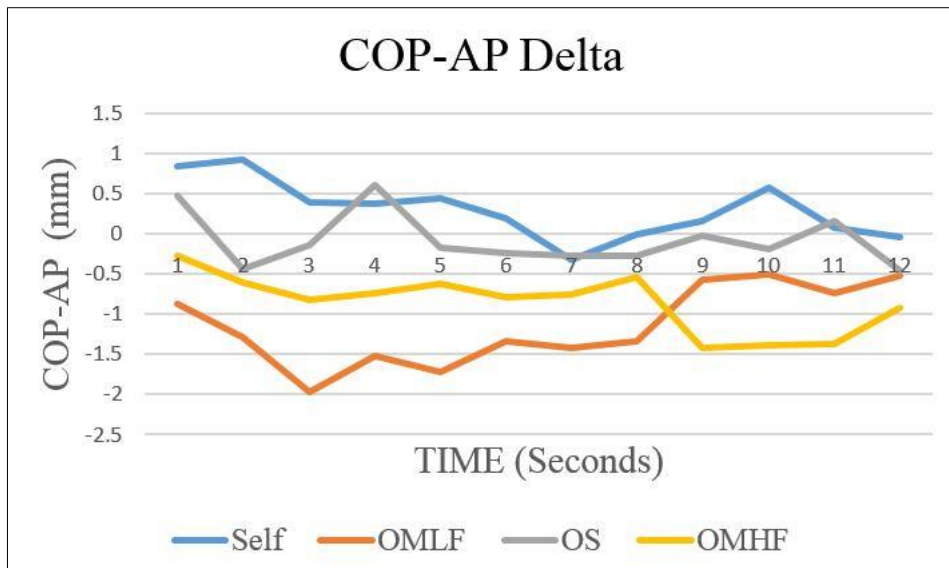


Figure 5: COP-AP delta results. Significant interaction between perspective (Self, OMLF, OS, OMHF) and time (1-12 sec).

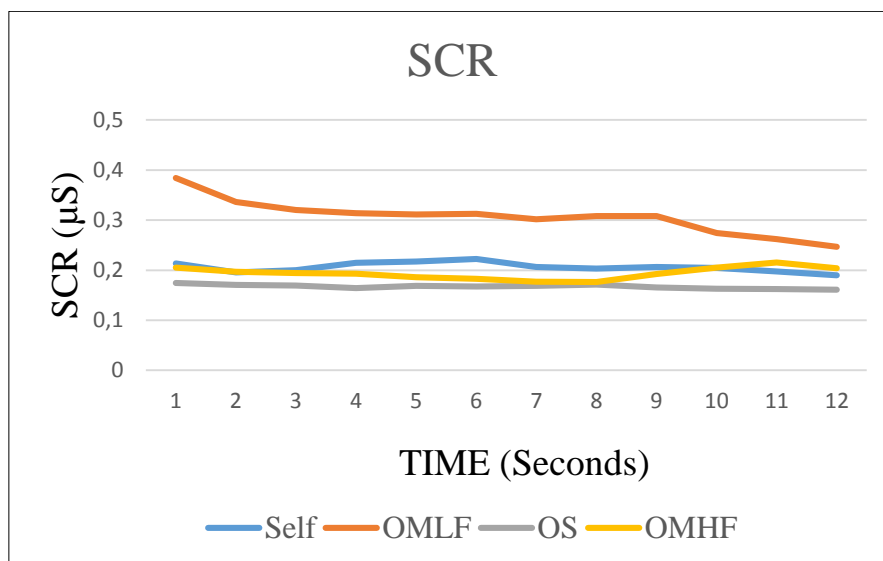


Figure 6: SCR results. Significant interaction between perspective (Self, OMLF, OS, OMHF) and time (1-12 sec).

3. Experiment 3: Empathy or Ownership? Evidence of corticospinal modulation during pain observation.

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In the third experiment of the thesis, we analyzed the self / other perspective in empathy for pain task as we did in the previous two experiments. Here we focused the attention on the corticospinal system responses during the observation of others' pain. The mere observation of painful stimuli administered on the actor's body produces a fine-grained modulation of corticospinal system in the observer (freezing-effect; Avenanti *et al.*, 2005), comparable to the direct experience of painful stimuli. Previous studies (Avenanti *et al.*, 2005, 2006, 2009; 2010) have shown an inhibition of the observer's corticospinal system when the hand that receives a painful stimulus is presented in first-person perspective. However, it is crucial to ask what happens when the observed hand is presented in third-person perspective, the one in which, in everyday life, we perceive the others' body parts (Ruby and Decety, 2001, Saxe *et al.*, 2006).

In this study, we investigated if the inhibition of the corticospinal system during the observation of the hand receiving painful stimuli is due to an empathic resonance with another individual (representing the physiological basis of empathy) or to the incorporation of the observed hand in the observer's sensorimotor system (representing the physiological counterpart embodiment phenomenon, related to the sense of body-ownership). We know that by simply looking at a fake hand, whenever positioned in a body-congruent egocentric perspective, may lead the subjects to experience it as part of their own body. In the present study, we compared the empathy and the ownership hypothesis, by manipulating, during observation-conditions, the perspective of the view of a hand model receiving pain. Similar results in both the first-person and third-person perspectives would confirm the empathy hypothesis; a different result in the first-person perspective (where the embodiment occurs) would confirm the body-ownership hypothesis.

Therefore, we used transcranial magnetic stimulation (TMS) to record changes in corticospinal motor representations of the hand, while subjects observed videos showing (i) a

needle penetrating or (ii) a Q-tip touching the model's hand, presented either in a first-person or in a third-person perspective. Motor evoked potentials (MEPs) were recorded from the right first dorsal interosseous hand muscle. Here, the perspective-taking paradigm (i.e., to adopt the formerly used perspectives: Self, OMLF, OS, OMHF) was not applied, because a previous study had evidenced the inefficacy to give different instructions on the corticospinal response (Avenanti, Paluello, Bufalari, & Aglioti, 2006). Results showed that compared to the third-person perspective, a significantly greater reduction of the mean MEPs amplitude (freezing-effect) was found when the hand model receiving pain (needle-penetration) was presented in an egocentric perspective.

In conclusion, this finding suggests that the freezing effect during pain observation can be better explained by the body-ownership than by the empathy hypothesis.

**Empathy or Ownership? Evidence from corticospinal modulation during pain
observation**

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Abbreviated title. Empathy or Ownership?

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Abstract

Recent studies show that motor responses similar to those present in one's own pain (freezing-effect) occur as a result of pain-observation in others. This finding has been interpreted as the physiological basis of empathy. Alternatively, it can represent the physiological counterpart of an embodiment phenomenon related to the sense of body-ownership. We compared the empathy and the ownership hypotheses by manipulating the perspective of the observed hand-model receiving pain so that it could be a first-person perspective, the one in which embodiment occurs, or a third-person perspective, the one in which we usually perceive the others. Motor evoked potentials (MEPs) by TMS on M1 were recorded from FDI muscle, while subjects observed video-clips showing a) a needle penetrating or b) a Q-tip touching a hand-model, presented either in first-person or in third-person perspective. We found that a pain-specific inhibition of MEPs amplitude (a significantly greater MEPs reduction in the 'pain' compared to the 'touch' conditions) only pertains to the first-person perspective and it is related to the strength of the self-reported embodiment. We interpreted this corticospinal modulation according to an "affective" conception of body-ownership, suggesting that the body I feel as my own is the body I care more about.

INTRODUCTION

In the first decade of the 21st century, the mirror neurons paradigm (Rizzolatti, Cattaneo, Fabbri-Destro, & Rozzi, 2014) has exercised a strong influence in cognitive neuroscience and, from the domain of action where it was discovered, a “mirror-matching” simulation mechanism has been extended to others domains, including emotional experience (Gallese, 2003; Keysers et al., 2004; Singer et al., 2004). According to this mechanism, the emotional state of an individual activates corresponding representations in another individual observing that state. In a seminal paper, Avenanti and colleagues (Avenanti, Buetti, Galati, & Aglioti, 2005) demonstrated that motor responses, similar to those present in one’s own pain (i.e. freezing-effect; occur as a result of pain observation in others. Consistent with the “mirror-matching” simulation theory, this finding has been interpreted as the physiological basis of empathy for other’s pain (Singer & Frith, 2005).

In the Avenanti and colleagues (2005) paper, as well as in a series of further papers, different variables have been manipulated, such as the stimulus category (e.g., hand vs tomato; Avenanti et al., 2005), the recorded muscle (e.g., FDI vs ADM; (Avenanti et al., 2005), the observed limb (e.g., hand vs foot; Avenanti *et al.*, 2005, 2006), the stimulus intensity (e.g., hand penetrated by a needle vs hand pricked; (Avenanti et al., 2006), the observed hand congruency (e.g., right vs left; Avenanti, Minio-Paluello, Sforza, et al., 2009), the observed hand race (e.g., in-group vs out-group; Avenanti et al., 2010). However, to the best of our knowledge, the stimulus presentation perspective has never been investigated and the hand model has been always presented in a first-person perspective.

In recent years, the increasing interest for the concept of body-ownership (i.e. the belief that a specific body part belongs to one’s own body; e.g., Tsakiris, 2010) pays specific attention to the relation between the perspective through which a body-part is observed and the possibility for the subjects to experience it as part of their own body (i.e., embodiment phenomenon).

Converging evidence, coming from experimental manipulations in healthy subjects (e.g., rubber-hand-illusion; Costantini and Haggard, 2007) and pathological conditions after brain damage (e.g., delusion of body-ownership; Garbarini et al., 2013, 2014, 2015), shows that embodiment occurs only when the rubber/alien limb is located in a position coherent with the subjects' higher-order and pre-existing body representation, whenever it is perceived from a first-person perspective.

In the present study, we aimed at disentangling the empathy and the ownership hypothesis by manipulating the perspective of the observed hand model receiving pain, so that it could be a first-person perspective, the one in which embodiment occurs, or a third-person perspective, the one in which, in everyday life, we perceive the body parts of others (Ruby & Decety, 2001). If the pain-specific corticospinal modulation, found by Avenanti and colleagues' studies when stimuli were presented in a first-person perspective, also occurs in a third-person perspective, this would confirm the empathy for others' pain hypothesis. Alternatively, a perspective-dependent effect, only related to the first-person viewpoint, would suggest that this pain-specific corticospinal modulation represents the physiological counterpart of an embodiment phenomenon, related to the sense of body-ownership.

MATERIALS AND METHODS

Participants

Twenty participants took part in the experiment (12 women; mean age \pm s.d. = 24.3 ± 3.34 , range 20 - 36). Due to technical problems during MEPs recording, three subjects were excluded from the analysis, resulting in a sample of seventeen participants (10 women; mean age \pm s.d. = 24.12 ± 3.59 , range 20 - 36). All were right-handed according to the Standard Handedness Inventory (Oldfield, 1971), with normal or corrected-to-normal visual acuity. None of them had a history of neurological, major medical, or psychiatric disorders and they were free from any

contraindication to transcranial Magnetic Stimulation (TMS) (Rossi, Hallett, Rossini, & Pascual-Leone, 2009). Before starting the experimental session each participant was naïve as to the purposes of the study and signed an informed consent; information about the study purpose were provided only at the end of the experimental session. The experimental procedure was granted by ethical approval of Ethics Committee of the University of Turin and was carried out in accordance with the principles of the revised Helsinki Declaration (World Medical Association General Assembly, 2008). None of the participants reported discomfort or adverse effects during TMS acquisitions.

Stimuli

Four different color video-clips were used as experimental stimuli: (i) a right hand presented in first-person perspective deeply penetrated by a needle on the First Dorsal Interosseus (FDI) muscle (“Pain first-person”); (ii) a right hand presented in third-person perspective deeply penetrated by a needle on the FDI muscle (“Pain third-person”); (iii) a right hand presented in first-person perspective touched by a Q-tip on the FDI muscle (“Touch first-person”); (iv) a right hand presented in third-person perspective touched by a Q-tip on the FDI muscle (“Touch third-person”). Moreover two further video-clips were used as baseline condition: a) a dorsal view of a right hand presented in first-person perspective (“Baseline first-person”); b) a dorsal view of a right hand presented in third-person perspective (“Baseline third-person”).

TMS stimulation and EMG recording

TMS is a non-invasive brain stimulation technique extensively used in cognitive neuroscience (Miniussi, Harris, & Ruzzoli, 2013). In the present study, TMS pulses were administered using a Magstim Rapid2 stimulator (Magstim, Whitlan, Dyfed, Wales, UK) connected to a 70-mm

figure-of-eight coil positioned over the left primary motor cortex (M1) hand region. The coil was held tangentially to the scalp with the handle pointing backwards and laterally with a 45° angle to the midline. This orientation permits the lowest motor threshold, optimizing the stimulation (Brasil-Neto, Pascual-Leone, Valls-Sole, Cohen, & Hallett, 1992). Before the recording session the coil was moved in steps of 1 cm over the left motor cortex to determine the individual optimal position (OSP) from which maximal MEPs amplitudes were elicited in FDI. Once the OSP was found, the individual resting motor threshold (rMT) was determined as the lowest stimulus intensity that induced at least five MEPs (at list 50 μ V peak-to-peak amplitude) out of ten consecutive TMS pulses in the recorded muscle (Rossini et al., 2015). Mean rMT was 58% (ranging from 41% to 78%) of maximum stimulator intensity. During the recording session stimulation intensity was set at 115% of the rMT. MEPs were recorded from the FDI muscle of the participant's right hand. The registration of this muscle was selected because it is the same muscle penetrated by a needle or touched by a Q-tip in the presented video-clips. Electromyographic (EMG) activity was recorded by pairs of Ag–AgCl surface electrodes (11 mm diameter) (EL503) connected to a Biopac MP-150 electromyograph (Biopac Systems Inc., Santa Barbara, CA). They were placed in a classical belly-tendon montage: the active electrode over the muscle belly and the reference electrode over the associated joint or tendon. The ground was placed over the participant's left elbow. EMG signal was sampled (10 kHz), amplified, filtered with a 50 Hz notch filter and stored on a PC for off-line analysis.

Procedure

The experiment was carried out in a dimly illuminated room where participants were seated in a comfortable armchair with their head positioned on a fixed head rest. A single experimental session lasted 1 hour and 45 min approximately and each session was divided in two blocks. The task (see Figure 1) consisted in watching video-clips displayed on a 17-inch monitor

(resolution 1280 x 780 pixels, refresh frequency 60 Hz, background luminance of 0.5 cd/m²) placed at a distance of 80 cm. Participants were instructed to lay motionless on the armchair and to keep their hands in a prone position on a pillow, trying to relax the muscles as much as possible. TMS-induced MEPs from the right FDI muscle were acquired once for each video presentation at one of two possible time points: early time (200 ms after needle penetration / Q-tip touch) and late time (600 ms after needle penetration / Q-tip touch). These stimulation times correspond to the earliest and the latest stimulation times used in previous experiments (Avenanti et al., 2005, 2006, 2010; Avenanti, Minio-Paluello, Bufalari, & Aglioti, 2009; Avenanti, Minio-Paluello, Sforza, et al., 2009), where the TMS pulse was randomly triggered between 200 and 600 ms before the end of the video-clip. Here, we controlled the time variable, stimulating at two defined time points (early; late), since the literature recently evidenced two different phases in the functional modulation of the motor cortex: an earlier time of stimulation should evidence an orienting response; a later time of stimulation might represent motor resonance (Borgomaneri, Gazzola, & Avenanti, 2014). Each video-clip presentation was followed by 8200 ms of inter trial interval: a white fixation cross was presented for 7200 ms and was then replaced by a green cross (1000 ms) prompting the participant to watch the new video-clip. Each video-clip lasted 1800 ms. For each block, video-clips of each condition were presented 9 times in a random order resulting in a total of 72 trials (4 video-clips x 9 repetitions x 2 time points). Baseline measures of the corticospinal excitability were also assessed prior to and following the video presentations by means of two supplementary series of 12 MEPs. A static hand was randomly presented 6 times for each of the two perspectives (first-person and third-person). TMS stimulation was delivered 1424ms after stimulus onset. Thanks to these series of MEPs registrations, we checked for any corticospinal excitability change related to TMS per se between the beginning and the end of each experimental block; these MEPs average amplitudes were calculated to set individual baselines for data normalization. The stimulus-

presentation timing, EMG recording and TMS triggering, as well as stimuli randomization, were controlled by E-Prime V2.0 software (Psychology Software Tools Inc., Pittsburgh, PA, USA) running on a PC.

Self-report measures of Body-Ownership and Empathy

At the end of the two experimental blocks a body-ownership questionnaire was administered. An image representative for each experimental condition (Pain first-person, Pain third-person, Touch first-person, Touch third-person) was shown to participants along with an item-question referred to the condition depicted in the image: “*I felt as if I was perceiving the touch/penetration on my own hand*”. Participants were asked to answer using a seven points likert scale from -3 (i.e.: I don’t agree at all) to 3 (i.e.: I totally agree) measuring subjective agreement with the presented statements. At the end of the experiment, the Italian version (Bonino, Coco, & Tani, 2010) of the Interpersonal Reactivity Index (IRI) (Davis, 1983) was administered.

Data Analysis

In order to prevent contaminations of MEPs by background EMG activity, trials with any background activity greater than 50 μV in the 100 ms window preceding the TMS pulse were excluded from the MEPs analysis. EMG data were collected for 300 ms after the TMS pulse. Data were analyzed offline using AcqKnowledge software (Biopac Systems, Inc., Santa Barbara, CA) and Statistica Software 6.0 (StatSoft Inc., Tulsa). Averaged peak-to-peak amplitudes of MEPs recorded on FDI were computed separately for each condition (Pain first-person, Pain third-person, Touch first-person, Touch third-person) and for the two stimulation conditions (early and late). MEPs amplitudes deviating more than 2 standard deviations from the mean for

each condition and trials contaminated by muscular pre-activation were excluded from the analyses and considered as outliers (2%).

In order to control for the possible effect of TMS *per se* in modulating corticospinal excitability, a preliminary analysis was conducted by means of a 2x2x2 repeated measure ANOVA on the baseline mean raw MEPs values with perspective (first-person, third-person), block (first, second) and session (before, after the experimental block) as within subjects factors. In the main analysis of the physiological data, for each block, the MEPs values recorded from each experimental condition were averaged and normalized as percentage of the mean MEP value recorded from the baseline condition of each experimental block ($\text{MEP ratio} = \text{MEP}_{\text{obtained}} / \text{MEP}_{\text{baseline}} * 100$). Normalized data were entered into a 2x2x2 repeated measure ANOVA with perspective (first-person, third-person), time of stimulation (early, late) and valence of stimuli (pain, touch) as within-subjects factors. Post hoc comparisons were performed by means of Duncan test.

For the body-ownership questionnaire, the subjects' rating scores in each condition were averaged and entered into a 2x2 repeated measure ANOVA, with valence of stimuli (pain, touch) and perspective (first-person, third-person) as within experimental factors. Post hoc comparisons were carried out using the Duncan test. In order to examine whether a correlation existed between the physiological data and the subjective report, we also performed, in two different models for each time of stimulation (early or late), a linear regression where the normalized MEPs values were used as predictors for the body-ownership questionnaire ratings at each experimental condition (Pain first-person, Pain third-person, Touch first-person, Touch third-person). Finally, according to the Avenanti and colleagues' (2005) method, for correlation analyses with the scores obtained at the IRI subscales, we computed an index of MEPs amplitude change, as follows: MEPs amplitude during the pain condition minus amplitude during the corresponding (first-person or third-person) baseline condition divided by the

average of the same two conditions. For each pain condition (early pain first-person, early pain third-person, late pain first-person, late pain third-person), the obtained values were used to predict the scores obtained at the IRI subscales.

RESULTS

EMG Results

Preliminary analysis on the MEPs acquired during the baseline conditions showed neither significant main effects nor interactions. This means that a) non-specific perspective effects were absent [Perspective: $F(1,16) = 0.22$, $P = 0.64$]; b) the cortical excitability was unchanged in the second compared to the first experimental block [Block: $F(1,16) = 0.9$, $P = 0.76$]; c) TMS *per se* did not induce any change in corticospinal excitability [Session: $F(1,16) = 1.47$, $P = 0.24$]. Repeated measures ANOVA on normalized MEPs amplitudes revealed a significant interaction among perspective, time of stimulation and valence of stimuli [$F(1,16) = 4.4946$, $P < 0.05$] (see Figure 2). This indicates that a pain-specific inhibition of MEPs amplitude (i.e., a significantly greater MEPs reduction in the pain compared to the touch condition) only pertains to the late time of stimulation and to the first-person perspective (MEPs mean amplitude \pm s.d.: late touch first-person = 0.92 ± 0.49 ; late pain first-person = 0.67 ± 0.21 ; $P = 0.01$). No difference between pain and touch conditions was found at the early time of stimulation or when stimuli were presented in third-person perspective. Overall, the MEPs amplitude in the late first-person pain conditions was significantly lower with respect to all the other conditions ($P < 0.05$ for each post hoc comparison). It is interesting to note that a significant difference between first- and third-person perspective only pertains to the pain condition in the late time of stimulation (MEPs mean amplitude \pm s.d.: late pain first-person = 0.67 ± 0.21 ; late pain third-person = 0.86 ± 0.37 ; $P = 0.037$). No significant perspective effect was found in the early time of stimulation or for the touch conditions. Examples of MEPs recorded from the FDI muscle of a representative subject are shown in Figure 3.

Self-report measure results

The repeated measure ANOVA on the body-ownership scores showed a main effect of valence of stimuli [$F(1,16)= 7.33, P= 0.01$] and perspective [$F(1,16)= 11.87, P= 0.003$], suggesting a higher rating in pain compared to touch stimuli (mean \pm s.d.: Pain= -0.48 ± 2.28 ; Touch= -1.23 ± 1.88) and in first-person compared to third-person perspective (mean \pm s.d.: first-person= -0.08 ± 2.19 ; third-person = -1.63 ± 1.74). In the regression analyses, the normalized MEPs values, recorded at the late time of stimulation, significantly predict the ratings reported at the body-ownership questionnaire: the smaller the MEPs amplitude, the higher the self-reported embodiment over the observed hand model (linear regression body-ownership rating by MEPs amplitude: $r = -0.23; P = 0.05$; see Figure 4. Furthermore, no significant correlation between the index of MEPs amplitude change and the IRI questionnaire scores was found.

DISCUSSION

In the present study we asked whether pain-specific motor responses occurring during pain observation can represent, as previously suggested (Avenanti et al., 2005), the physiological basis of empathy or, alternatively, can be better explained by an embodiment mechanism related to the sense of body-ownership. To answer this question, we manipulated the perspective of the observed hand model receiving pain, while MEPs to single-pulse TMS on left M1 were recorded from the right FDI muscle. According to the Avenanti and colleagues studies, a pain-specific corticospinal modulation can be described as a significant decrease of the MEPs amplitude in pain compared to touch conditions. However, our results show that this motor response only pertains to the late time of stimulation and, most importantly, to the first-person perspective.

The evidences concerning the onset of the modulation of the corticospinal excitability after an observed action are rather contradictory. While some studies show that modulation of TMS-induced MEPs can occur 60-90 ms after the salient stimulus (Lepage, Saint-Amour, & Théoret,

2008), others studies fail to report this early modulation, suggesting that muscle-specific modulation can be induced only by late components of the mirror response (Cavallo, Heyes, Becchio, Bird, & Catmur, 2014). These findings have raised the intriguing hypothesis of a separation between early and late components of the mirror response (e.g. Candidi et al., 2014; for a review see Naish et al., 2014): an initial muscle-unspecific modulation would be followed by a later phase of modulation which would be muscle-specific (Romani, Cesari, Urgesi, Facchini, & Aglioti, 2005) and then closely related to a motor resonance mechanism (Borgomaneri et al., 2014). Our findings corroborate this two-stage hypothesis showing an effect of time on CS excitability.

The literature supporting the empathy for pain hypothesis, i.e., that the same neural mechanism underpinning the perception of physical pain can be involved in the observation of others' pain (e.g., Avenanti et al., 2005; Godinho et al., 2006; Lamm et al., 2011; Singer et al., 2004; Valeriani et al., 2008) also suggests that self-related variables, such as the proximity and the tangibility of the observed pain, can play a crucial role in determining the empathetic experience (e.g., Jackson et al., 2006; de Vignemont and Singer, 2006). Along this line of research, for instance, Mahayana and colleagues (Mahayana et al., 2014) have found, during the observation of others' pain, a significant corticospinal inhibition (i.e., reduction in MEPs amplitude) for stimuli presented in peripersonal space and not for stimuli presented in extrapersonal space. The authors interpreted this proximity-related response as a consequence of the misidentification of sensory information as being directly related to the observer. However, in the context of the empathy for pain hypothesis, the stimulus presentation perspective has never been investigated and the hand model has been always presented in a first-person perspective. However, to corroborate the empathy for others pain hypothesis, a pain-specific effect should also be found when the stimuli are presented in a third-person perspective, the one in which we usually perceive and interact with the body parts of others (e.g., Ruby and Decety, 2001).

The notion about the importance of the perspective through which a body-part is observed comes from the results of the embodiment-related literature, investigating the alterations of the sense of body ownership both in experimental manipulations in healthy subjects and in pathological conditions after brain damage. One of the more compelling demonstrations of the mechanisms subserving body ownership has been obtained in healthy participants by means of an experimental procedure known as the rubber hand illusion (e.g., Botvinick and Cohen, 1998). Essentially, watching a rubber hand being stroked while one's own unseen hand is stroked synchronously can lead to a sense of ownership over the rubber hand (as self-reported at the body-ownership questionnaire) and to a shift in the perceived position of the real hand (as measured by the proprioceptive drift). It has been demonstrated that simply looking at a fake hand being approached by the experimenter's hand, can lead the subjects to experience it as a part of their own body only when the hand is positioned in a body-congruent, first-person perspective (Ferri, Chiarelli, Merla, Gallese, & Costantini, 2013). Accordingly, previous studies have shown that the illusion effect disappears when the fake hand is rotated (i.e., it is perceived from a third-person perspective) or misaligned with respect to the subject's shoulder (Austen, Soto-Faraco, Enns, & Kingstone, 2004; Costantini & Haggard, 2007; Farnè, Pavani, Meneghello, & Làdavas, 2000; Lloyd, 2007; Pavani, Spence, & Driver, 2000). In brain damaged subjects, a monothematic delusion of body-ownership has been described where patients treat and care for the examiner's hand as if it was their own, showing a consistent embodiment of the alien hand in their own body schema (Garbarini et al., 2013, 2014, 2015; Pia, Garbarini, Fossataro, Fonia, & Berti, 2013). This delusion of ownership, although resembling the rubber hand illusion, is spontaneous and not induced by any experimental procedure. Interestingly, as for the rubber hand embodiment, this phenomenon occurs only when the alien hand is perceived in a first-person perspective and it is aligned with the patients' contralesional shoulder, exactly where it is normally expected to be. If the alien hand is

perceived from a third-person perspective or it is misaligned with respect to the patient's shoulder, the pathological embodiment does not occur and patients correctly identify their own hand.

According to a philosophical definition of the term "Embodiment", "*E is embodied if and only if some properties of E are processed in the same way as the properties of one's body*" (de Vignemont, 2010). In line with this definition, by recording the skin conductance response during noxious stimulations, previous studies on the rubber hand illusion in healthy subjects (Armel & Ramachandran, 2003; Ferri et al., 2013; Guterstam, Petkova, & Ehrsson, 2011) and on the pathological embodiment after brain damage (Garbarini et al., 2014), showed that an alien hand can be so deeply embedded into one's own somato-sensory experience as to elicit physiological reactions specific to the own hands. In the present study, we demonstrated that a motor response, comparable to that found when the subjects receive nociceptive stimuli on their own body (freezing-effect; Farina et al., 2003; Urban et al., 2004), also occurs when the nociceptive stimuli were delivered to someone's else hand, whenever it is perceived in a first-person perspective, automatically leading to a sort of embodiment (e.g. Ferri et al., 2013). On the one hand, our physiological data showed that the MEPs amplitude was significantly lower in the pain compared to the touch condition only in a first-person perspective; on the other hand, the behavioral data showed that the self-reported embodiment over the perceived hand model was significantly greater in a first-person compared to a third-person perspective and in the pain compared to the touch condition. Crucially, physiological and behavioral data were significantly correlated: the stronger the freezing effect, implicitly measured as a drop in the MEPs amplitude recorded from the FDI muscle, the stronger the embodiment sensation, explicitly reported at the body-ownership questionnaire. This also suggests a mutual interaction between our conscious beliefs about the body and the physiological mechanisms subserving the body image.

It is worth noting that the perspective-dependent effect we describe only pertains to the pain condition at the late time of stimulation. A previous study, investigating the perspective effect on motor imagery, showed a greater facilitation of MEPs recorded from FDI in third-person imagery, where the action was clearly attributable to another person, with respect to first-person imagery (Fourkas, Avenanti, Urgesi, & Aglioti, 2006). Together with our results, these findings showed lower values when MEPs were recorded in first-person compared to third-person perspective. Thus, it was crucial to investigate the presence of a non-specific perspective effect. However, we did not find a significant perspective effect either at the early time of stimulation or in the touch condition. Furthermore, no difference was found between the baseline values recorded when the hand model was presented in a first-person and in a third-person perspective, suggesting absence of a non-specific perspective effect.

The key finding of the present study is that a pain-specific inhibition of MEPs amplitude (i.e., a significantly greater MEPs reduction in pain compared to touch conditions) only occurs in a first-person perspective. On the contrary, no difference between pain and touch conditions was found when stimuli were presented in a third-person perspective. Crucially, the corticospinal excitability was directly related to the extent to which the subjects reported, while observing the hand model being penetrated, to feel “as if” their own hand was penetrated. On the contrary, unlike previous studies (Avenanti et al., 2005), no significant correlation between the index of MEPs amplitude change and the empathetic traits, as reported at the IRI questionnaire, was found, at least in our sample. Taken together, these findings suggest that the motor response of the onlooker can be better interpreted referring to the concept of body-ownership than to the empathy for others’ pain hypothesis. In particular, these data are suggestive of an “affective” conception of body-ownership (de Vignemont, 2014), indicating that the body I feel as my own is the body I care more about, the one to which I react when under threat.

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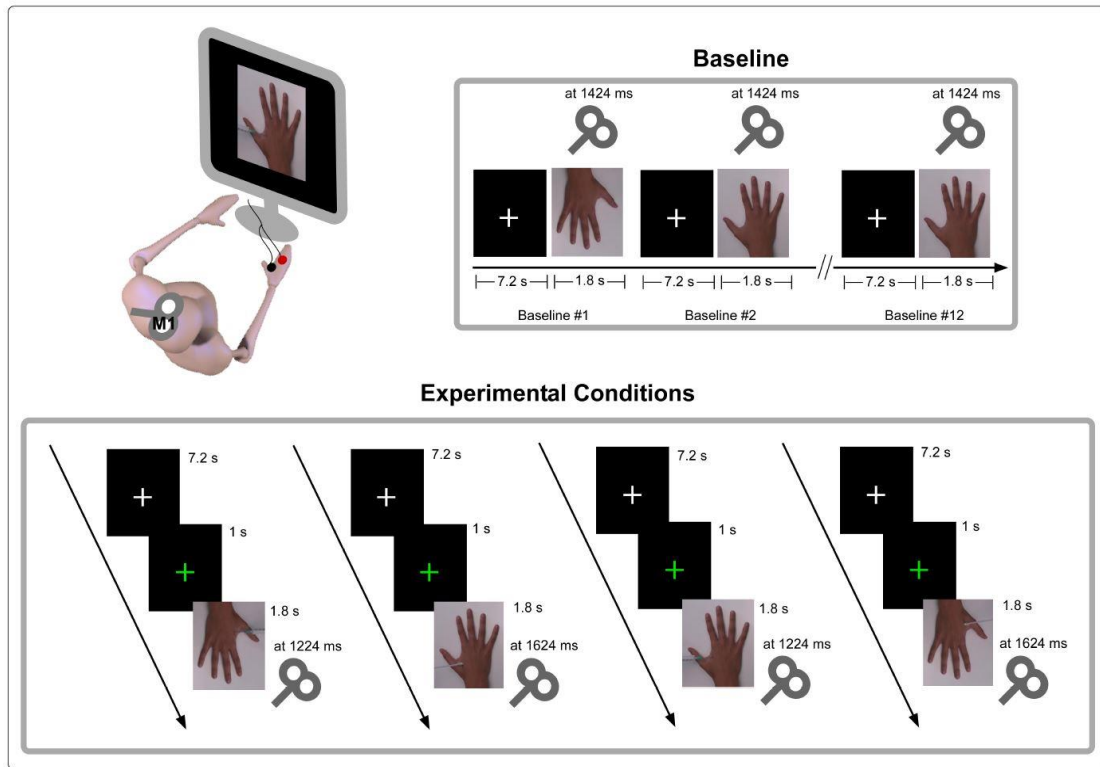


Fig. 1 Schematic representation of the experimental protocol and design. Top panel, on the left side, shows a graphic representation of the experimental setting: a subject watching the video-stimuli, presenting hand models in a first- or in a third-person perspective, while TMS pulses were delivered over the left M1. Top panel, on the right side, shows the stimuli presented in the baseline conditions: a static hand was randomly presented either in the first- or in the third-person perspective. TMS pulses were delivered 1424 ms after stimulus onset. Bottom panel shows the stimuli presented in the experimental conditions: the hand-model, presented in a first- or in a third-person perspective, penetrated by a needle or touched by a Q-tip. TMS pulses were delivered at two possible time points: early time (200 ms after needle penetration / Q-tip touch; i.e., at 1224 ms after stimulus onset) and late time (600 ms after needle penetration / Q-tip touch; i.e., at 1624 ms after stimulus onset).

Significant Interaction
“Perspective*Time of stimulation*Valence of stimuli”

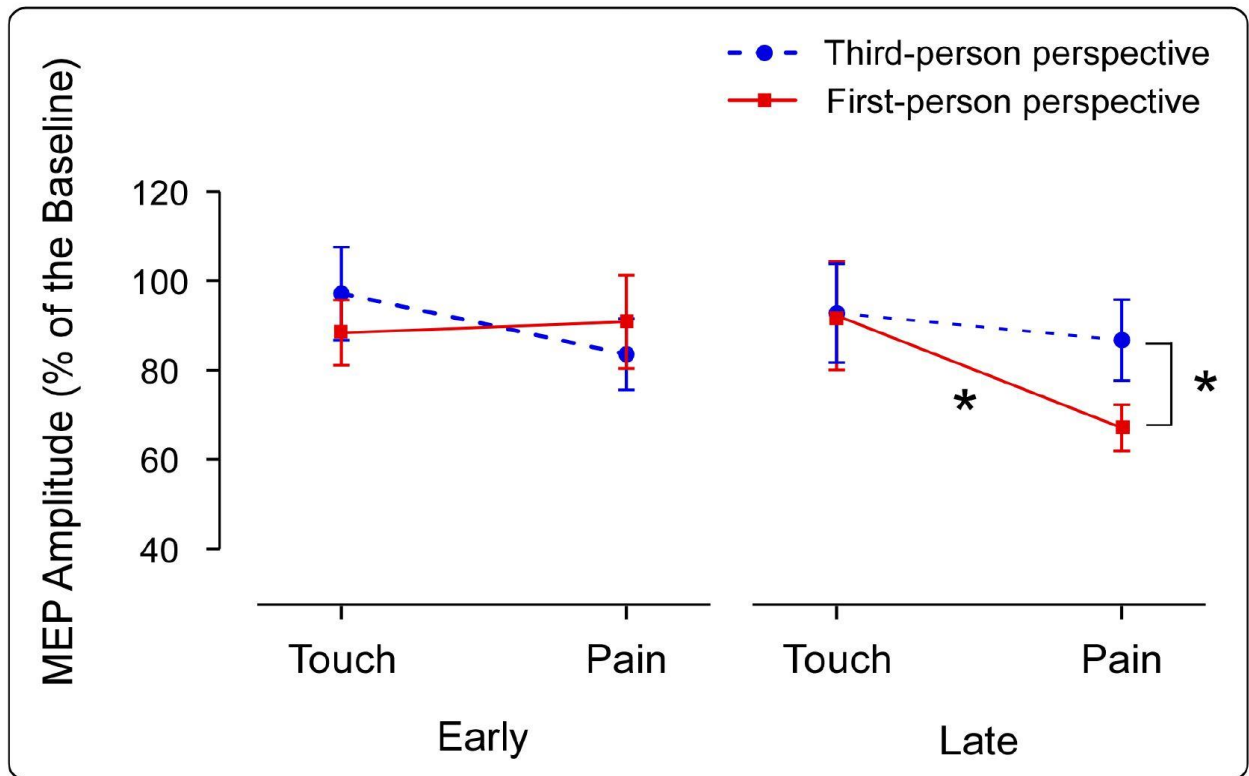


Fig. 2 MEPs ANOVA results. Significant interaction among perspective, time of stimulation and valence of stimuli. MEPs amplitudes are expressed as percentage of the baseline in the four experimental conditions (Pain first-person, Pain third-person, Touch first-person, Touch third-person) and in the two times of stimulation (Early, Late). Error bars indicate sem. (*P<0.05).

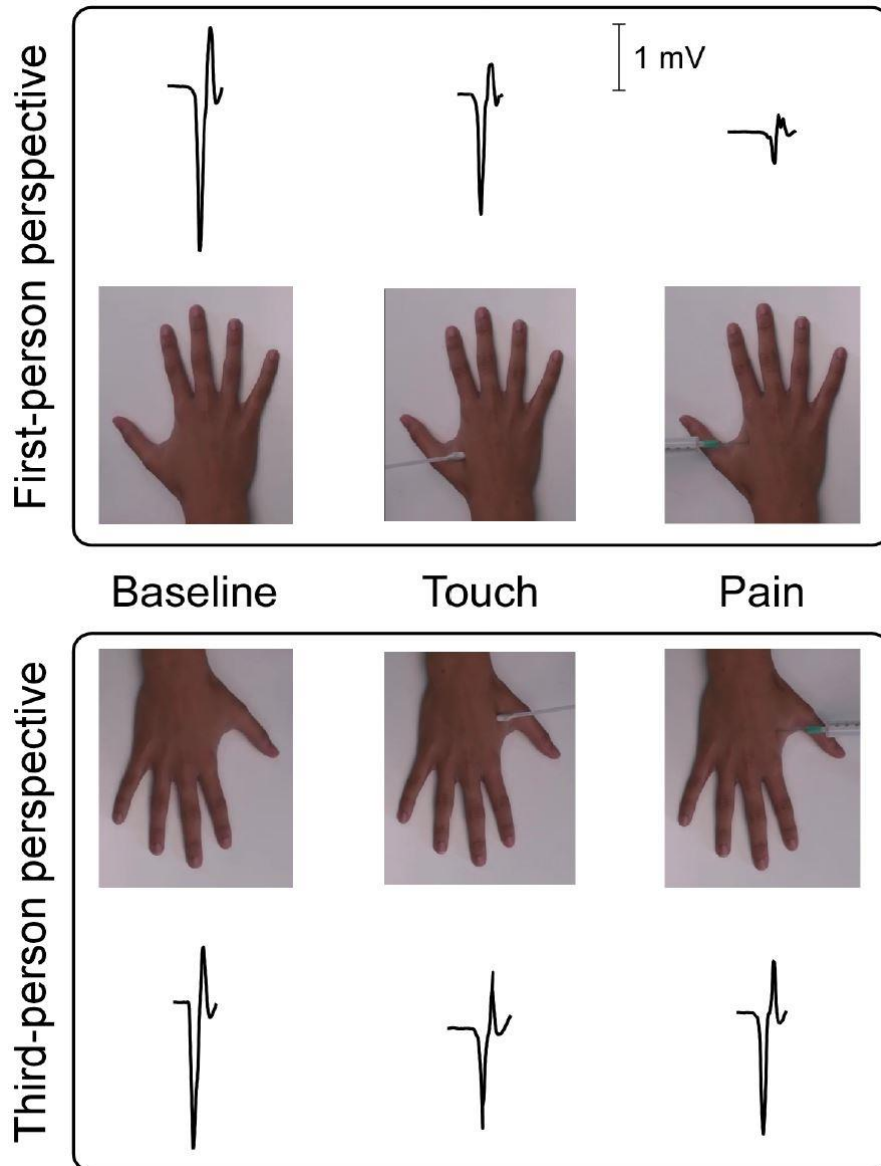


Fig. 3 Raw MEPs amplitudes recorded from FDI muscle in one representative subject during different experimental conditions at the late time of stimulation.

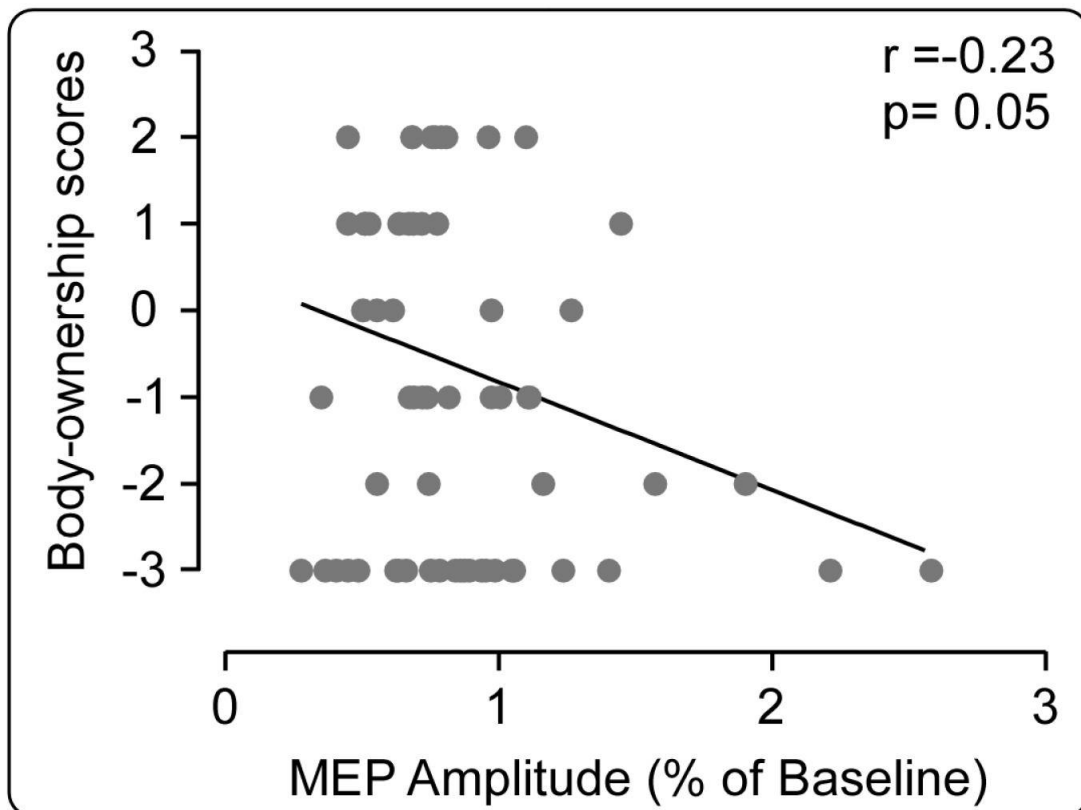


Fig. 4 Linear regression “body-ownership scores by MEP” results. The MEPs amplitude, at the late of stimulation, was used as independent variable to predict the sensation of body-ownership over the hand model reported on a 7 points Likert scale.

4. Experiment 4: Pain anticipation induces freezing effects as in the actual pain: evidence from corticospinal modulation during classical conditioning paradigm

Fossataro Carlotta*, Bucchioni Giulia*, D'Agata Federico, Mouras Harold, Krystkowiak Pierre and Garbarini Francesca. *In preparation*

In this last experiment, we focused on the anticipation of a painful response. In several studies, it has been shown that pain expectancy shares common neural pathways with actual pain. Moreover, previous studies evidenced that the neural network activated during the observation of others' pain and the neural circuits activated during the own experience of pain anticipation seem to be partially overlapped (Morrison *et al.*, 2004). According to these authors, a possible interpretation of empathy for pain is that it might represent the pain anticipation in the self. For instance, Porro and colleagues (Porro *et al.*, 2003) have approached the study of pain anticipation by using fMRI, but this technique did not allow to clarify the role of the primary motor cortex and of the corticospinal modulation during pain expectancy.

In order to verify this hypothesis, in this study, by using a classical conditioning paradigm, we investigated whether the expectancy for aversive stimuli could affect the motor cortex excitability. We took advantage from the freezing effect known to accompany the actual pain; i.e., the inhibitory modulation of the motor pathway to the muscle adjacent to the painful area. Participants undergone spTMS over the primary left motor cortex, while MEPs were recorded from the abductor pollicis brevis (APB) and the abductor digiti minimi (ADM) muscle of the right hand. We employed a classical conditioning paradigm in which visual and auditory stimuli (colored squares and sounds) were conditioned by pairing aversive stimuli (electric shocks delivered to the right digit V, Unconditioned Stimuli - US), giving rise to three conditions, depending on the presented stimuli: conditioned stimuli paired with US (CS+); conditioned stimuli not paired with US (CS-); neutral stimuli (N).

It was hypothesized the mere anticipation of the painful stimulus delivering may induce a significant inhibition in the sensorimotor system, as it happens during the direct exposure to

painful stimuli. More specifically, if the pain anticipation may induce changes in the corticospinal excitability, we predicted that a significant decrease in the MEPs amplitude (freezing effect), with respect to the baseline N condition, should be present not only in CS+ condition (when the actual pain was present) but also in CS- condition (when only the pain expectancy was present). The MEPs amplitude in both CS+ and CS- was significantly lower than in N condition (freezing effect). Moreover, the freezing effect in CS+ and CS- with respect to N condition, although present in both muscles, was greater in APB. Our findings is in agreement with the notion according to the human brain a strong predictive nature, suggest that actual pain is not necessary in order to induce corticospinal modulation occurrence and rather the pain expectancy would be sufficient. Finally, the anticipatory response recorded in the corticospinal system might represent the defensive preparation of the organism to the exposure to a dangerous event, likely during the observation of a painful event.

Pain anticipation induces freezing effects as in the actual pain: evidence from corticospinal modulation during classical conditioning paradigm

Fossataro Carlotta*, Bucchioni Giulia*, D'Agata Federico, Mouras Harold, Krystkowiak Pierre and Garbarini Francesca. *In preparation*

ABSTRACT

Previous studies show that a peripheral painful stimulation induces the inhibition of motor responses (freezing-effect) in the corticospinal system. Moreover, pain anticipation has been shown to share common neural pathways with the actual pain. The role of the primary motor cortex and the corticospinal excitability in pain anticipation is still largely unknown. Here, by using a classical conditioning paradigm, we aimed at testing whether pain expectancy affect corticospinal excitability. Transcranial magnetic stimulation was applied over the primary left motor cortex, while motor evoked potentials (MEPs) were recorded from the APB and the ADM right hand muscles. Visual and auditory stimuli were conditioned by pairing aversive stimuli (i.e. peripheral electric shocks). If pain anticipation is able to induce changes in the corticospinal excitability, we predicted that a significant decrease in the MEPs amplitude (i.e., freezing effect), with respect to the neutral condition (N condition), should be present not only when the peripheral electric shocks and the actual pain was present (CS+ condition) but also when only the pain expectancy was present (CS- condition). We recorded significantly lower MEPs amplitude (freezing effect) in APB muscle for both the CS+ and CS- with respect to N condition. Our results evidence the influence of pain anticipation in the primary motor cortex and in the corticospinal system, highlighting the adaptive defensive role of the motor system not only when the individual receive an actual noxious stimulation but also when he is going to receive it.

INTRODUCTION

The International Association for the Study of Pain (IASP) defines pain as an “unpleasant subjective, sensory and emotional experience associated with actual or potential tissue damage, or described in term of such damage” (Tracey & Mantyh, 2007). Pain is an highly subjective experience, is not linearly related to a nociceptive drive or input and is not a

synonymous of nociception (i.e. activity in the peripheral and central nervous system elicited by mechanical, thermal or chemical stimuli having the potential to inflict tissue damage (Sherrington, 1906)). Indeed, many people report pain experiences also in absence of tissue damage or any other pathophysiological cause and usually there is no way to distinguish their experience from that due to a tissue damage (e.g. chronic pain state) (Bogduk and Merskey, 1994; Tracey and Mantyh, 2007). In the last decade several studies, aimed to understand how noxious stimuli are processed by the cortex and how the pain experience may emerge from this processing, has been published describing a putative pain specific network of cortical areas, originally called Neuromatrix (Melzack, 1989) and recently named Pain Matrix (Ploghaus *et al.*, 1999). Hence, the primary (S1) and secondary (S2) somatosensory cortices, Insula, Anterior cingulate cortex (ACC) has been considered the structures involved not only in the perception of actual pain (Apkarian *et al.*, 2005; Buchel *et al.*, 2002; Ingvar, 1999; Peyron *et al.*, 2000; Ploghaus *et al.*, 1999; Rainville, 2002; Tracey and Mantyh, 2007), but also in experiencing empathy for pain (Avenanti *et al.*, 2005; Singer *et al.*, 2004) and social rejection (Eisenberger and Lieberman, 2004; Eisenberger, 2012; Eisenberger *et al.*, 2003). Despite such studies, there are compelling evidences to consider this pattern of brain activation, commonly labeled as the Pain Matrix, be unspecific to pain. Indeed, similar brain responses can be elicited by non-nociceptive sensory stimuli and better explained by a combination of multimodal and somatosensory-specificity neural activity (Iannetti and Mouraux, 2010; Iannetti *et al.*, 2013; Legrain *et al.*, 2011; Liang *et al.*, 2013; Mouraux *et al.*, 2011).

A closely link between somatosensory/nociceptive input and motor system activity has been supported by different studies (Cash *et al.*, 2015; Sailer *et al.*, 2004). For instance, when a part of our body comes in contact with noxious stimuli, we usually withdraw the affected body part from the source of pain. Such adaptive withdrawal response is the result of reflex circuits and has the evolutionary advantage of protecting the hand to further damage, facilitating the escape (Clarke and Harris, 2004; Sherrington, 1910). Furthermore, different avoidance behaviors may be adopted when peoples are exposed to pain experiences. For instance, chronic pain patients tend to limit or inhibit movements because of the pain fear (Crombez *et al.*, 2012). Moreover, it has been shown that the electric stimulation of the primary motor cortex (M1) may have a therapeutic function: it is efficacious in pain reducing (Cruccu *et al.*, 2007), suggesting that the motor system might influence somatosensory sensations (García-Larrea *et al.*, 1999; Peyron *et al.*, 1995, 2000). Other evidences supporting the link between motor system and pain

came from studies in which condition of pain were induced experimentally leading to find that painful peripheral stimulations are able to inhibit the motor cortex. Indeed, motor-evoked potentials (MEPs) to transcranial magnetic stimulation (TMS) over the primary motor cortex (M1), recorded from the participant hand where painful cutaneous stimuli were delivered, highlighted the inhibition of the corticospinal system excitability (Farina *et al.*, 2001, 2003; Le Pera *et al.*, 2001; Svensson *et al.*, 2003; Urban *et al.*, 2004). Moreover, in the last decade, it has been found that the mere observation of painful stimuli delivered to a hand model produces a fine-grained modulation of the observer's corticospinal system (Avenanti *et al.*, 2005, 2009, 2010; Bufalari *et al.*, 2007; Minio-Paluello *et al.*, 2006; Singer and Frith, 2005; Valeriani *et al.*, 2008) as it happens during a real painful stimulation. Although, this result has been interpreted as the physiological basis of empathy, we recently found that a pain-specific corticospinal modulation occurs only when noxious stimuli were delivered to hand model presented in first person perspective. In line with an "affective" conception of body-ownership according to which the body I feel as my own is the body I care about, we offer an alternative interpretation of such corticospinal modulation during observation of pain as physiological counterpart of an embodiment phenomenon related to the sense of body-ownership (De Vignemont, 2014) (Bucchioni *et al.*, under review).

Nowadays, there are compelling evidences that the interconnected network of cortical and subcortical regions participating in the processing of noxious stimuli undergo a complex Top-down modulation by cognitive, affective and motivational process (Legrain *et al.*, 2012). Indeed, attention (Van Damme *et al.*, 2010), pain catastrophising (Sullivan *et al.*, 2001), space representation (Sambo and Iannetti, 2013; Sambo, Forster, *et al.*, 2012; Sambo, Liang, *et al.*, 2012) bodily ownership (Garbarini *et al.*, 2014; Pia *et al.*, 2013) and Hypnosis (Kupers *et al.*, 2005; Vanhaudenhuyse *et al.*, 2014), are examples of top down process may significantly modulate not only pain perception (Wiech *et al.*, 2008) but also defensive behaviors (Sambo and Iannetti, 2013) and empathy for pain (Goubert *et al.*, 2005; Hein and Singer, 2008). Moreover, an important adaptive capacity is the ability to predict the likelihood of an aversive event among these factors, the expectation of a painful event (i.e., pain anticipation), associated with fear and anxiety plays an important role in pain perception (Ploghaus *et al.*, 2003). In several neuroimaging studies, it has been shown the anticipation of pain shares common neural pathways with the actual pain (Fairhurst *et al.*, 2007; Koyama *et al.* 2005; Porro *et al.*, 2003;

Wang *et al.*, 2008). Anyway, none fMRI study, permitted to clarify the role of the M1 and of the corticospinal modulation during pain expectancy.

Moreover, previous studies evidenced also the neural network activated during the observation of others' pain and those circuits activated during the first hand experience of pain anticipation seem to be partially overlapped (Morrison *et al.*, 2004). Taking in account the new interpretations about the shared representations of pain, the neural circuits activated during the observation of others' pain might represent the neural basis of a threat avoidance mechanism (like freezing behavior) (Decety, 2015) and the activation of these circuits might co-occur with defending response to prevent dangerous events (Avenanti & Aglioti, 2006). Along these lines, a possible interpretation of empathy for pain is that it might represent the pain anticipation in oneself. Furthermore, new findings report a first-person pain-specific corticospinal inhibition during the observation of a hand-model undergoing a painful stimulation (Bucchioni *et al.*, under review). These results might also be interpreted under the light of pain anticipation: the observer is might preparing himself in receiving a real painful stimulation, anticipating it.

In the present work, using a classical conditioning paradigm, it was investigated the modulation of the corticospinal excitability, focusing on the freezing effect registration during an electrical painful stimulus anticipation. It was hypothesized the mere anticipation of the painful stimulus may induce a significant inhibition in the sensorimotor system, as it happens during the direct exposure to painful stimuli. Specifically, here it was employed a classical conditioning paradigm (Pavlov & Anrep, 1927) in which visual and auditory stimuli (i.e., colored squares and sounds) were conditioned by pairing aversive stimuli (i.e. electric shocks delivered to the right digit V, Unconditioned Stimuli - US), giving rise to three conditions, depending on the presented stimuli: conditioned stimuli paired with US (CS+); conditioned stimuli not paired with US (CS-); neutral stimuli (N). This paradigm has previously showed to be effective in modulating Skin Conductance Response (SCR) (Masi *et al.*, 2014): SCR responses were higher for the CS+ condition compares to the N condition, but crucially SCR responses were higher also for the presentation of the CS- condition compared to the N condition. In the present research, SCR and MEPs were collected. We predicted that whether the pain anticipation induce changes in the corticospinal excitability, significant decrease in the MEPs amplitude (i.e., freezing effect), with respect to the baseline N condition, should be present not only in CS+ condition (when the actual pain was present) but also in CS- condition (when only the pain expectancy was present).

MATERIALS AND METHODS

Participants

Twenty-one healthy volunteers (19-29 years, mean \pm SD 22.6 ± 2.43 ; 10 females) participated in the study. All participants were right-handed, as assessed with the Edinburgh Handedness Inventory (Oldfield, 1971), naïve to the experimental procedure and before taking part in the study they gave written informed consent. None of them had a history of neurological, major medical, or psychiatric disorders and they were free from any contraindication to TMS (Rossi *et al.*, 2009). The experimental procedure was approved by local ethics committee and performed in line with the Declaration of Helsinki (1964).

Stimulation and Recordings

Magnetic Stimulation. MEPs were elicited by a single pulse transcranial magnetic stimulation (TMS) (Magstim Rapid2; Magstim Co. Ltd, Whitland, UK) with a figure-of-eight-shaped coil positioned over the left motor cortex (M1, hand area). In order to determine the optimal position able to elicit the greatest amplitude MEP with the lowest stimulation intensity, the coil was held tangentially to the scalp and moved over the left hemisphere with the handle pointing backwards at 45° from the midline (Brasil-Neto *et al.*, 1992). By moving the coil in step of 1 cm over the left motor cortex the optimal point able to activate the selected muscle was found then the coil was fixed and held by a mechanical arm. The intensity of magnetic pulses was set at 115% of the resting motor threshold (mean \pm SD: $63.9\% \pm 8.24\%$, range 54-78% of the maximum stimulator output), defined as the lower intensity of the stimulator output able to elicit five MEPs of ten consecutive pulses with an amplitude of at least $50\mu\text{V}$ (Rossini *et al.*, 1994).

Electromyography recording. Electromyogram (EMG) activity was simultaneously recorded (MP150, Biopac System, USA), from the right Abductor Digiti Minimi muscle (ADM) and the Abductor Pollicis Brevis muscle (APB), using two pairs of bipolar surface electrodes with the active electrode over the muscle belly and the reference electrode over the associated joint or tendon. Signals were amplified and digitalized with a sample rate of 10 kHz, band-stop filtered at 50 Hz and stored for off line analysis (see Figure 1).

Skin Conductance Response recording. SCR was recorded continuously (MP150, Biopac System, USA). Two Ag-AgCl electrodes with constant voltage (0.5 V) were attached to the participant's left hand on digit IV and III. Signal was amplified and digitalized with a sample rate of 1 kHz, band-stop filtered at 50 Hz and stored for off line analysis.

Electrical Stimulation. Transcutaneous electrical stimuli consisted in constant current square-wave pulses (DS7A, Digitimer) delivered to the right digit V, using a surface bipolar electrode attached with a Velcro strap. The stimulus duration was 200 μ s and the delivering came ~50ms first the TMS pulse. Stimulus intensity was adjusted, in each participant, such that stimulation was deemed "painful but tolerable", mean stimulus intensities were 34.82 ± 10.63 mA, range 20-48 mA.

Procedure

The experiment was programmed by using E-prime presentation software V2.0 (Psychology Software Tool Inc., USA) in order to control sequence, timing and duration of the stimuli and to trigger TMS pulses, EMG and SCR recording and electrical stimulation delivering. Participants were seated comfortable in front of a PC screen (17-inch monitor; resolution 1280x720 pixels; refresh frequency 60 Hz) at a distance of 80 cm, with the head restrained by a comfortable pillow wrapping around the neck and supported by a fixed head rest, and with their forearm resting on a pillow in order to avoid any muscles contractions.

The experiment consisted of two separate blocks with a break of 20 min from each other in order to minimized habituation. In each block a total of 40 stimuli were presented in a pseudorandom order, 20 out of them were neutral stimuli (N), 15 were conditioned stimuli paired with unconditioned stimuli (i.e. CS+) and 5 were conditioned stimuli unpaired with unconditioned stimuli (i.e. CS-). It is important to note that the pseudorandom sequence was generated firstly, so that the CS- stimuli were never presented in the first five trials and secondly, in order to have no more than two equal stimuli in consecutive trials. In the N condition a visual stimulus (i.e. a blue square) attended by an auditory stimulus (i.e. a tone "bee") were presented. In the CS+ condition a visual stimulus (i.e. green square) attended by an auditory stimulus (i.e. a white noise "zzz") were presented paired with an electrical stimulus (i.e. US) on the digit V of the right hand. In the CS- a visual stimulus (i.e. green square) attended by an auditory stimulus (i.e. a white noise "zzz") were not paired in order to compare the physiological

responses (i.e. SCR and MEP) elicited by the CS in the absence of US. The two auditory stimuli were significantly different in term of frequency (“zzz”: 9957Hz; “bee”:10097Hz). All visual stimuli were presented for 4000 ms on a black background, attended by auditory stimuli of 1000ms, and spaced out by a fixation cross with a variable jittering (12000-16000ms), chosen in order to have a variable time stimuli presentation. According to condition visual stimuli were followed i) by a TMS pulse, in N trials; ii) by and electric shock followed after 50ms by a TMS pulse, in CS+ trials; iii) by a TMS pulse, in CS- trials (see Figure 2).

In order to asses baseline corticospinal excitability before and after each blocks a total of ten baselines with a fixation cross of 1050ms in the center of the screen were presented, five out of them were paired with an electrical shock on the digit V, the other ten were unpaired and used as baseline MEP values to normalized data.

Self-report measure. After the experiment all participants completed the trait scale (Y1) and the state scale (Y2) of the State-Trait-Anxiety-Inventory (STAI) (Spielberger *et al.*, 2010; Weiner & Craighead, 2010). The STAI is a questionnaire of 20 items for assessing trait anxiety and 20 for state anxiety. State anxiety scale includes either item related to the presence (e.g. “I am tense; I am worried”) either to the absence of anxiety (e.g. “I feel calm; I feel secure.”). Trait anxiety scale includes item related to the presence (e.g. “I worry too much over something that really doesn’t matter”) either to the absence of anxiety (e.g. “I am content; I am a steady person”). All items are rated on a 4-point scale in terms of how often participants fell as described from 1 indicating “Almost Never” to 4 indicating “Almost Always” (items indicating absence of anxiety are reversed scored). Higher scores indicate greater anxiety. Moreover, participants were asked to answer by means of a Likert scale from -5 (i.e. I am not agree at all) to +5 (i-e- I am totally agree) about their agreement with some statement referred to the experimental condition: a) when the green square was presented I expect it would happen to me something positive; b) when the blue square was presented I expect it would happen to me something positive; c) when the “zzz” noise was presented I expect it would happen to me something negative; d) when the “bee” noise was presented I expect it would happen to me something negative.

DATA ANALYSIS

SCR analysis

SCR data were analyzed offline. For each subject and each experimental condition, the average peak-to-peak amplitude was extracted (as a difference between the minimum and the maximum value after the trigger coding for the stimulus delivering). Then, to obtain comparable measure among subjects, the peak-to-peak responses were normalized within subject and converted into Z-scores (Garbarini *et al.*, 2014; Romano *et al.*, 2014). In order to test the effect of fear conditioning on the skin conductance response was performed a one-way repeated measure ANOVA with ‘condition’ (three levels: ‘N’, ‘CS+’ and ‘CS-’) as within subject factors. Post hoc comparisons were carried out by means of the Duncan test.

MEP analysis

EMG data were analyzed offline. By visual inspection, the absence of any voluntary contraction in the time window of 100ms before the TMS pulse was verified by monitoring the EMG activity online and all trials with any activity greater than 50 μ V were excluded from analysis. For each subject and separately for each experimental condition the average peak-to-peak MEPs’ amplitude was extracted separately for ADM and APB. For each muscle all trials in which the MEP amplitude were ± 2 SD of the mean amplitude were identified as outliers and excluded from the analysis.

Firstly, in order to test the effect of the electrical stimulation per-se and the TMS on the corticospinal excitability, on the MEPs amplitude of the first and the last block of baseline was performed a 3x2 repeated measure ANOVA with ‘Pain’ (two level: ‘pain’; ‘no-pain’), ‘Muscle’ (two levels: ‘APB’; ‘ADM’) and ‘Block’ (two levels: ‘block1’; ‘block2’) as within subject factors. Secondly, in order to test the effect of fear conditioning on the corticospinal excitability, MEPs amplitude in each experimental condition expressed as percentage of the baseline, were analyzed by means of a 2x3 repeated measure ANOVA with ‘Muscle’ (two levels: ‘APB’; ‘ADM’) and ‘Condition’ (three levels: ‘N’, ‘CS+’ and ‘CS-’) as within subject factors. Post hoc comparisons were carried out by means of the Duncan test.

Correlation Analysis

Pearson correlation was used to investigate whether a) self-report measure of Trait-Anxiety (STAI) and b) skin conductance responses were significantly correlated with amplitude change of MEP recorded from each muscle. In order to perform correlation analysis an index of MEP amplitude change respect to the baseline was compute as follow: amplitude during each experimental condition minus amplitude during baseline condition divided by the average of the same two conditions.

RESULTS

Skin conductance results

We found a significant effect of condition ($F_{1,20}=69.88$, $p=0.000001$) (see Figure 3) suggesting that the amplitude of the SCR was different between conditions. Particularly, the SCR amplitude was significantly greater not only in CS+ trials compared to all other conditions (mean \pm s.d.: CS+ = 0.65 ± 0.27 ; N= -0.48 ± 0.17 ; $p=0.000061$), but crucially SCR amplitude in CS- trials was significantly greater compared to N trials (mean \pm s.d.: CS- = -0.15 ± 0.34 ; N= -0.48 ± 0.17 ; $p=0.001918$). This data show that when participants learned the associations between conditioned stimulus and the unconditioned stimulus the physiological enhancement of the SCR amplitude typically triggered by the unconditioned stimuli can be elicited by the conditioned stimulus, suggesting that only the expectancy to be able to receive painful stimuli can induced an enhancement of the SCR.

MEPs results

Mean MEP values in the two baseline blocks were comparable, the ANOVA revealed main effect of both 'Pain' ($F_{1,20}=35.27$, $p=0.000008$) indicating that the MEPs' amplitude was minor in pain trials than in no-pain trials; and 'Muscle' ($F_{1,20}=11.95$, $p=0.002$) indicating that MEPs amplitude was higher in APB than in ADM. Moreover, the interaction between these two factor was significant ($F_{1,20}=31.34$, $p=0.00002$) (see Figure 4). This in line with previous study (Urban *et al.*, 2004) indicates that there is a different pain modulation according to the recorded muscles and particularly that the corticospinal inhibition induced by pain was higher in APB (mean \pm s.d.: no-pain= 903.57 ± 584.29 ; pain= 287.17 ± 207.27) than in ADM (mean \pm s.d.:

no-pain= 402.93 ± 316.33 ; pain= 283.76 ± 226.55). Crucially, we observed no effect of block ($F_{1,20}=0.5$, $p=0.48$), this indicates that the difference between the baseline blocks at the beginning and the one at the end of the experiment was not significant, therefore no change in corticospinal excitability was induced by TMS per se during the experiment and for this reason MEP amplitude in each experimental condition were expressed as percentage of a unique baseline for both blocks.

ANOVA on the MEPs values in each experimental condition showed a main effect of both 'muscle' ($F_{1,20}=36.7$; $p=0.000006$), indicating greater MEPs mean amplitudes recorded from ADM muscle than for APB muscle, and 'condition' ($F_{1,20}=10.41$; $p=0.0002$), indicating that the MEPs amplitude in both CS+ and CS- was significantly lower than in N condition (freezing effect). Moreover the interaction between this two factors was significant ($F_{1,20}=4.72$; $p=0.014$) (see Figure 5), indicating that there is a difference corticospinal modulation according to the muscle recorded. Post-hoc analysis (Duncan) showed that in APB the difference between all conditions was significant: MEPs amplitude in CS+ trials was significantly lower compared to the amplitude in CS- trials (mean \pm s.d.: CS+= 0.30 ± 0.19 ; CS-= 0.52 ± 0.23 ; $p=0.000149$). MEPs amplitude in CS+ trials was significantly lower compared to MEPs values in N trials (CS+= 0.30 ± 0.19 ; N= 0.64 ± 0.23 ; $p=0.000061$), but crucially there was a significant decrease MEPs amplitude in CS- trials compared to N trials (mean \pm s.d.: CS-= 0.52 ± 0.23 ; N= 0.64 ± 0.23 ; $p=0.01$). In ADM muscle, despite the difference between MEPs amplitude in CS+ and CS- trials was not significant (mean \pm s.d.: CS+= 0.69 ± 0.42 ; CS-= 0.75 ± 0.29 ; $p=0.25$), both CS+ (mean \pm s.d.: CS+= 0.69 ± 0.42 ; N= 0.84 ± 0.26 ; $p=0.004$) and CS- MEPs amplitudes were significantly lower compared to the N condition (mean \pm s.d.: CS-= 0.75 ± 0.29 ; N= 0.84 ± 0.26 ; $p=0.05$). The significant reductions of the mean MEPs amplitude in CS- condition compared to the mean MEPs amplitude in N condition, in both muscle, suggest that the expectancy of being able to receive painful stimuli can induce a significant modulation of the corticospinal excitability.

Correlation results

SCR and MEPs amplitude correlations. We found a significant negative correlation between mean SCR amplitude and mean MEPs amplitude only in APB ($r= -0.4$; $p<0.0001$) (see Figure 6), but not in ADM ($r= -0.11$; $p=0.2$). This indicates that when participants receive

painful stimuli (CS+ condition) they have higher SCR response and lower MEPs values compared to N condition, but also when participants expect to be able to receive painful stimuli (CS- condition) they show higher SCRs values and lower MEPs amplitude values compare to neutral condition.

STAI and MEPs amplitude correlations. A significant negative correlation was found between STAI trait scale (Y1) and mean MEPs amplitude in CS- condition in both muscles (ADM: $r = -0.5$; $p = 0.01$, Figure 7 A; APB: $r = -0.5$; $p = 0.03$, Figure 7 B). These results showed that in participants with a higher level of trait anxiety, are recorded lower MEPs amplitude values in the CS- condition. A significant negative correlation was also found between the STAI state scale (Y2) and mean MEPs amplitude in CS- condition for the ADM muscle ($r = -0.5$; $p = 0.02$, Figure 7 C).

DISCUSSION

In the present study, it has been hypothesized that the mere anticipation of delivered painful stimulus may induce a significant inhibition in the sensorimotor system, as it happens during the exposure to painful stimuli (Farina *et al.*, 2001; Le Pera *et al.*, 2001; Urban *et al.*, 2004). In order to verify this hypothesis, we employed a classical conditioning paradigm where electrical painful stimuli were coupled with visual and auditory stimuli while SCR was collected and MEPs induced by single-pulse TMS placed on left M1 were recorded from the right APB and ADM muscles. SCR results are in agreement with previous study (Masi *et al.*, 2014) confirming that SCR is a good physiological correlate of conditioning. More specifically, in the present study, conditioning was used as a mean to show evidence of increasing of SCR signal amplitude recorded not only for the actual painful stimulation but also for the anticipation of a painful event. According to previous neuroimaging studies, similar regions are involved in the anticipation of pain and in the actual pain (Porro *et al.*, 2003; Wang *et al.*, 2008); but none of these studies has yet clarified the involvement of the M1 and of the corticospinal system in pain anticipation. Dubé and Mercier (2011) have previously attempted to study pain expectancy in the corticospinal system, but they didn't succeed in recording it probably because, as the authors reported, the intensity of the noxious stimulation used in their study was too weak to induce pain anticipation (they used painful stimuli inducing "low-to-moderate short-lasting phasic pain") and the pain expectation induced in their experimental setting was not so intense

as that felt in a real situation. In our study, we replicated previous results confirming the electrical painful peripheral stimulation induces a corticospinal inhibition (Urban *et al.*, 2004). Moreover, the MEPs amplitude reduction was also demonstrated to be a good physiological correlate of conditioning allowing evidencing the effect of pain anticipation in the M1 and in the corticospinal system. Furthermore, a significant negative correlation was found between the two physiological parameters recorded in the present study. When the subject received a painful stimulation (CS+) and when he was expecting to receive it (CS-): more the SCR amplitude augmented, more the MEP amplitude was reduced. Moreover, a significant negative correlation was also found between the STAI trait scale (Y1) and mean MEPs amplitude during the pain expectancy condition (CS-): a higher level of trait anxiety was predictive of lower MEPs amplitude values. This means that during pain anticipation, in individuals with higher trait anxiety the MEPs amplitude resulted to be more inhibited compared to less anxious individuals.

Several evidences are given in support of the existence of a link between pain and motor system (Crombez *et al.*, 1999; Cruccu *et al.*, 2007; Peyron *et al.*, 2000). Withdrawal and freezing behaviors in response to painful stimuli are showed to be helpful reflexes with a self-protective aim for the individual undergoing the painful stimulation (Dworkin, 2007). Moreover the observation of painful stimuli induces freezing responses in the observer (Avenanti *et al.*, 2005) similar to those recorded during a real painful stimulation (Farina *et al.*, 2001; Urban *et al.*, 2004). It has been suggested that the observation of other's pain may reflect the anticipation of pain in oneself (Avenanti & Aglioti, 2006; Singer & Frith, 2005). In fact it has been previously shown that pain anticipation shares circuits not only with the actual pain (Porro *et al.*, 2003; Wang *et al.*, 2008), but also with the network activated during the observation of other's pain and empathy for pain (Morrison *et al.*, 2004). Along these lines of research, the registration of the anticipatory response in the corticospinal system might represent the defensive preparation of the organism to the exposure to a dangerous event, likely during the observation of a painful event (Avenanti *et al.*, 2005, 2006; Decety, 2015). Indeed, the embodiment for other's pain in the onlooker corticospinal system may prepare the organism to freeze or to escape (Avenanti & Aglioti, 2006; Buccioni *et al.*, under review).

In conclusion, the present research permits to highlight the adaptive function of the pain expectancy in motor system: to anticipate the consequences of aversive stimuli such as pain allows the mobilization of the organism's resources to prepare the defensive action (fight or flight; Keay & Bandler, 2002) or to reduce the influence of the painful event (freezing behavior;

Fanselow & Helmstetter, 1988). In the present study, for the first time we evidenced the involvement of the corticospinal system and the M1 on the anticipation of peripheral noxious stimuli, confirming the existing link between pain and action system (Avenanti & Aglioti, 2006).

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FIGURE AND CAPTIONS

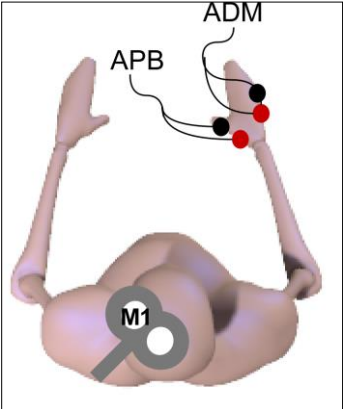


Figure 1. Schematic representation of the experimental protocol. Single-pulse TMS delivered over the participant’s left M1 and MEPs recorded from the APB and ADM muscles.

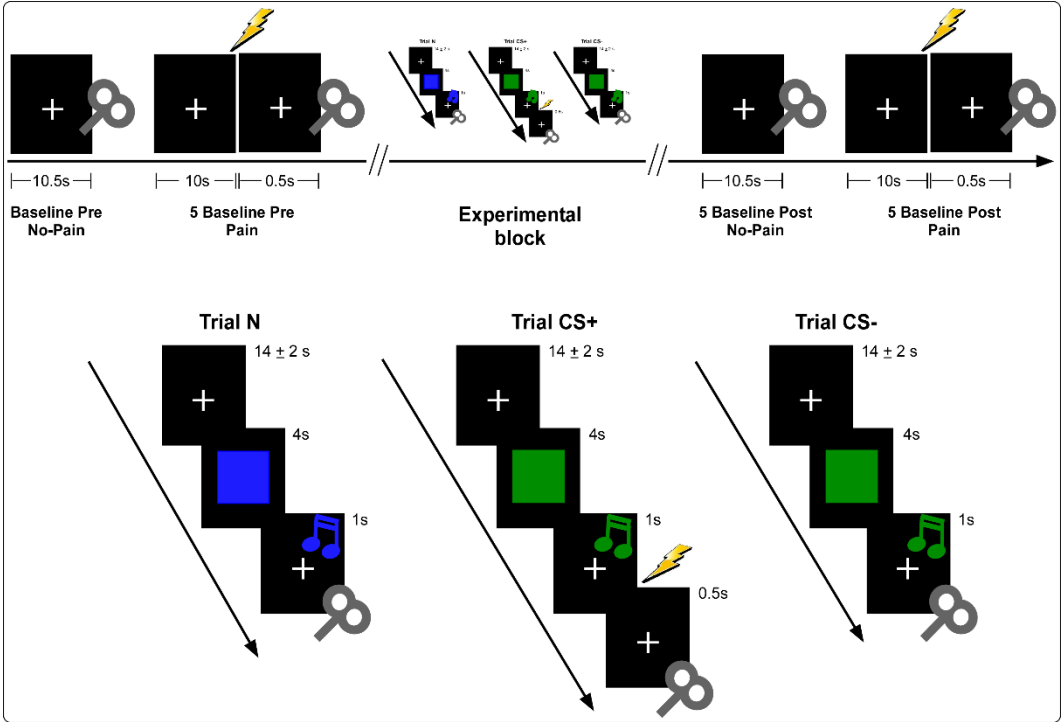


Figure 2. Schematic representation of the experimental protocol and design. Top panel on the right and on the left side shows the stimuli presented in the baseline conditions: a fixation cross was always presented associated with TMS stimulation in ten trials; in five of these, the TMS stimulation was associated to an electrical painful stimulation. Bottom panel shows the stimuli presented in the experimental conditions: a blue square and a “bee” noise associated with the TMS stimulation (N condition); green square and a “zzz” noise associated with TMS and electrical painful stimulation (CS+ condition); green square and a “zzz” noise associated with TMS stimulation (CS- condition).

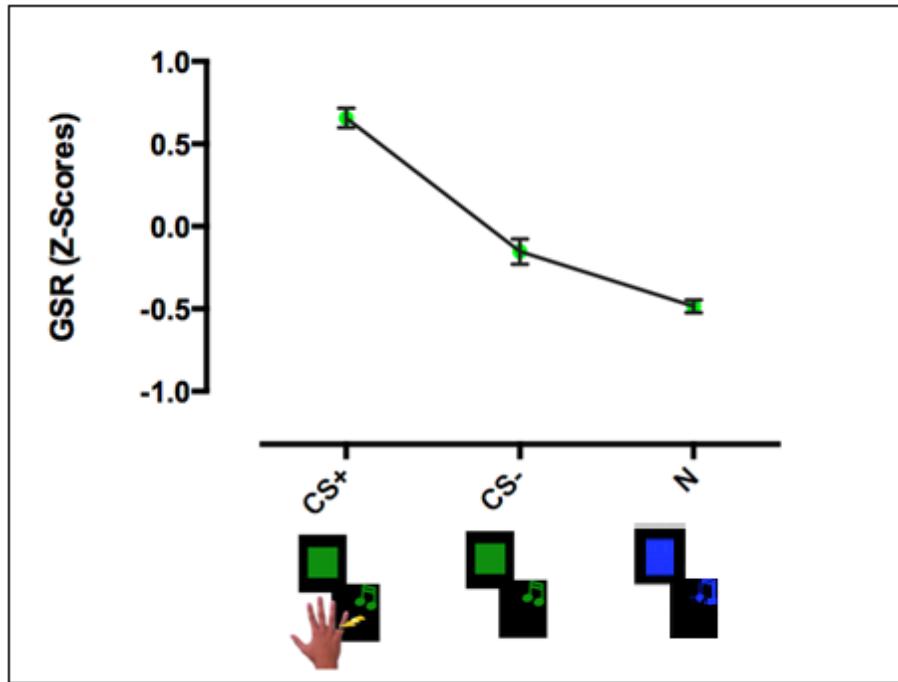


Figure 3. SCR ANOVA results. Significant effect of condition (CS+; CS-; N). Error bars indicate sem. (* $P < 0.05$).

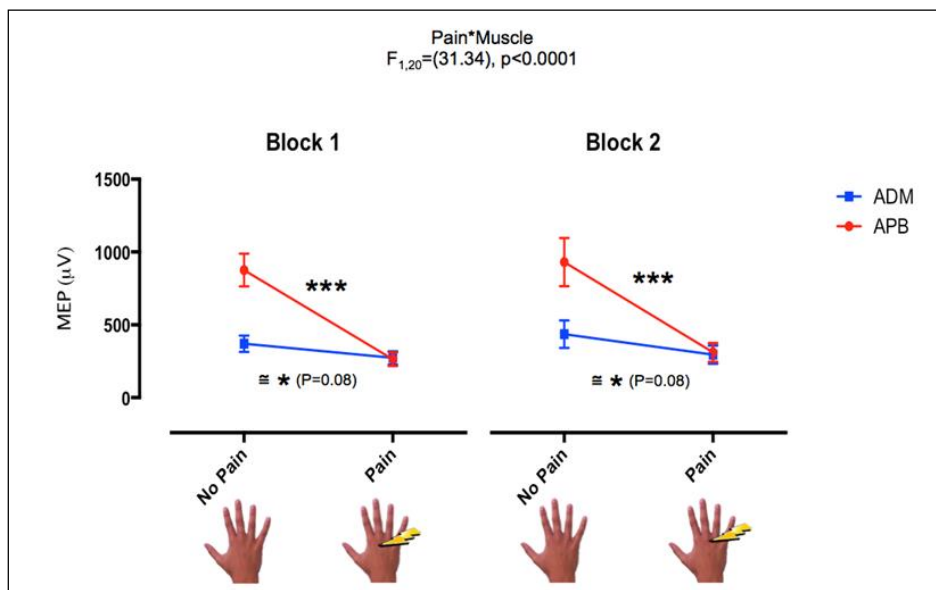


Figure 4. MEPs ANOVA Baseline results. Significant interaction between Pain (Pain; No-pain) and Muscle (ABP; ADM). Error bars indicate sem. (* $P < 0.05$).

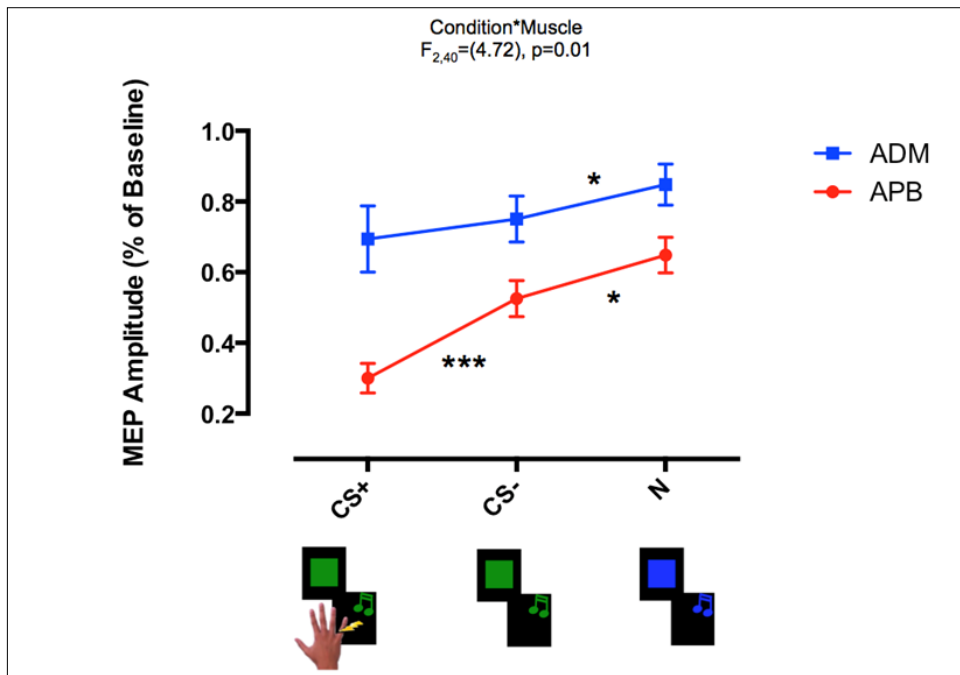


Figure 5. MEPs ANOVA results. Significant interaction between muscle (APB; ADM) and condition (CS+; CS-; N). Error bars indicate sem. (* $P < 0.05$).

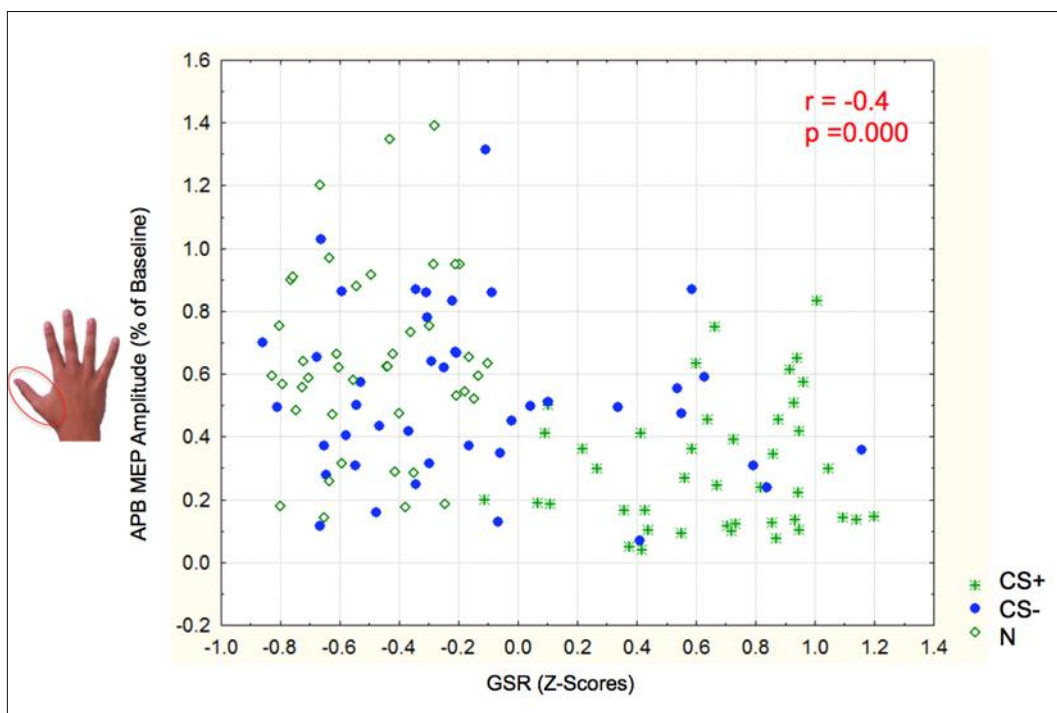


Figure 6. Correlation between SCR and MEPs amplitude. Significant negative correlation between SCR mean amplitude and mean MEPs amplitude in APB muscle.

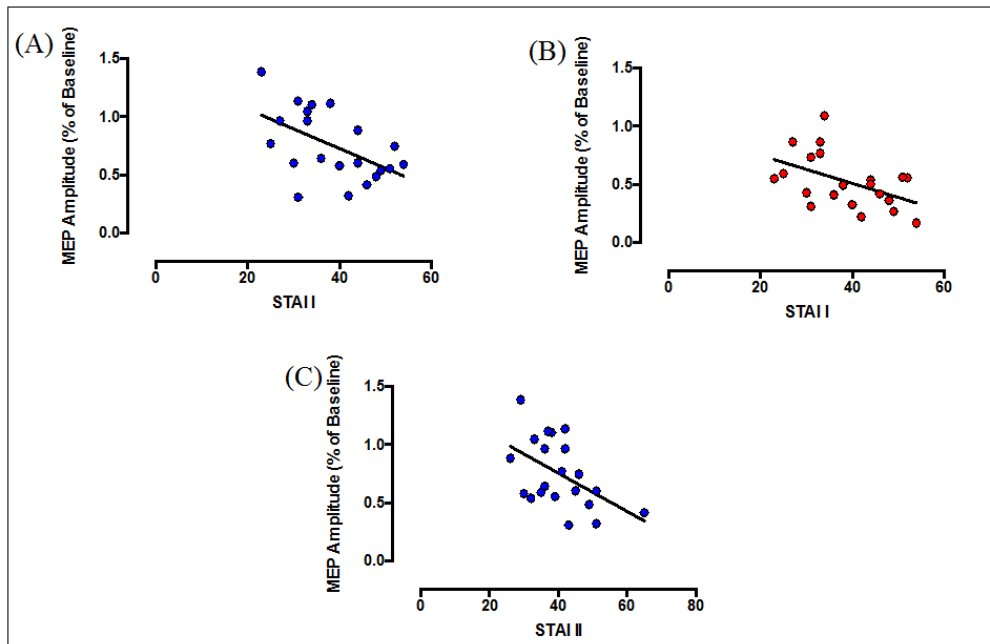


Figure 7. Correlations among STAI Y1 and STAI Y2 results questionnaires and mean MEPs amplitudes for the CS- condition in ABP and ADM muscles. (A) Significant negative correlation between STAI Y1 and ADM MEPs mean amplitudes in the CS- condition; (B) Significant negative correlation between STAI Y1 and APB MEPs mean amplitudes in the CS- condition; (C) Significant negative correlation between STAI Y2 and ADM MEPs mean amplitudes in the CS- condition.

CHAPTER IV
DISCUSSION &
PERSPECTIVES

Pain is an unpleasant sensory and emotional experience (IASP) and it has an evolutionary defensive value leading the individual to avoid it, once the consequence of a painful event is learned (Pavlov, 1927). Moreover, the observation of other persons undergoing a painful stimulation may induce in the observer automatic motor reactions inducing him to help (prosocial behavior) or to avoid (self-protective behavior) the person in pain (Goubert *et al.*, 2009). In addition, pain anticipation may elicit adaptive avoidance reactions to the incoming painful event (Ploghaus *et al.*, 2003). Indeed, it seems that not only the direct experience of pain, but also pain observation and pain anticipation might induce motor reactions both in the witness of others' painful events and in the person who is about to receive a painful stimulation. More specifically the main objective of the present thesis was to study approach and defensive (withdrawal and freezing) motor responses that may occur in the observer of a painful situation and it may also occur in the person undergoing painful stimulation during the anticipation of a painful event.

Even if previous studies have already investigated the above described phenomena there are still some points to be elucidated (Lelard *et al.*, 2013; Avenanti *et al.*, 2005; Dubé & Mercier, 2011). Notably, it remains still unclear which direction tends to adopt the empathizer (approach / withdrawal or freezing behavior) when he faces the target individual in pain and if the observer's reaction changes according to the level of proximity and familiarity felt with the empathic target. Indeed, our understanding of other's experience is modulated by different intra- and interpersonal factors. In order to answer to these questions, the first group of studies revealed the influence of the self / other perspective and the impact that different kinds of relationship representing the "other" have on the empathizer's behavior during an empathy for pain task. Specifically, we firstly analyzed the observer's pain ratings and reactions times given in response of the presentation of painful visual stimuli in a perspective-taking task (experiment 1). Later, we applied the same perspective-taking task associated to the presentation of painful visual stimuli in order to investigate approach / avoidance and freezing postural responses (experiment 2). In the third experiment (experiment 3), the corticospinal freezing effect during the observation of a model-hand undergoing a painful stimulation was studied; the model-hand was presented in first- (self) or third- (other) person perspective. Previous studies show evidences that the neural network activated during the observation of others' pain and those circuits activated during the first hand experience of pain anticipation seem to be partially

overlapped. Indeed, in the last study (experiment 4), we investigated the corticospinal freezing effect during the anticipation of painful stimuli in oneself.

In the following sections, the results obtained in the present thesis will be summarized and discussed with reference to study already published and future perspectives will be presented.

1. The effect of the self / other perspective on onlooker's behavior during the observation of painful stimuli

The adoption of the “self” or the “other” perspective results to be crucial for a successful empathic behavior. Decety and Jackson (2004) suggested that empathy is made up by three different “vital components”. The authors argue that the *affective sharing* between the self and the other permits to share representations between the self and the other. The *self / other awareness* keeps separated the self and the other identities without any confusion and *mental flexibility* and *self-regulation* permit to adopt the other perspective keeping a conscious self / other distinction of the two perspectives. In the first part of the thesis, we have shown the approach / avoidance and freezing reactions that the witness of painful events has. In the first two experiments (experiment 1 and 2), we recorded the observer's responses in two perspective-taking tasks where we manipulated high-level cognitive variables involved in empathy and theory of mind (Decety & Lamm, 2007). In this case, top-down influences mainly modulate the observer's response. Then, we analyzed the freezing effect in the observer of painful events manipulating low-level cognitive variables (Decety & Lamm, 2007), only showing the visual painful and non-painful stimuli in two different perspectives without explicitly ask to participants to adopt the “self” or the “other” perspective. In this second task, bottom-up influences mainly modulate the observer's response.

1.1. The influence of perspective-taking in behavioral and postural responses during the observation of painful stimuli

Previous studies had attempted to investigate the different role of intrapersonal and interpersonal factors influencing the empathic response. In particular some researches attempted to study the degree of proximity felt by the empathizer for the empathic target (Montalan *et al.*, 2012; Cheng *et al.*, 2010). Anyway, some points about the modulation of the empathic responses were still to be clarified: notably, if the observed person in pain is familiar / unfamiliar to the onlooker and the kind of this relationship. This effect was investigated applying a previously validated perspective-taking empathy for pain experimental paradigm (Jackson *et al.*, 2005; 2006) where we varied the type of relationship between the observer and the empathic target. Participants were asked to rate the intensity of pain in different painful and non-painful situations represented in the images adopting different perspectives: their own perspective (Self), their most loved person perspective (OMLF), their most hated person perspective (OMHF) and a stranger person perspective (OS). Results showed that pain ratings were higher when participants adopted OMLF person perspective in a painful condition and pain ratings were significantly higher in the same OMLF perspective in female participants compared to males ones. Moreover, the lower pain ratings were recorded during the most hated person (OMHF) perspective. RTs resulted to be quicker for the observer's most loved person (OMLF) and himself (Self) and the participants tended to be slower in the painful condition. These results confirmed previous studies: the empathic response is more important for a person whose is part of our group (Montalan *et al.*, 2012) and that we love (Cheng *et al.*, 2010) compared to a stranger. Furthermore, it was evidenced the role of the hated person in empathy for pain.

Previous studies have investigated the intergroup harm and pain in social psychology paradigms. Cikara *et al.*, (2011; 2014) focused on the reactions the participants had when they were faced to the out-group harm and the out-group pain. Specifically they found the antipathy felt for the "out-group" led to empathize less with the "out-group" itself and also to feel pleasure when the "out-group" felt pain (Cikara *et al.*, 2014). Along these lines, our behavioral results could be in agreement with previous studies that investigated counter-empathic responses (i.e., discrepant emotional responses often recorded when there is no concordance of the emotional

state between the observer and the observed person) and “Schadenfreude” responses (i.e., the pleasure derived from the others’ misfortunes) (Yamada *et al.*, 2011). Indeed, it seems to be not always useful to empathize with the other, especially when the observer and the observed person are in a context of competition (Lanzetta & Englis 1989).

This first study (experiment 1; Bucchioni *et al.*, 2015) gave initial information about the elaboration of painful visual stimuli according to the self and the other perspectives and to the familiar relationship the empathizer had with the observed person. Anyway, this study did not present clear evidence of the behavioral approach / avoidance and freezing reactions co-occurring during an empathy for pain task. Indeed, in the second study (experiment 2), we investigated the posturographic COP’s parameters when the participant was faced to the previously presented visual stimuli (painful, non-painful) (Jackson *et al.*, 2005; 2006), in the same perspective-taking task as the one used in the experiment 1 (Bucchioni *et al.*, 2015). The posturographic parameters analyzed were the following: the displacement in the anteroposterior axis (COP-AP), which allows to study the approach / avoidance displacement and the length of the COP sway path in the AP axis (path[COP]-AP) that allows to study the freezing behavior.

The analysis of these parameters has evidenced a significant COP-AP backward greater displacement for the OMLF perspective compared to the Self and the OMHF perspectives during the presentation of painful images. Moreover, the COP-AP averaged position was shifted backwards for the presentation of painful visual stimuli, compared to the observation of non-painful ones. This result was specifically recorded for the OMLF and the OMHF perspectives and suggests that familiarity plays an important role in modulating the observer’s approach / avoidance responses according to the valence of the presented stimuli. The delta COP-AP (subtraction between painful and non-painful visual stimuli) highlighted a significant backward COP-AP displacement for the OMLF perspective compared to all other perspectives at the 3rd second of painful visual stimuli presentation. These results evidenced a general greater withdrawal behavior when it was adopted the perspective of the loved person in a painful context compared to the other perspectives.

Batson *et al.* (2003) report that self / other perspective-taking may lead to two forms of empathic reaction: (i) other-oriented response (sympathy and compassion); (ii) self-oriented response (personal distress). Along these lines, Goubert *et al.*, (2005) and Singer and Klimecki (2014) empathy for pain models (see Figure 21 for an integration of the two models) suggest that the empathizer’s withdrawal behavior (we recorded it in the OMLF perspective), is

associated with the affective response of empathic personal distress. In agreement with these theories, it is possible that when the loved person in pain is observed, the observer-empathizer tends to move away in order to protect himself from this emotionally negative charged situation. This observer's reaction occurs in order to avoid the negative feelings of empathic personal distress arising in the observer when his loved person is in pain.

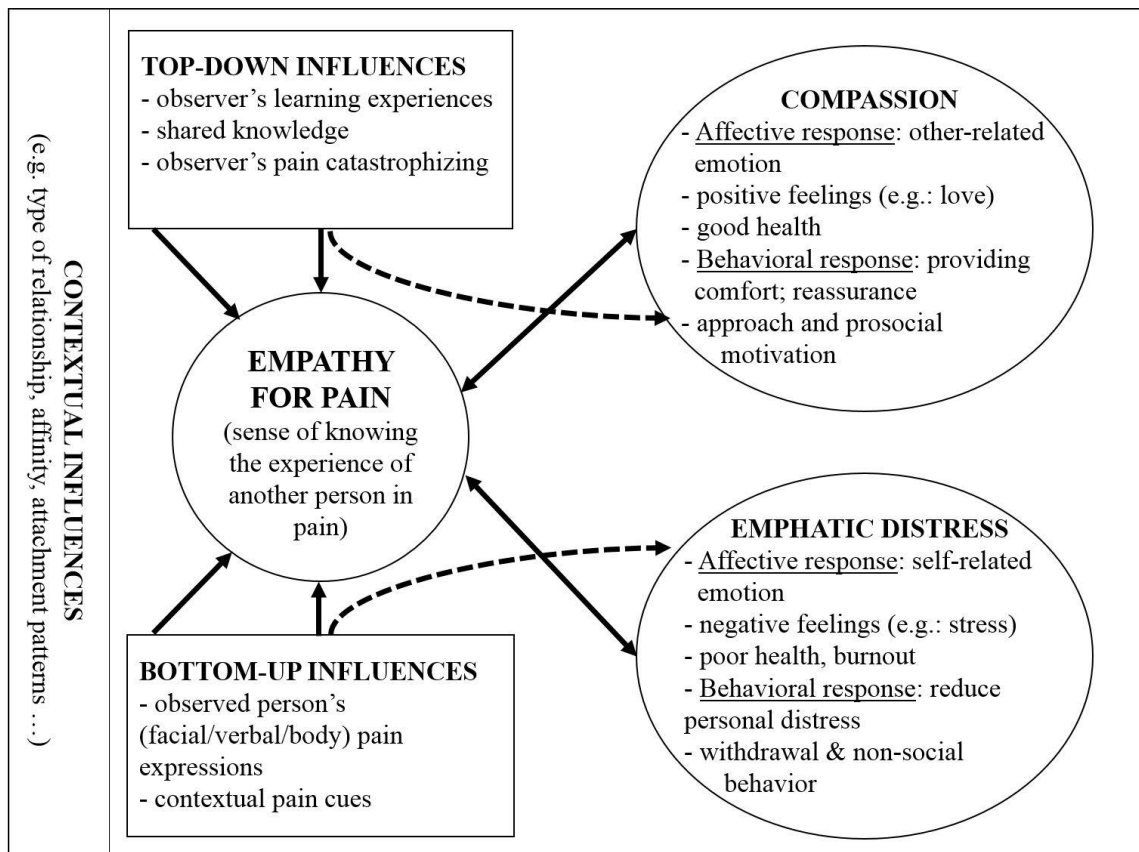


Figure 21. Integration of Goubert et al., (2005) and Singer and Klimecki (2014) models. The authors in their models differentiate between two empathic reactions to the others' suffering: compassion (or sympathy) and empathic personal distress (realized by GB).

In our study, the withdrawal position recorded by postural parameters is in agreement with Tullet *et al.* (2012) results. The authors evidenced that the right frontal EEG asymmetry, normally associated with negative emotions and withdrawal motivation (Harmon-Jones & Allen, 1997) may be related to empathic reactions. Moreover, this relation was mediated by feelings of sadness and personal distress (Tullet *et al.*, 2012). Theories about empathy for pain support the adaptive value of this process. It is possible that the observation of other persons in painful situations induces the observer to anticipate the consequence of the painful events for himself (Morrison *et al.*, 2004) and to avoid adaptively the dangerous situation. The adaptive

role of the empathic pain is supported by recent theories hypothesizing the shared representations network may be better activated by pain-related cues, inducing the observer to adopt self-protective survival behaviors, to avoid danger and threat (Iannetti & Mouraux, 2010; Decety, 2015).

On the contrary, when the most hated person (OMHF) perspective is adopted, the observer tends to assume a more forwarded COP-AP averaged position during the observation of painful stimuli compared to the position adopted for all the other perspectives (at least for the first 8 seconds of stimuli presentation). These results are in agreement with Cikara *et al.* (2014) results: individuals tend to empathize less with whom that belongs to the “out-group” and this is due to the antipathy felt for the same “out-group”. According to Goubert *et al.* (2005) and Singer and Klimecki (2014) theories it is possible that the observer’s more forwarded position recorded for the OMHF might be associated with the absence of empathic distress in the onlooker.

In accordance with a previous study, we recorded greater amplitude of the SCR for the OMLF perspective (Vico *et al.*, 2010). The SCR measure presents the evidence of sweating variations highlighting situations emotionally salient to the individual (Sequeira *et al.*, 2009) and SCR is sensitive to attention and memory (Dawson *et al.*, 2007). Along these lines, we have recorded higher SCR amplitude for the OMLF perspective in accordance with previous studies reporting that to look at the loved person induces a greater positive emotional arousal, which does not depend on the effect of familiarity (Vico *et al.*, 2010; Cacioppo *et al.*, 2012; Guerra *et al.*, 2012). Indeed, we recorded a different response for the other familiar perspectives (Self, OMHF). Anyway, our study did not present evidence of any modulation of SCR response for the valence of the presented painful and non-painful visual stimuli. Lelard *et al.* (2013) also did not record this SCR magnitude increase associated to painful visual stimuli.

Anyway, the EMG highlighted the difference in valence between painful and non-painful stimuli. A significant greater activation of the Tibialis anterior muscle was recorded during the presentation of painful images. The activation of this muscle represents the adoption of a stiffen freezing-strategy. Moreover, this muscle was more activated during the Self perspective. These results are suggestive of the adoption a freezing defensive behavior in the Self perspective, as it was recorded in Lelard *et al.* (2013) study, but the path[COP]-AP data recorded in the Self perspective did not allow us to get conclusion in this direction. Instead, it was recorded a freezing effect for the stranger perspective (OS) compared to the Self and the

OMLF perspectives, but again no interaction with the valence (painful and non-painful) of stimuli was recorded; indeed it was evidenced a generalized freezing effect for this perspective.

In this study, we used the same battery of visual stimuli in the different perspective-taking conditions and in each block we only varied the perspective-taking instructions. This suggests that in this experimental paradigm high-level cognitive variables are investigated (Decety & Lamm, 2007) and in this case the empathic response is mainly modulated by a top-down control.

Limits and future perspectives

The studies described above have some limitations, which give the opportunity to rise further questions and suggest future perspectives. A limit concerning the first two studies (experiment 1 and 2) and generally studies investigating empathy (Dziobek *et al.*, 2008), is that non-controlled factors such as the social desirability may influence the participant's responses. In fact, in both of our first two studies the behavioral pain ratings collected in the observer, were higher for the most loved person (OMLF perspective) rather than for the oneself (Self perspective). This result is in contrast with previous perspective-taking studies that found an egocentric bias (e.g., Epley *et al.*, 2004) and suggested this bias might help to do a distinction between the self and the other (Cialdini *et al.*, 2007). The greater response recorded for the OMLF perspective seems instead to be supported by Aron *et al.* (1991, 1992) theories: the closer the relationship between two individuals, the more they are integrated into a Self-perspective (Bucchioni *et al.*, 2015). In line with the behavioral data, we recorded a greater withdrawal response for the OMLF perspective also for the postural and physiological parameters (experiment 2). Anyway, the pattern of postural and physiological responses collected in our study for the other perspective-taking conditions (Self, OS and OMHF) highlights significant limits that need to be clarified by future researches.

For the Self perspective, there is a lack of coherence between the postural and physiological parameters. The COP's parameters do not permit to conclude if the participant adopted a freezing strategy when he was imaging his own body part undergoing painful event. Instead the EMG of the Tibialis Anterior suggests the adoption of a stiffen strategy for this perspective, as in Lelard *et al.* (2013) study. Moreover, for the observation of painful stimuli it was also recorded a greater EMG contraction supporting the hypothesis that the dimension of

valence (painful or not painful) is associated with approach avoidance and freezing behaviors (Bradley & Lang, 2000). Otherwise, concerning the OS perspective, it was recorded a generalized freezing effect not influenced by the valence of the presented stimuli. For instance, it has been recorded that children have a freezing reaction when they are faced to a stranger (Buss *et al.*, 2004). Anyway, this reaction may be influenced by the effect that children considered the presence of the stranger as a threatening situation *per se*. Obrist (1981) defined the immobility as an attentive posture; indeed, it is possible that the OS perspective may simply induces a state of “attentive immobility” (Marks, 1987) in the observer due to the non-familiar perspective (Curran, 2004). Concerning the most hated person perspective (OMHF), we did not found a clear pattern of activation of postural and physiological responses. The COP-AP averaged position recorded during the presentation of painful images was more forwarded for the OMHF perspective compared to the other perspectives. Anyway, this COP-AP averaged position was not forwarded with respect to the baseline position in each second of picture presentation, probably because of the influence of the painful visual stimuli, which have a negative valence (Cacioppo, Priester & Bermson, 1993). Moreover, the delta COP-AP averaged position for the OMHF perspective results to be shifted backwards, further indicating the influence of painful stimuli on the COP-AP averaged position for this perspective.

Indeed, these data do not permit to achieve certain conclusions about the postural and physiological responses to the exposure of painful visual stimuli in the Self, OS and OMHF perspectives. Furthermore, it is important to evidence that the correlations between postural and physiological and self-report responses are not informative. This limits the possibility to evaluate the postural and physiological responses under the influences of the empathic and anxious observer’s disposition. All these limits are at least in part due to the presence in the experiment 2 of a huge number of variables levels. Indeed, for future studies we suggest to increment the effect size of the sample or to reduce the number of variables (Lenth, 2003).

Postural control task allows to study the natural approach / avoidance and freezing whole body reactions and it is not influenced by the task instructions. Anyway, this experimental context may limit the external validity of the results and does not allow us to make sure conclusions about the observer’s intentions in a real life context. In fact, an alternative possible explanation to our findings is that the witness of other’s painful events may not have the intention to avoid the empathic target, but he may prepare himself to a forward step. In fact, according to Naugle *et al.*, 2011 in the initial part of a forward step preparation it was recorded

a greater backward displacement of the COP in order to facilitate a forward step. In this case, the interpretation of the observer's response will be no more a self-directed behavior, but an other-oriented pro-social response (Goubert *et al.* 2005; Singer & Klimecki, 2014). Furthermore, an eventual empathic approach response would be best supported by studies linking the empathy to the altruism (Batson *et al.*, 2002; Eisenberg *et al.*, 2001) and by the influence that the altruism depends also on the degree of proximity and familiarity felt with the empathic target (Hamilton, 1964).

Along these lines, In order to disentangle these hypothesis, we suggest to do a step initiation task that investigates the observer's step forward (approach) and backward (avoidance) in front of painful and non-painful visual stimuli in the Self, OMLF and OS perspectives. We suggest to present only three perspectives in order to reduce the number of variables. This may allow a better interpretation of our OMLF perspective results. Indeed, this new experiment would permit to know if the witness of the loved person in pain tends to approach (associated to compassion and to a pro-social behavior; Batson *et al.*, 2002) or to avoid (associate to personal distress and self-directed behavior; Grynberg, & Maurage, 2014) the loved person in pain. Postural parameters and response time will be analyzed. These measures will include the COP AP and ML displacements, postural immobility (i.e., the length of path[COP]-AP recorded starting from the onset of picture presentation until 250ms), the RT, the movement time (i.e., time interval between the reaction time and the final position of the COP) and the peak velocity (i.e., maximum speed during the step execution). These and others parameters have been previously recorded in Stins *et al.*, (2011) experiment. Self-report measures will be administered to the subjects including the Interpersonal Reactivity index (IRI; Davis, 1983) and the 20-item Self-Report Altruism scale (SRA) (Rushton *et al.*, 1981). These self-report measures will give information about participant's empathic and altruistic dispositions.

We propose that if the faster forwarded response is recorded when the observer of painful images adopts the OMLF perspectives, compared to the Self and OS perspectives, this would suggest that the observer apply a pro-social behavior (Kemp, & Guastella, 2010) and that the loved person perspective would specifically influence this onlooker's pro-social reaction (Hamilton, 1964). The approach pro-social behavior may not necessarily be directed by a real intention to help the individual in a painful situation. It is also possible that the observer adopted a pro-social behavior in order to reduce his own feelings of personal distress. This can

be considered an emotion regulation strategy: the observer of other's painful events may avoid to be overwhelmed by feelings of personal distress engaging himself to help the empathic target (Cameron & Payne, 2011; Tullet et. al, 2012). Self-report measures analysis will be helpful in order to define the nature of this altruistic behavior. On the other hand, it is also possible to record a withdrawal behavior of the OMLF perspective, as we recorded in the experiment 2. This response should be represented by a faster observer's backward response for the OMLF perspective during the observation of painful stimuli. Indeed the withdrawal behavior associated to the feeling of personal distress would be confirmed, underling that empathy is not necessarily associated with pro-social behaviors (Singer & Lamm, 2009). This response would evidence the possibility that the witness of other's pain has in avoiding a helpful interaction as often happens in real-life situations (Cesario *et al.*, 2010). For the Self perspective we would expect to record a lower length of path[COP]-AP, and a reduced postural mobility confirming the presence of a freezing strategy when the observer is faced to painful images adopting a first-person perspective (Lelard *et al.*, 2013; experiment 3: Bucchioni *et al.*, under review).

1.2. The influence of self / other perspective during the observation of painful stimuli in the corticospinal system

In the third study (experiment 3; Bucchioni *et al.*, under review) we investigated the role of the self / other perspective in the corticospinal system during the observation of painful and non-painful stimuli. According to the "mirror-matching" simulation mechanism, the emotional state of an individual activates corresponding representations in another individual observing that state (Gallese, 2003; Keysers *et al.*, 2004). Recent studies showed that motor responses similar to those present in one's own pain (freezing-effect) occur as a result of pain observation in others. In agreement with the "mirror-matching" simulation theory, this finding has been interpreted as the physiological basis of empathy (Avenanti *et al.*, 2005, 2006, 2009, 2010). Alternatively, we suggest that it can represent the physiological counterpart of an embodiment phenomenon, related to the sense of body-ownership (Ehrsson *et al.*, 2004). In recent years, the increasing interest for the concept of body-ownership (i.e., the belief that a specific body part belongs to one's own body) pays specific attention to the relation between the perspective through which a body-part is observed and the possibility for the subjects to experience it as

part of their own body (i.e. embodiment phenomenon) (Costantini and Haggard, 2007; Ferri *et al.*, 2003). In the present study, we aimed at disentangling the empathy and the ownership hypothesis, by manipulating the perspective of the observed hand model receiving pain, so that it could be a first-person perspective, the one in which embodiment occurs, or a third-person perspective, the one in which, in everyday life, we perceive the body parts of others. Specifically, we investigated the pain-specific freezing effect that occurs as result of pain observation in the onlooker (Avenanti *et al.*, 2005) which is similar to the freezing response recorded in one's own pain (Farina *et al.*, 2001; Urban *et al.*, 2004). In this experiment, we used the experimental paradigm previously validated by Avenanti *et al.* (2005) in the first-person perspective: participants had to watch video-clips of a hand-model deeply penetrated by a needle or touched by a Q-tip. Moreover, to study in the onlooker the influence of the self / other perspective during the observations of painful situations, we presented the hand model in the first- (representing the self) and the third-person (representing the other) perspectives. We used a low-level cognitive task because Avenanti *et al.*, (2006) showed only stimulus features modulate the freezing effect recorded in the corticospinal system. In fact the authors found that the task demand (to ask participants to adopt the self or the other perspective) had no effect in modulating the corticospinal excitability. Our results confirmed the registration of the freezing effect found in the observer's corticospinal system during the observation of a needle penetrating a hand model (Avenanti *et al.*, 2005; 2006; 2009; 2010). Moreover, we presented the evidences that this pain-specific freezing effect is perspective-dependant: it only pertains to the first-person perspective, the one in which embodiment occurs. No effects was found when stimuli were presented in third-person perspective, the one we perceive the body parts of others. Indeed this paradigm, where we manipulated low-level cognitive variables and where bottom-up influences, mainly modulated the observer's response, seems to allow to evidence in the corticospinal system a self-defensive freezing response, supporting the adaptive role of other's pain observation (Decety, 2015; Yamada & Decety 2009). Furthermore, the MEPs amplitude significantly predicts the extent to what the participants reported in the body-ownership questionnaire. In fact, while the participants were observing the hand model penetrated by a needle, they felt to feel "as if" their own hand was penetrated. That is, the greater was the subjects' self-report the lower was the MEPs amplitude, representing a freezing effect.

The present findings suggest that the freezing effect during pain observation can be better explained by the body-ownership than by the empathy for others' pain hypothesis.

Converging evidence, coming from experimental manipulations in healthy subjects (e.g., rubber-hand-illusion; Botvinick & Cohen, 1998) and pathological conditions after brain damage (e.g., delusion of body-ownership; Garbarini *et al.*, 2013; 2014; 2015), show that the embodiment phenomenon occurs only when the rubber / alien limb is located in a position coherent with the subjects' higher-order and pre-existing body representation, whenever it is perceived from a body-congruent first-person perspective. The data discussed here are suggestive of an "affective" conception of body-ownership (De Vignemont, 2014), indicating that the body I feel as my own is the body I care more about, the one to which I react when under threat.

Limits and future perspectives

In the third experiment of the thesis (Bucchioni, *et al.*, under review), we manipulated the perspective of the observed hand-model receiving pain and we found a pain-specific corticospinal modulation only when stimuli were presented in a first-person perspective, automatically leading to a sort of embodiment. No modulation effect was found in a third-person perspective, the one in which we usually perceive the others. In order to confirm these results, we suggest to investigate the influence of the self / other distinction on the perspective-dependent effect recorded in our study. A possibility is to create a "virtual lesion" of the self / other discrimination by using repetitive TMS (rTMS) over the right inferior parietal lobule (IPL). The subjects undergoing this procedure would be temporarily and selectively inhibited to distinguish the self from the other (Uddin *et al.*, 2006). Indeed, we would organize the experiment in two blocks where the participant would be exposed to an experiment similar to experiment 3 (Bucchioni *et al.*, under review). The main difference of this new experiment is that before the second experimental block, a "virtual lesion" will be performed by means of rTMS over the right IPL. In this new experiment, each subject would be stimulated by 20 min of rTMS at 1 Hz over the right IPL. As described earlier in experiment 3, after the temporary inhibition of the self / other distinction we might expect to find no difference in the corticospinal modulation for the first- and the third- person perspectives during the pain-observation.

Moreover, there is possibility that confounding factors may limit the internal validity of our study. As reported before, the study of corticospinal responses during the observation of painful images are considered as a low-level cognitive task, were mainly bottom-up influences

acts in attuning the participants' response (Avenanti *et al.*, 2006). This suggests that the visual features of the presented stimuli mainly influence the corticospinal freezing effect. If the corticospinal modulation is influenced by the specific characteristics of the observed stimuli, it is possible that we did not record a pain-specific freezing effect in the third-person (the "other") perspective, because of the observed features of the stimuli per se. The use of whole body (with the face blanked out) as stimuli would highlight possible empathic response in the observer's corticospinal system. Indeed in the new experiment, the MEPs amplitudes would be collected from the participant's right FDI muscle during the observation of video-clips depicting a whole body penetrated by a needle in the FDI muscle. We suggest that the observation of a whole body model presented in the third-person perspective would give the evidence of the empathic corticospinal pain-specific freezing effect in the onlooker. In fact, human body posture conveys salient messages, whose perception is at the foreground of social interactions (Borhani *et al.*, 2015). Moreover, processing of others' body emotional state quickly prepares the organism to implement response action (Borgomaneri *et al.*, 2015; Ekman & Davidson, 1994; Lang, 1995). Indeed, we suggest that the observation of a whole body undergoing painful stimulation, would eventually permit to highlight the corticospinal empathic modulation during a low-level cognitive task in the observer.

1.3. Conclusions

In the first part of this thesis, we found that the observation of painful visual stimuli has on the onlooker's behavior. We recorded different defensive responses according to the "self" and the "other" perspective adopted by the observer. The freezing effect recorded in the corticospinal system (experiment 3 Bucchioni *et al.*, under review) is specific for the first-person perspective and it is suggestive of an "affective" conception of body-ownership, referring to a basic sense of the self. In previous study, Lelard *et al.* (2013) used a paradigm similar to our perspective-taking postural task (experiment 2). The authors asked the participants to imagine themselves in a painful situation, and they recorded a whole body freezing response in the self-perspective during the observation of painful situation. Unfortunately, our paradigm (experiment 2) did not clearly highlighted freezing response in this condition. However, we suggest that when the self-perspective is adopted during the observation of painful

event, at least in a low-level cognitive task (when the bottom-up influences mainly modulate the observer's response), a freezing defensive behavior is recorded. In fact, when the observer adopts the self-perspective, an immediate personal involvement is required (Lamm *et al.*, 2008) and an increased activation is evidenced in cerebral regions associated with defensive behaviors (Jackson *et al.*, 2006; Iannetti & Mouraux, 2010). Future studies have to further investigate the self-perspective response during the observation of a painful event in high-level cognitive tasks. Moreover, we recorded a greater withdrawal behavior when is adopted the most loved person perspective during the observation of painful stimuli. This response was recorded in a high-level cognitive task (experiment 2), where top-down influences are predominant. This behavior suggests an empathic self-directed response represented by the adoption of a self-protective behavior in order to avoid feelings of personal distress elicited by the observation of the loved person in pain (Goubert *et al.*, 2005; Singer & Klimecki, 2014).

Overall, these findings suggest that different defensive behaviors are implemented in response to the pain observation in others and these responses are mainly grounded in the self. Self-protective mechanisms during pain observation in others are implemented through the freezing effect and the withdrawal behavior. Specifically the freezing effect recorded in the corticospinal system during the pain observation in others might be conveyed by an embodiment phenomenon. Instead, the withdrawal postural reactions recorded when the observers were imagining the loved person in painful situation, evidence a self-directed empathic response. Further investigations of the approach and the defensive behaviors occurring during the observation of other's pain would better clarify the nature of these mechanisms and in which conditions they eventually led the witness to help the others.

2. Pain anticipation in the corticospinal system

In the last part of the thesis, we focused on the study of the pain anticipation in the corticospinal system. In fact, some evidences support the existence of link between motor system and pain (Sailer, *et al.*, 2002). For instance, when a hand touches hot objects, the heat-pain activates a reflex of withdrawal response (Dworkin, 2007). This adaptive unconscious behavior prevents the hand to further damage, which facilitate the escape. Moreover, studies showed that it is possible to highlight the inhibition of the corticospinal system excitability when painful stimuli are delivered to the participant's hand and the presentation of these

aversive stimuli are associated with the stimulation of M1 via TMS (Farina *et al.*, 2003; Urban *et al.*, 2004). This freezing effect may be induced by different kinds of painful peripheral stimulation (Farina *et al.*, 2001; Farina *et al.*, 2003; Le Pera *et al.*, 2001; Svensson *et al.*, 2003; Urban *et al.*, 2004). It is well known that pain expectancy shares common neural pathway with the actual pain (Fairhurst *et al.*, 2007; Koyama *et al.*, 2005; Porro *et al.*, 2003; Wang *et al.*, 2008). Anyway, no fMRI study up until now has attempted to clarify the role of the M1 and the corticospinal modulation during pain expectancy. Indeed, by using a classical conditioning paradigm, we investigated whether the pain anticipation for aversive stimuli would affect the motor cortex excitability. We applied single-pulse TMS over the left M1 while the participant received electric shocks delivered to the right digit V, and MEPs were recorded from the APB and the ADM right hand muscles. We manipulated the participant's pain expectancy by using the classical conditioning paradigm including the presentation of visual and auditory stimuli conditioned by pairing the aversive and painful electric shock.

We recorded a significant decrease in MEPs amplitude not only during the CS+ condition (i.e., conditioned visual and auditory stimuli were associated to the painful electrical shock) but also the CS- condition (i.e., the condition in which conditioned visual and auditory stimuli were not associated to the painful electrical shock) compared to the baseline neutral stimuli (N condition). SCR results were in agreement with the MEPs amplitude response and with previous studies (Masi *et al.*, 2014): SCR was higher for CS+ condition (when actual pain was present) compared to N condition and crucially SCR amplitude was greater for the CS- condition (when only the pain expectancy was present) compared to N baseline condition. SCR is a good physiological correlate of fear conditioning (Büchel & Dolan, 2000), signaling threat, and in this case the anticipation of threat. Furthermore, a significant negative correlation was found between the two physiological parameters recorded in the present study. In fact when the subject received a painful stimulation (CS-) and when he was expecting to receive it (CS-): the more the SCR amplitude augmented the more the MEP amplitude decreased.

Our results corroborate the notion of pain as a highly subjective sensory experience, which is influenced by the relation among the expectations of the incoming stimuli and the real sensorial stimulations (Koyama *et al.*, 2005). Top-down cognitive processes including pain catastrophizing, learning experiences (e.g., habituation or pain avoidance) and attentive processes are important in the modulation of pain perception (Sambo *et al.*, 2010). Indeed, the expectation of a painful event (i.e., pain anticipation), associated with fear and anxiety plays an important role in the pain perception (Ploghaus *et al.*, 2003).

Moreover, our results suggest that the pain anticipation of aversive stimuli affects the motor cortex excitability, inducing a preparatory defensive freezing behavior to what is perceived as the incoming aversive stimuli. These findings further supported the evidence of involvement of circuits in the elaboration of painful stimuli that is, the so called “Pain Matrix” (SI, SII, AI, ACC) are no more considered as specifically involved in the elaboration of painful stimuli. Rather this network may generally be activated by the direct or indirect exposure to threatening situations and it is adaptively associated with withdrawal behaviors (Iannetti & Mouraux, 2010).

Along these lines of research, the registration of the freezing anticipatory response in the corticospinal system recorded in the present study might represent the defensive preparation of the organism to dangerous event exposure. The activation of these circuits might co-occur with defending response to prevent dangerous events (Avenanti & Aglioti, 2006).

Limits and future perspectives

In the last experiment of the thesis, we investigated the pain anticipation of the corticospinal system when the participant was directly undergone to a peripheral painful stimulation. Results showed that both MEPs and SCR amplitudes were modulated by the anticipation of an electric painful shock delivered over the participant’s right hand. These results confirmed the Urban *et al.* (2004) study: during the direct peripheral painful stimulation a reduction of corticospinal excitability (freezing effect) is recorded. It remains unclear if the freezing effect recorded during the first-hand anticipation of a painful event might also occurs in the observer of other individuals undergoing the same situation. In fact it has been previously shown that pain anticipation shares circuits not only with the actual pain (Porro *et al.*, 2003; Wang *et al.*, 2008), but also with the network activated during the observation of other’s pain and during the empathy for pain (Morrison *et al.*, 2004). Along these lines of research, the registration of the anticipatory response in the corticospinal system might represent the defensive preparation of the organism to the exposure to a dangerous event, likely during the observation of a painful event (Avenanti *et al.*, 2005, 2006; Decety, 2015). In Olsson *et al.* (2007), SCR responses and fMRI activations were recorded when participants were directly exposed to painful stimulation during a classical conditioning task (similar to that we used in experiment 4) and when participants were looking at a video displaying a model undergoing painful stimuli during the same classical conditioning task. Olsson *et al.*, (2007) found that

similar neural mechanisms and similar SCR responses occurring during a direct anticipation of painful stimuli and during the observation of painful stimuli in others. We suggest application of the same paradigm used by Olsson *et al.*, (2007) during the registration of the corticospinal excitability. The presentation of a whole body in the third-person perspective in the observed video clip (as in Olsson *et al.*, 2007) would probably allow the registration of a freezing effect during the anticipation of aversive painful events in others, as it happens in a first-hand experience of pain anticipation. In fact, previous studies showed that the feature of the observed stimuli mainly modulates the freezing effect recorded during the observation of others' painful events, and this response is not influenced by the task demand (Avenanti *et al.*, 2006). We suggest that the observation of a whole body undergoing a potential painful stimulation would probably convey clearer information about "the other", allowing to study the others' pain anticipation in the observer's corticospinal system. Otherwise, if the freezing effect due to the anticipation of a painful event would be recorded only in the first-hand experience of pain anticipation, the predominant role of the self-perspective during the elaboration of actual or potential painful stimuli (Jackson *et al.*, 2006) will be confirmed.

3. General conclusion

The main aim of this thesis was to study approach / avoidance and freezing behaviors that may occur during the observation of other's pain and during the first-hand anticipation of pain in oneself. Pain, which is an unpleasant sensory and affective experience, conveys an important defensive message, inducing the individual, once he learned the consequences of painful events, to avoid them (Pavlov, 1927). Overall, the results of the studies discussed herein provided converging evidences with regard to the occurrence of withdrawal and freezing defensive behaviors during the observation of other individuals undergoing painful events and during the anticipation of pain in oneself. Both in our studies during pain observation and in the study of pain anticipation we showed that self-protective mechanisms are implemented through the freezing effect and the withdrawal behavior. During high-level cognitive tasks where mainly top down influences are modulated, withdrawal behaviors emerge when it is imagined a loved "other" performing a painful action. This response suggests self-protective strategies are adopted in the observer in order to avoid feelings of personal distress due to the observed situation. The freezing effect recorded in the corticospinal system is specific for the observation

of the first-person perspective and it is suggestive of an “affective” conception of body-ownership suggesting that “the body I feel as my own is the body I care more about” when it is exposed to a dangerous event. Moreover, the self-protective mechanism seems to be particularly sensitive to potentially dangerous events for the self. In our last experiment, we recorded the freezing effect not only for the actual pain but also for the anticipation of the pain in the self. Overall, these results prove with strong evidences the adaptive role of empathy for pain, pain observation and pain anticipation mechanisms revealing humans adopt defensive behaviors both when they are directly exposed to pain and when they are exposed to others’ pain observation.

CHAPTER V

APPENDIX

In this section is reported a study made in collaboration with the members of the LNFP and the STAPS laboratories in Amiens.

Influence of postural threat on postural responses to aversive visual stimuli

Lelard Thierry, Krystkowiak Pierre, Montalan Benoit, Longin Estelle, Bucchioni Giulia, Ahmaidi, Said, Godefroy Olivier & Mouras Harold. (2014). *Behavioural brain research*, 266, 137-145.

Several studies show that posture can be influenced by the presentation of emotional stimuli (Azevedo *et al.*, 2005; Facchinetti *et al.*, 2006). The objective of the preset study was to determine if the postural threat condition might influence the postural and the physiological reactions to visual aversive stimuli. Thirty-two participants had to stay as firm as possible on a posturographic platform looking at aversive and neutral visual stimuli. These images have been presented in two different conditions: in presence (80 cm in high) or absence of postural threat (on the floor). Here we studied the Center of Pressure (COP) displacement, the Electrodermal Activity (EDA), Heart Rate (HR) and Electromyographic (EMG) variations. HR and the EDA results showed that the subject's emotional state varies as a function of postural threat condition. The anteroposterior (AP) displacement was shifted more backwards during the presentation of aversive stimuli compared to neutral ones in absence (-0,65 vs 0,90mm) and presence (-0,00 vs 0,89 mm) of postural threat. The COP-AP path resulted to be inferior during the presentation of aversive stimuli compared to neutral stimuli in presence of postural threat (16,26 vs 174,66 mm). Our results evidence a withdrawal behavior in response to aversive stimuli compared to neutral ones both in absence and in presence of a postural threat condition.



Research report

Influence of postural threat on postural responses to aversive visual stimuli



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Recent research has shown that emotion influences postural control. The objective of the present study was to establish whether or not postural threat influences postural and physiological responses to aversive visual stimuli. In order to investigate the coupling between emotional reactions, motivated behavior and postural responses, we studied the displacement of the subject's center of pressure (COP) and the changes in electrodermal activity (EDA), heart rate (HR) and postural muscle activation. Thirty-two participants (15 males, 17 females; mean \pm SD age: 21.4 ± 2.3) viewed affective and neutral pictures while standing still on a force platform in the presence or absence of postural threat. The HR and EDA data revealed that the emotional state varied as a function of the postural condition. The mean displacement in the anteroposterior (AP) axis was more rearwards in response to aversive stimuli than in response to neutral stimuli, in both the absence of postural threat (-0.65 mm and $+0.90$ mm for aversive and neutral stimuli, respectively) and the presence of postural threat (-0.00 mm vs. $+0.89$ mm, respectively). An aversive stimulus was associated with a shorter AP COP sway path than a neutral stimulus in the presence of a postural threat (167.26 mm vs. 174.66 mm for aversive and neutral stimuli, respectively) but not in the latter's absence (155.85 mm vs. 154.48 mm, respectively). Our results evidenced withdrawal behavior in response to an aversive stimulus (relative to a neutral stimulus) in the absence of postural threat. Withdrawal behavior was attenuated (but nevertheless active) in the presence of a postural threat.

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1. Introduction

Although relationships between emotional and motor systems were even described by Darwin [1], they were not extensively characterized until quite recently. Darwin argued that emotion induces an adaptation of behavioral response as a function of the triggering environmental context. Thus, automatic responses triggered by emotional stimuli have an impact on a species' survival and reproduction [2] and can be viewed as instinctual responses [3]. It is now thought that interaction between the motor and limbic systems explains why emotion influences behavior. It is generally assumed that emotional processes affect behavioral responses at central, cognitive and motor preparation stages [4–6]. Recent research has suggested that emotions influence motor processes [7–10]. Thanks to the development of neuroimaging techniques, the interrelation between behavior and emotion can now be investigated by gathering neuroanatomical data regarding the interface between limbic and motor circuits. For example, the basal ganglia are involved in the physiological expression of emotions [11] as well as in involuntary movements (such as gait and posture).

Behavioral responses appear to be context-dependent, as illustrated by the example of the defensive response to an aversive stimulus (e.g. fear behavior in animals). The defensive behavior system can produce different responses (e.g. freezing or aggression) as a function of the level of fear, which in turn may depend on the proximity and intensity of the triggering stimulus [12–14]. Some studies in humans have demonstrated that behavior changes according to the degree of postural threat and so lend support to the environmental effect hypothesis. In several previous studies, the environment in which a postural task was performed was modulated by having participants stand on an elevated surface 80 cm above the ground (either at the edge of some distance away [15–23]). Given that a fall from that height would be likely to cause some degree of injury or discomfort, the researchers hypothesized that standing at the edge of an elevated surface would increase a participant's will to control postural sway. This emotional state was referred to as “fall anxiety” or “postural anxiety” [22,23] and can be assessed by monitoring postural responses. When the participant was standing on the elevated surface, increased skin conductance evidenced a change in psychophysiological state [24,25] – presumably because the participant felt less confident, less stable and more anxious of falling when standing at that height [26,27]. Even though the biomechanical constraints for maintaining postural balance on the stable, elevated surface and on the ground were the same, the results evidenced a change in the balance control strategy. When standing on the edge of an elevated surface, both young and older adults changed their postural control strategy as a function of the level of fear experienced [15–21,23]. Hence, the subjects were generally found to sway less in the postural threat condition than when standing on the ground. Although bipedal stance on the elevated surface was stable and safe, the subjects nevertheless sought to minimize the amount of sway in this condition. This was interpreted as the adoption of a “freezing” behavior in response to postural anxiety. Indeed, a number of researchers have studied the relationship between “freezing” behavior and postural threat by monitoring displacements of the whole-body center of mass (COM) or center of pressure (COP) and mean changes in leg and ankle muscle activation [16,21]. These studies showed that the amplitude, area and standard deviation of COP displacements decreased as the postural threat increased. Significant increases in mean tibialis anterior (TA) activity and muscle co-activation (described as increased muscle stiffness) were observed with increased postural threat.

Recent research has shown that the presentation of emotionally evocative pictures affects various physiological responses and

postural indices. Most of these studies used a set of color pictures selected from the International Affective Picture System (the IAPS, encompassing semantic categories such as a mutilation, threat, erotica [28]) to induce an emotional state and then assessed the latter's incidence on approach-withdrawal motor behavior. Lang et al. [28] explored psychophysiological, behavioral and neurophysiological reactions in subjects viewing these visual affective stimuli [29]. In terms of subjective judgment, the highest agreement was obtained for pictures depicting mutilation, which are consistently rated as evoking disgust by both men and women [29]. In terms of physiological changes, electrodermal activity (EDA, reflecting sympathetic nervous system activity) was greater when viewing pleasant or unpleasant pictures than when viewing neutral pictures. These findings suggest that a measurable change in EDA merely corresponds to high motivational activation and may not be sufficient to differentiate between responses to appetitive and defensive stimuli. The heart rate (HR) responds specifically to pleasant and unpleasant visual stimuli [30]. Greater initial heart rate deceleration was associated with viewing unpleasant contents [31]; this suggests that regardless of arousal, unpleasant contents prompt greater initial attention (relative to appetitive stimuli). However, only a few recent studies have focused on the effects of emotion on the postural control system [22,32–37]. Several researchers have argued that behavior is governed by motivational circuits as a function of the stimuli's valence and intensity, with pleasant events (appetitive stimuli) triggering an approach response and unpleasant events (defensive stimuli) triggering withdrawal [22,28,29,38,39]. In humans, motor responses and related postural responses to an emotional stimulus have been mainly observed during the presentation of aversive visual stimuli (i.e. stimuli corresponding to unpleasant, highly arousing situations [22]). In most previous studies, the motor response corresponded to a freezing strategy.

Postural changes and body movements can be described posturographically by analyzing the trajectory of the center of pressure (COP [41–43]). The mean COP position in the anteroposterior axis is a good index for objectively studying approach-withdrawal behaviors [40]. Although human postural responses to visual emotional stimuli have been described in several recent studies, the findings showed two types of defensive responses [32–34,36]. All the studies clearly showed that the presentation of unpleasant pictures had an effect on postural control. However, the posturographic responses to unpleasant pictures differed from one study to another and thus were interpreted differently. Some researchers have described withdrawal behavior in response to unpleasant visual stimuli [34], while others have evidenced a freezing strategy [24,32,33,36] by evidencing a smaller COP area, shorter COP path or standard deviation of the COP displacement. There is some evidence to suggest that evaluation of the valence of a stimulus occurs immediately (without the need for attention), leading to an automatic response [40,44]. However, to the best of our knowledge, the effect of the environmental context on physiological and postural automatic responses to an aversive stimulus has not previously been studied.

The objective of the present study was to better characterize the effect of the environmental context (through induction of a postural threat) on the body's postural and physiological responses to aversive stimuli. Our starting hypothesis was that (i) postural threat would influence the postural behavior generally encountered in response to an aversive visual stimulus and (ii) this influence would be evidenced by changes in the COP trajectory (e.g. reduced COP area and COP path), while the mean COP position remained unchanged. Mean muscle activity and physiological indices were also recorded, in order to understand the putative mechanisms through which changes in ankle stiffness and muscle activation are achieved during presentation of aversive visual stimuli and in the

presence and absence of postural threat. We further hypothesized that the arousal of the experimental condition would influence (i) EDA (with the highest values in the most-aroused condition, i.e. postural threat and presentation of aversive stimuli); (ii) HR (with the lowest values in the most-aroused condition) and (iii) electromyographic (EMG) activity of the TA and the soleus (SO) muscles (with the highest values in the most-aroused condition).

2. Materials and methods

2.1. Participants

Thirty-two right-handed, healthy participants (15 males, 17 females; mean \pm SD age: 21.4 ± 2.3) were included in the study and gave their informed, written consent to participation. All the participants were students recruited from the Jules Verne University of Picardy (Amiens, France) and received financial compensation for their participation. The experimental procedures complied with the ethical standards of the Declaration of Helsinki and were approved by the local ethical committee (Comité de Protection des Personnes Nord-Ouest II, Amiens, France).

2.2. Stimulus materials

Ten pictures from the IAPS [28] were presented on a screen (projected picture size: 120 cm) using a video projector, while participants stood barefoot in the middle of a force platform (Satel, Blagnac, France) located 2 m from the screen. We selected five aversive pictures representing mutilation scenes (IAPS 3000, 3010, 3060, 3080 and 3100) and five neutral pictures representing household objects (IAPS 7001, 7004, 7009, 7150 and 7025). The emotional pictures were matched for mean luminance (104.5 ± 32.1 Cd/m² for mutilation and 107.4 ± 32.0 Cd/m² for neutral pictures, $p=0.90$). Increased arousal is a good index of increased activation of the motivational system and more intense affective responses [45,46]. Moreover, the arousal level produced by a given stimulus may influence postural responses [22].

2.3. Data collection

Participants were asked to maintain a comfortable, quiet, bipedal stance, with their arms by their side, a heel-to-heel distance of around 3 cm and each foot pointing outwards by 30° relative to the AP axis (i.e. forming an angle of 60° between the long axes of the feet). Stimulus presentation was controlled by a computer running E-Prime software (Psychology Software Tools, Inc., Pittsburgh, PA). Posturographic and physiologic data were recorded simultaneously at a rate of 1000 Hz using a Biopac MP150 system (Biopac Inc., Goleta, CA, USA). Analog data from three strain gauges were recorded and movements of the COP in the anteroposterior (AP) and mediolateral (ML) axes were computed with AcqKnowledge software (Biopac Inc., Goleta, CA, USA). Electromyography of the TA and SO muscles, electrocardiography (ECG) and EDA measurements were also performed. The EMG data were collected using bipolar Ag/AgCl surface electrodes (EL503, 8 mm diameter, Biopac Inc., Goleta, CA, USA) positioned on the left leg only. Prior to electrode positioning, the skin was slightly abraded and cleaned with an alcohol solution (in order to keep the inter-electrode impedance below 5 k Ω). To ensure good electrical contact, an electrolyte gel was applied between the skin and the electrodes. For the TA, the electrodes were placed (2 cm apart, center-to-center) over the belly of the muscle. For the SO, the electrodes were placed vertically 2 cm below the insertion of the gastrocnemii on the Achilles tendon. The HR was recorded with a standard lead II electrocardiogram using three disposable electrodes (EL503, Biopac Inc., Goleta, CA, USA). Electrodermal activity

was recorded with two Ag/AgCl electrodes (GSR100C, Biopac Inc., Goleta, CA, USA) filled with isotonic paste and attached to the volar surface of the index and middle fingers of the participant's hand. A constant-voltage device was used to apply 0.5 V across the electrodes.

2.4. Procedure

First, participants were asked to produce maximal voluntary contractions (MVCs) of the SO and the TA. The subjects were in a sitting position (with the knee and ankle joints at angles of 90°) and were instructed to produce three maximal plantarflexions and three maximal dorsiflexions. Each MVC was sustained for 5–8 s. To facilitate maximum force production during the sustained contraction, verbal encouragement was given by the experimenter and participants were able to see the degree of electromyographic activity on a computer screen.

All postural tests were performed in a quiet room with no fluctuations in sound or light levels. Neutral and aversive visual stimuli were presented in random order. For each picture, a trigger corresponding to each type of emotional stimulus was sent to a Biopac MP150 system (Biopac Inc., Goleta, CA, USA). For each trial, stimulus presentation was preceded by a fixation cross for 0.5 s. The stimulus was presented for 12 s and the inter-stimulus interval was 2 s (Fig. 1b). There were two experimental conditions (Fig. 1a), with the participant standing (i) at ground level (no postural threat) or (ii) 80 cm above the ground (postural threat). The order of performance of the two experimental conditions was randomized. The force plate was positioned on the ground or on a table, depending on the postural threat condition. When the platform was positioned at the edge of the table, we checked that it was stable and rigid. When the subject stood in the middle of the force platform, his/her toes were 5 cm from the edge of the table. It is important

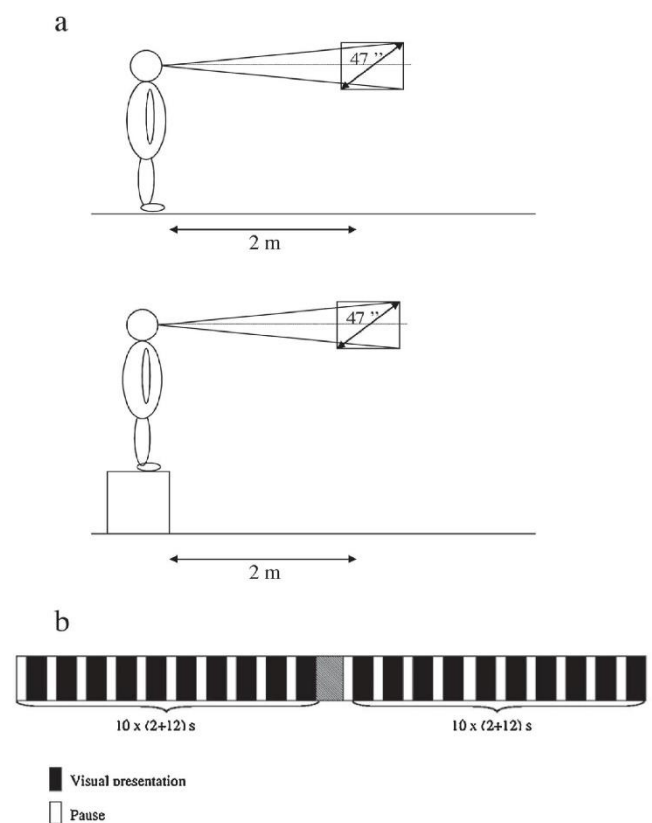


Fig. 1. The experimental design (a) and timeline (b).

to note that the same pictures were shown in both postural threat conditions.

2.5. Data analysis

For each subject, data for both neutral and aversive stimuli (the outcome measures) were averaged for the five trials lasting 12 s each. The following indices were calculated for each test: (i) the mean COP displacement in the AP axis (COP-AP, in mm, corresponding to the difference between the COP position during the stimulus presentation and the COP position one second before stimulus onset), (ii) the length of the COP sway path in the AP axis (path [COP]-AP, in mm); (iii) the area encompassed by COP displacements (COP-area in mm², corresponding to the surface area of the confidence ellipse containing 90% of the sampled COP positions); (iv) the standard deviation of the COP displacement in the AP axis direction (COP-SDAP, in mm); and (v) the standard deviation of the COP displacement in the ML direction (COP-SDML, in mm). As reported above, the calculation of these postural indices [42] can reflect approach-withdrawal behavior or the adoption of a freezing strategy. The level of muscle activation was quantified by calculating the root mean square (RMS) of raw data over 500 ms, with a sliding time window. The RMS-TA and RMS-SO corresponded to the level of activation of the TA and SO muscles, respectively (expressed as a percentage of the MVC). On the basis of the RMS from the 500 ms sliding window for the SO and TA, a peak value was determined and used to normalize RMS values. To evaluate the level of leg muscle activation, the RMS data recorded from the TA during the postural measurements were divided by the corresponding RMS data recorded during a plantar MVC and expressed as a percentage. Likewise, the RMS data recorded from the SO during the postural measurements were divided by the corresponding RMS data recorded during maximal dorsiflexion and expressed as a percentage [47,48].

A TA-SO co-activation index was also calculated: $(\sum_{i=1}^{i=12} (\text{RMS low}_1/\text{RMS high}_1)(\text{RMS low}_i + \text{RMS high}_i))$ [48,49]. The HR (in bpm) was calculated from the ECG signal using Acq-Knowledge Software (Biopac Inc., Goleta, CA, USA). The EDA signal was integrated over the 12 s of stimulus presentation and expressed in arbitrary units. In order to study the effect of postural threat on the postural response to an aversive stimulus, the 12 s means of these postural and physiologic data were analyzed in a 2 (postural threat/no postural threat) \times 2 (aversive stimulus/neutral stimulus) repeated-measures analysis of variance (ANOVA). Intercondition differences were tested in a pairwise Student's *t*-test. The threshold for statistical significance was set to $p < 0.05$ for all analyses.

A time course analysis was also performed (by averaging the data over a 1 s sliding window), in order to determine the onset time of any differences in COP-AP, HR and EDA. The time course data were also recalculated as a percentage of the initial value over

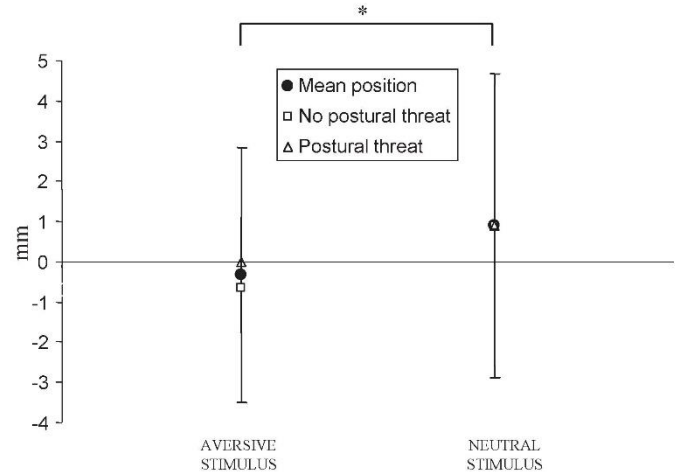


Fig. 2. Mean \pm SD anteroposterior COP position as a function of the stimulus (aversive vs. neutral) and postural threat (absence vs. presence). Significant differences are indicated as follows: * $p < 0.05$ when comparing aversive and neutral stimuli.

the first second, to describe changes over time after the first second. In order to study the time-domain responses to aversive stimuli and postural threat, the data were analyzed in a 2 (postural threat/no postural threat) \times 2 (aversive stimulus/neutral stimulus) \times 12 (time (s)) repeated-measures ANOVA. Again, intercondition differences were tested in a pairwise Student's *t*-test and the threshold for statistical significance was set to $p < 0.05$ for all analyses. We calculated the observed power using SPSS software (version 17.0.1, SPSS Inc., Chicago, IL).

3. Results

The data on postural and physiological responses to aversive visual stimuli are summarized in Table 1.

3.1. Postural responses to emotional visual stimuli and postural threat

A two-way ANOVA demonstrated a significant effect of stimulus on COP-AP [$F(1, 32) = 6.71, p < 0.05, \text{power} = 0.709$], due to a rearward COP-AP displacement for mutilation stimuli ($M = -0.32 \pm 3.16$ mm) and a forward position for neutral stimuli ($M = 0.90 \pm 3.79$ mm). These results are illustrated in Fig. 2. As reported in Table 1, this difference was observed in both the absence of postural threat (-0.65 vs. $+0.90$ mm for mutilation stimuli and neutral stimuli, respectively; $p < 0.05$) and its presence (-0.001 vs. $+0.89$ mm, respectively; $p = 0.19$).

A two-way ANOVA revealed a significant effect of postural threat on path [COP]-AP [$F(1, 32) = 16.44; p < 0.001, \text{power} = 0.976$].

Table 1
Postural and physiologic responses to neutral visual stimuli (household objects) or aversive visual stimuli (mutilation scenes) in the absence or presence of postural threat.

	No postural threat		Postural threat	
	Neutral	Mutilation	Neutral	Mutilation
COP-AP (mm)	0.90 (2.25)	-0.65 (3.59)	0.89 (2.82)	-0.001 (2.09)
Path [COP]-AP (mm)	154.48 (38.28)	155.85 (38.17)	174.66 (49.5)	167.26 (47.29)
COP-area (mm ²)	131.59 (107.95)	140.89 (113.59)	140.66 (120.50)	122.33 (89.46)
COP-SD _{AP} (mm)	3.29 (1.37)	3.48 (1.90)	3.20 (1.36)	3.23 (1.14)
COP-SD _{ML} (mm)	2.52 (1.04)	2.91 (2.31)	3.02 (1.74)	3.04 (2.32)
HR (bpm)	91.93 (14.85)	91.03 (14.49)	93.60 (19.99)	92.17 (20.27)
EDA (UA)	165.16 (114.44)	168.71 (109.85)	188.41 (127.27)	191.77 (130.30)
RMSTA (%)	2.68 (3.46)	2.70 (3.42)	4.05 (6.52)	4.72 (8.27)
RMSO (%)	3.87 (4.75)	3.83 (4.83)	5.53 (6.84)	5.48 (6.86)
Index of coactivation (UA)	102.9 (64.4)	104.1 (65.0)	141.9 (164.6)	148.6 (177.2)

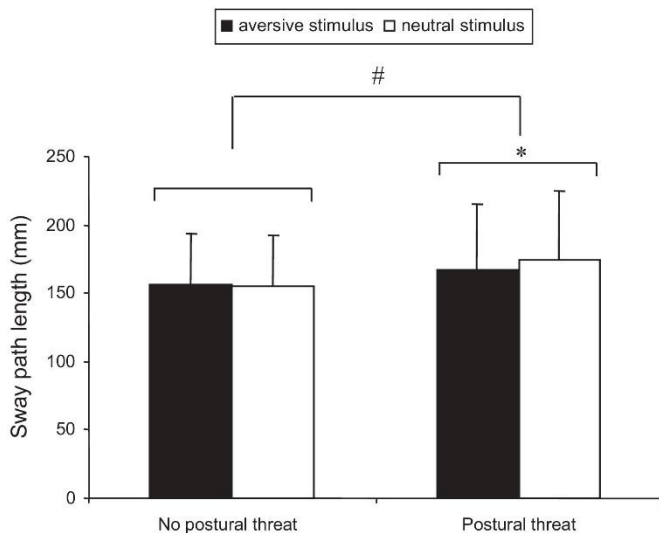


Fig. 3. Mean \pm SD anteroposterior COP path length (path-AP) as a function of the stimulus (aversive vs. neutral) and postural threat (absence vs. presence). Significant differences are indicated as follows: * $p < 0.05$ when comparing aversive and neutral stimuli; # $p < 0.05$ when comparing the absence and presence of postural threat.

These results (Fig. 3) were due to a lower path [COP]-AP in the absence of postural threat ($M = 155.1 \pm 37.9$ mm) than in its presence ($M = 171.0 \pm 48.2$ mm).

A two-way ANOVA also revealed a significant interaction effect between postural threat and stimulus for path [COP]-AP [$F(1, 31) = 7.3, p < 0.05, \text{power} = 0.745$]. To further characterize this interaction and determine whether path [COP]-AP varied according to the presence or absence of postural threat, the response to neutral and aversive stimuli were compared in each of the two experimental conditions. There were no significant differences between the responses to aversive and neutral stimuli in the absence of postural threat ($M = 155.9 \pm 38.2$ mm vs. 154.5 ± 38.3 mm). In the presence of postural threat, path [COP]-AP was significantly lower during the presentation of the aversive stimuli than during presentation of the neutral stimuli ($M = 167.3 \pm 47.3$ mm vs. 174.7 ± 49.5 mm, respectively; $p < 0.01$).

No significant inter-condition differences were reported for COP area (Table 1).

3.2. Physiologic responses to emotional visual stimuli and postural threat

A significant effect of the type of emotional visual stimuli was observed for EDA [$F(1, 32) = 6.8, p < 0.05, \text{power} = 0.715$]. This was due to lower EDA for neutral stimuli ($M = 176.8 \pm 118.6$) than for aversive stimuli ($M = 180.2 \pm 122.3$). A significant effect of postural threat on EDA was also observed [$F(1, 32) = 13.5, p < 0.001, \text{power} = 0.944$]. This effect was due to a lower EDA in the absence of postural threat ($M = 166.9 \pm 111.4$) than in the presence of a postural threat ($M = 190.1 \pm 127.9$).

3.3. Electromyographic responses to emotional visual stimuli and postural threat

Although the mean RMS-TA was lower in the absence of postural threat ($M = 3.32 \pm 2.02\%$ vs. $M = 4.40 \pm 4.45\%$), this difference did not achieve statistical significance [$F(1, 31) = 3.35; p = 0.12$]. However, a significant effect of postural threat on RMS-SO was observed [$F(1, 31) = 5.24, p < 0.05, \text{power} = 0.60$], due to a lower RMS-SO in the absence of postural threat ($M = 5.38 \pm 2.19\%$) than in the latter's presence ($M = 8.79 \pm 8.93\%$). These results are illustrated in Fig. 4.

No significant inter-condition differences in EMG co-activation index were observed. However, we observed a non-significant trend toward a greater co-activation index in the postural threat condition (mainly when viewing mutilation scenes; Table 1).

3.4. Time course analysis

The time-course of postural (COP-AP) and physiological responses (EDA and HR) was analyzed (Fig. 5). Values and associated t -test p values are reported in Table 2 for each time window analysis. The COP-AP was located significantly further back in response to aversive stimuli [$F = 5.91, p < 0.05, \text{power} = 0.65$] and a time-domain effect was observed [$F = 6.5, p < 0.001, \text{power} = 0.99$]. Post hoc tests revealed that when comparing aversive and neutral stimuli, the postural differences started to appear after 3 s. The EDA was significantly higher in response to aversive stimuli [$F = 5.9, p < 0.05, \text{power} = 0.65$] and postural threat [$F = 13.76, p < 0.001, \text{power} = 0.95$], and a time-domain effect was reported [$F = 3.5, p < 0.01, \text{power} = 0.96$]. Again, post hoc tests revealed that differences between aversive and neutral stimuli appeared after the 3 s.

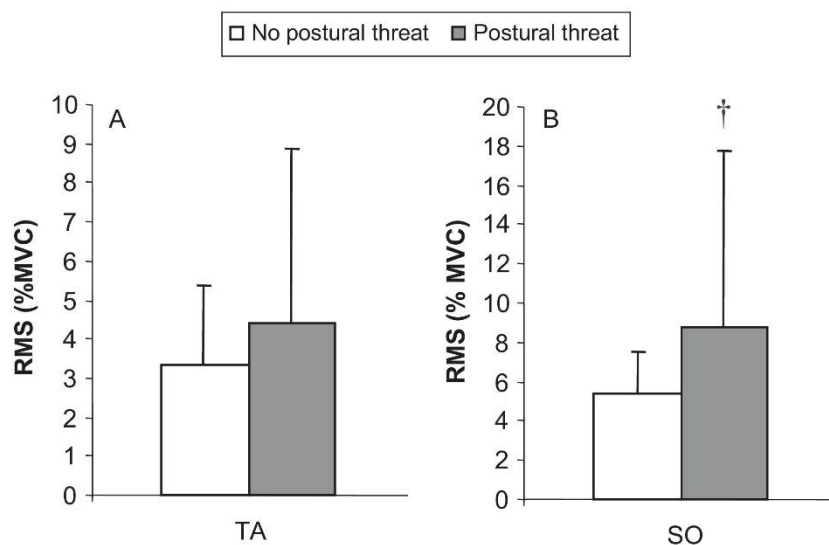


Fig. 4. Mean \pm SD EMG signals for the SO and TA muscles. (A) The RMS as a percentage of the MVC for the TA, as a function of postural threat (absence vs. presence) and (B) the RMS as a percentage of the MVC for the SO, as a function of postural threat (absence vs. presence). Significant differences are indicated as follows: † $p < 0.05$.

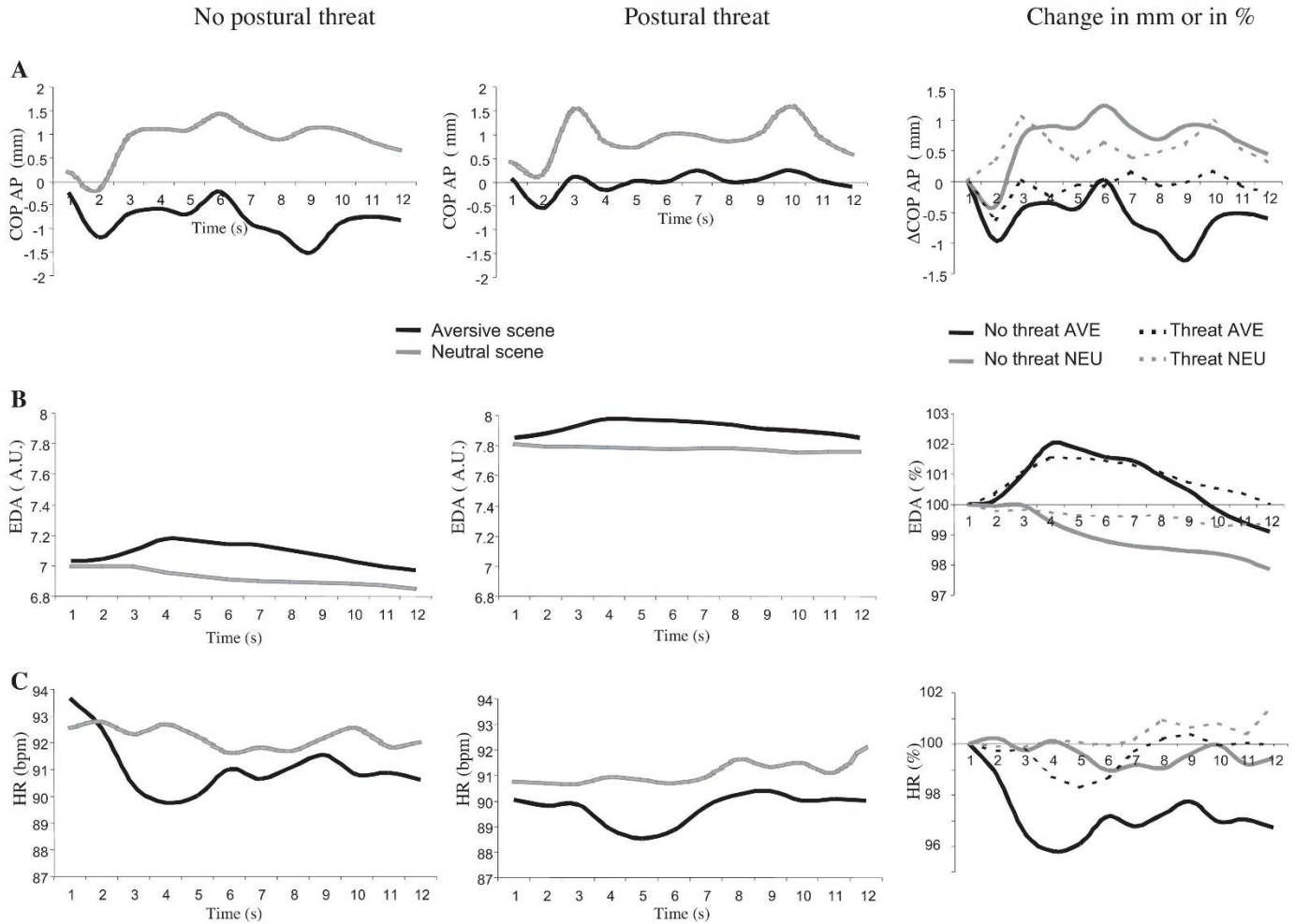


Fig. 5. The changes over time in postural and physiological responses for each visual stimulus (aversive (AVE) vs. neutral (NEU)) and each experimental condition (absence vs. presence of postural threat), in mm or as a percentage over the first second ([0–1]). (A) The mean anteroposterior COP position (COP-AP) value over time in a 1 s sliding window, (B) the mean EDA over time in a 1 s sliding window and (C) the mean HR over time in a 1 s sliding window.

Table 2
Mean PosAP (mm), HR (bpm), EDA (A.U.) values over time in a 1s sliding window in response to aversive (AVE) and neutral (NEU) scenes in absence or presence of the postural threat. T-test was calculated to compare AVE and NEU responses in both conditions of postural threat.

		[0–1]	[1–2]	[2–3]	[3–4]	[4–5]	[5–6]	[6–7]	[7–8]	[8–9]	[9–10]	[10–11]	[11–12]
COP-AP	AVE	-0.23	-1.19	-0.68	-0.59	-0.68	-0.21	-0.87	-1.10	-1.52	-0.87	-0.75	-0.83
	NEU	0.22	-0.19	0.95	1.12	1.10	1.45	1.10	0.90	1.14	1.10	0.86	0.66
	<i>p</i>	0.39	0.08	0.02	0.09	0.10	0.12	0.08	0.08	0.02	0.02	0.08	0.10
COP-AP	AVE	0.08	-0.54	0.11	-0.16	0.03	0.00	0.26	0.01	0.07	0.26	0.02	-0.10
	NEU	0.36	0.72	1.43	1.04	0.71	1.01	0.74	0.84	0.97	1.35	0.92	0.68
	<i>p</i>	0.47	0.02	0.03	0.04	0.30	0.16	0.54	0.40	0.32	0.27	0.40	0.45
HR (bpm)	AVE	93.67	92.55	90.43	89.76	90.02	91.01	90.65	91.10	91.56	90.83	90.90	90.63
	NEU	92.58	92.79	92.35	92.70	92.27	91.63	91.83	91.71	92.21	92.56	91.87	92.05
	<i>p</i>	0.23	0.76	0.07	0.02	0.06	0.59	0.14	0.53	0.47	0.30	0.24	0.09
HR (bpm)	AVE	90.05	89.82	89.85	88.91	88.54	88.86	89.80	90.25	90.40	90.02	90.09	90.02
	NEU	90.77	90.70	90.67	90.94	90.86	90.72	90.93	91.65	91.35	91.51	91.13	92.09
	<i>p</i>	0.49	0.41	0.22	0.02	0.01	0.05	0.22	0.08	0.25	0.05	0.19	0.05
EDA	AVE	7.04	7.05	7.10	7.17	7.17	7.14	7.14	7.10	7.07	7.03	6.99	6.97
	NEU	7.00	7.00	7.00	6.96	6.93	6.92	6.90	6.90	6.89	6.89	6.87	6.85
	<i>p</i>	0.60	0.47	0.16	0.02	0.01	0.00	0.00	0.01	0.02	0.07	0.10	0.10
EDA	AVE	7.85	7.88	7.93	7.98	7.97	7.97	7.96	7.94	7.91	7.90	7.88	7.85
	NEU	7.81	7.80	7.80	7.79	7.78	7.78	7.78	7.78	7.77	7.76	7.76	7.76
	<i>p</i>	0.53	0.18	0.03	0.01	0.01	0.02	0.02	0.03	0.04	0.03	0.07	0.18

The HR was significantly lower in response to aversive stimulus [$F=9.03$, $p<0.01$, power = 0.83].

4. Discussion

In the present work, we studied the postural, physiologic and EMG responses to aversive stimuli in the presence and absence of postural threat. As expected, we found that viewing aversive pictures (randomized among an equivalent number of neutral pictures) induced postural changes (though not always in the ways described in the literature). We also studied how postural threat would modulate postural and physiological responses to aversive visual stimuli (relative to neutral stimuli).

4.1. Defensive responses to aversive stimulus

The present study's first objective was to confirm that aversive stimuli induced defensive responses (independently of postural threat). Physiological data confirm the effect of the arousal manipulated by aversive pictures and postural threat on the mean level of EDA and the change over time in the EDA and HR. Our results suggest that EDA adapts slowly, whereas HR adapts rapidly. In agreement with the literature, we demonstrated an effect of aversive pictures on physiological responses (greater EDA and a lower HR in the time course analysis). This indicates co-activation of sympathetic and parasympathetic systems in response to an aversive visual stimulus [50–52]. Indeed, we found evidence of a decrease in HR (lasting about 2 s; Fig. 5C) following presentation of an aversive visual stimulus [51–56] – confirming that disgust is accompanied by a heightened parasympathetic response. We also observed that induction of disgust is accompanied by an increase in EDA (lasting about 7 s; Fig. 5B), which usually reflects increased sympathetic activation [50–54,56,57].

In the present study, the “defensive” postural response to aversive stimuli was characterized by a rearwards mean COP displacement. The observation of withdrawal behavior in response to aversive stimuli (i) supports the hypothesis whereby the individual tries to avoid unpleasant events and (ii) confirms the involvement of motivational circuits, as previously described [22,33,36,38,40]. However, this aspect needs to be examined further because the properties of the defensive postural response differ somewhat from one study to another and from those used in the present study. The above-cited studies employed a wide range of aversive stimuli (fear, disgust, etc.) even though it is known that a given category of visual stimulus can produce a specific behavioral response. In contrast, our present work used only mutilation scenes as aversive stimuli because they are highly arousing. Indeed, pictures depicting mutilation are consistently rated as evoking disgust by both men and women [29]. In contrast to the present work, the majority of previous studies have found that humans adopt a freezing strategy in response to aversive visual pictures [32,33,36]. Here, we observed a greater significant rearward COP displacement in response to aversive stimuli (relative to neutral stimuli). This phenomenon had previously been reported in a group of women only [34]. We also performed a two (male/female) \times two (postural threat/no postural threat) \times two (aversive stimuli/neutral stimulus) ANOVA but did not observe a gender effect (data not shown). Recently, Eerland et al. [40] also reported withdrawal behavior in response to emotional stimuli. The lack of agreement with our present results can be attributed to several methodological differences, including the randomization of pictures during the presentation. Two literature studies having focused on mutilation scenes [32,33] reported a freezing posture during presentation of an aversive visual stimulus for 3 s in a single block with no interstimulus interval; participants were therefore asked to watch a block of aversive stimuli for 72 s

(24 images) or 48 s (16 images). In contrast, our participants could not predict what kind of picture would be displayed after the 2 s inter-stimulus interval (a black screen). The importance of randomizing picture categories when measuring the valence of a specific picture has been highlighted [40]. Importantly (and in contrast to previous studies), we used a small number of trials so that the risk of fatigue and habituation was mitigated. As suggested by Eerland et al. [40] and given that only a slight rearward COP displacement was observed in the present study, rearward COP displacement in previous research may have been masked by factors related to long exposure times (i.e. postural imbalance, picture complexity, etc.). Moreover, the results of our time-domain analyses also indicated that postural responses appear early (as demonstrated by a difference of 2 or 3 s in the postural response to aversive vs. neutral stimuli).

4.2. The effect of postural threat

One novel aspect of this study was its evaluation of the effect of postural threat on emotional responses to an aversive stimulus. By using the same method in the presence and absence of postural threat, we sought to avoid or reduce the inter-study disparities observed in the literature. As we had hypothesized, EDA was higher when aversive visual stimuli were presented and was higher still when postural threat was added. Indeed, elevated EDA (which has been previously described in response to a high-arousal situation [22]) indicated that the participants did not perceive the postural threat as trivial. This result is in agreement with previous work in which greater EDA corresponded to an altered psychophysiological state when the subject was standing on an elevated surface [24,25]. Participants with greater EDA felt less confident, more fearful of falling, more anxious and less stable when standing on the elevated surface [26]. After performance of a balance task in the postural-threat condition, the participants' perceptions of anxiety (as recorded by a 16-item questionnaire) were 30% more intense [27]. The emotional state encountered during postural threat is therefore referred to as “postural anxiety”. As initially hypothesized, the HR was lower (albeit not significantly) in the postural threat condition and decreased further in response to aversive visual stimuli (the most-aroused condition).

Unexpectedly, we did not observe any freezing strategies (as would have been reflected by less sway) in response to postural threat [15–21,23]. The absence of postural freezing might have been due to the fact that participants were faced with an intermediate (rather than extreme) postural threat, since our participants were standing with their toes 5 cm away from the edge of the table [16]. Moreover, in the present study, trends for postural and EMG data are consistent with those reported by Carpenter et al. [16] in the context of an intermediate postural threat. In Carpenter et al.'s study, an increase in the EMG co-activation index was accompanied by (i) a non-significant increase in muscle activity for the most arousing experimental condition and (ii) a non-significant trend toward freezing for an intermediate level of threat.

The presence of an interaction between the stimulus and postural threat was a primary outcome criterion for the postural data but was only observed for path [COP]-AP. In the absence of postural threat, the response to an aversive stimulus was similar to the response to a neutral stimulus. In contrast, presentation of an aversive stimulus in the postural-threat condition was associated with a lower path [COP]-AP than presentation of a neutral stimulus. The lower extent of AP postural sway may correspond to a freezing strategy in the most-aroused condition (i.e. with both an aversive stimulus and postural threat). Unexpectedly, we did not observe a stimulus \times postural threat interaction for COP-AP. However, the mean position of COP-AP corresponded to greater rearward motion for aversive stimulation in the absence of postural threat

(−0.648 mm) than in the postural threat condition (−0.001 mm). A reduction in the rearward position of the mean COP-AP over a 12 s period is also consistent with the presence of a freezing strategy in the most-aroused condition. Previous studies have indicated that motor and cortical responses to perturbed balance can be modulated by postural threat [24–27]. These changes specifically affect late responses and not early responses [25]. The literature data emphasize the fact that some important survival responses (i.e. fall avoidance) cannot be canceled out by environmental constraints. Interestingly, our present results were quite similar (in as much as the response could not be abolished) when looking at the change over time in the COP-AP in response to emotional stimuli in the presence or absence of postural threat (Fig. 5). Even though no interactions were observed, the *t*-tests (Table 2) revealed that only the early postural response to an aversive stimulus differed from the response to a neutral stimulus in the postural threat condition, whereas both early and late postural responses differed when comparing aversive and neutral stimuli in the absence of postural threat. However, our study provides evidence that the motor response to emotional stimuli may differ as a function of environmental parameters or the subject's emotional state (e.g. postural anxiety). These may also be confounding factors that may explain the observed inter-study disparities in postural responses to arousing pictures [40].

In conclusion, we observed a rearward COP displacement in response to aversive visual stimuli (a mutilation scene) independently of postural threat condition. A previous study reported that pictures representing attacks and pictures of human mutilation prompted the strongest evidence of defensive activation [31]. To the best of our knowledge, this is the first time that a rearward COP position has been observed in participants of both genders.

Deeply anchored defensive responses have an important role in the survival of the species. As has been suggested for dual cognitive tasks, individuals may be required to give priority to a postural task [56] at the expense of an emotional task. Aversive stimuli are the most threatening emotional stimuli from a survival perspective, and instinctual responses to aversive stimuli are reflex responses that have evolved to facilitate the survival of individuals and species [31,58,59]. From an evolutionary perspective, instinctual responses to aversive stimulation must be maintained whatever the environmental context. Our present results showed that postural threat involves specific responses, including a tendency to show freezing behavior when highly aroused. However, early-onset responses (such as the automatic responses triggered by emotion) appeared to be unaffected (Fig. 5). Further studies are now needed to more clearly explain why some studies have reported a postural response indicative of flight, whereas others have found signs of freezing. The present study demonstrates the value of time-domain analyses of physiological and postural signals, with a view to better understanding the interaction between emotion and motion. Environmental conditions appear to affect late-onset responses but not early-onset instinctive and reflex responses. More research is also needed to establish whether a highly accentuated postural threat can significantly inhibit the rearwards postural response observed here for a moderate postural threat.

Acknowledgments

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